Viral Agglutination of Saponin-Lysed Chicken Erythrocytes. (17387)

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The results of recent studies concerning the mechanism of hemagglutination by viruses have brought out certain facts pertaining to the presence, on or within the surface structure of agglutinable erythrocytes, of receptor-like substances which can be removed by appropriate procedures. It has been established,[1] for example, that treatment of human group O erythrocytes with a filtrate of a broth culture of *Vibrio comma* results in a suspension of cells which have lost their capacity to adsorb or be agglutinated by influenza virus. It appears that substances with such receptor-destroying properties produce no drastic alteration of the red cell architecture. This is evidenced by the fact that treatment of a suspension of chicken erythrocytes with influenza virus renders the cells receptor-free without producing any obvious change in the appearance of the suspension or in the morphology of the component cells. On the other hand, normal chicken erythrocytes which have been lysed, or partially lysed, by mechanical trauma, water, or dilute saponin retain the ability to adsorb[2,3] and be agglutinated[4] by influenza virus. A consideration of these facts raised the question as to whether a hemolytic agent such as saponin has no effect on the surface receptors or whether additional "internal" or "nuclear" receptors are exposed by the lytic process. The present paper provides information in support of the former possibility in that it is shown that chicken erythrocytes rendered inagglutinable by treatment with influenza virus are not agglutinated by the virus after lysis in dilute saponin. Experiments in this connection were prefaced by a quantitative study of the agglutinability of saponin-lysed normal erythrocytes by the virus. Methods were developed for the preparation and use of suspensions of lysed cells in agglutination and inhibition titrations.

Preparation of suspensions of lysed cells. It was observed that if chicken erythrocytes were lysed with saponin by appropriate procedures, resulting suspensions were homogeneous, colorless, and opaque. Examination in a wet mount by ordinary or phase microscopy revealed the presence of particles which superficially resembled erythrocyte nuclei. The periphery, however, was not discrete and had a coarsely granular appearance. A considerable amount of amorphous extranuclear material, apparently stroma, was seen in heavily stained films.

Lysed cells can be used in agglutination (CCA) and -inhibition (CCAI) titrations, set up according to the Salk method,[5] only if the suspensions are prepared properly. It was found that almost every step in the procedure is critical. The ratio of number of cells to volume of saponin is of some importance, but of more importance are the concentration of saponin and the time the cells are allowed to remain in the saponin before centrifugation and washing. The lysing procedure that was adopted is as follows: one ml of adequately washed and packed chicken erythrocytes are added to 100 ml of 0.125% saponin. The cells are allowed to lyse for 6 minutes with constant shaking by hand. The suspensions are centrifuged at low speed (1800 R.P.M.), and are washed twice and resuspended in 0.9% NaCl.

Suspensions of lysed cells prepared in this manner were used in the early phases of the work. CCA and CCAI tests were not easy to read, however, in that the pellets and agglutination patterns were colorless. This situation was remedied by the addition of 2-3 ml of a 0.5% aqueous methyl green solu-

[1] Burnet, F. M., *Brit. J. Exp. Path.*, 1946, **27**, 228.

[2] Hirst, G. K., *J. Exp. Med.*, 1942, **76**, 195.

[3] Lajmanovich, S., and Mittleman, N., *Rev. Soc. Argentina Biol.*, 1946, **22**, 339.

[4] Carlisle, H. N., and Elrod, R. P., *Soc. Am. Bact. Proc. Meetings*, 1949, **2**, 92.

[5] Salk, M. E., *J. Immunol.*, 1944, **49**, 87.

tion to the 100 ml of saponin used for lysis. In this manner, the erythrocytes were lysed and stained simultaneously. The use of methyl green stained suspensions definitely facilitated the reading of agglutination and inhibition titrations. Pellets were blue-green in color and the supernatant was colorless. The staining procedure did not affect the agglutinability of the particles. It appears that methyl green does not have the sensitizing effect in this reaction that it does in certain other agglutination systems.[6]

Use of lysed cells in CCA and CCAI tests. Suspensions of lysed erythrocytes were used to measure the CCA capacity of allantoic fluids containing the PR8 and Lee strains of influenza virus. Antisera against these agents prepared in chickens and rabbits were utilized in the CCAI titrations. All tests were set up according to the method of Salk.[5] When methyl green stained suspensions were used, pellets, rings and positive patterns were as discrete, and end-points were as definite and readable as in parallel control titrations in which normal erythrocytes were used. However, since the particles in the suspensions of lysed cells were smaller than erythrocytes, they sedimented more slowly, and a longer time was required for good pellet formation. All tests were read after storage overnight at approximately 4°C.

The end-points of agglutination titrations were governed by the concentrations of the suspension of lysed cells. As the concentration was decreased, the agglutination titer of virus preparations increased. Since this relationship between concentration and titer existed, suspensions of lysed cells could be adjusted with saline so that they yielded the same end-points as were obtained in parallel titrations of virus against normal erythrocytes. And, if such adjusted suspensions were used in inhibition titrations, end-points were the same as when erythrocytes were used. It was determined that suspensions of lysed cells which gave these similar titers contained 2.5 times as many particles per unit volume as did the 0.25% erythrocyte suspension used in the control tests. The significance of this observation is being considered and will be discussed in a later communication.

Experiments with receptor-free erythrocytes. From the results obtained with normal erythrocytes it appeared that saponin had no effect on the receptors concerned in the agglutination of the cells by influenza virus. The possibility existed, however, that the surface receptors were destroyed and that additional receptors located within the cell were exposed by the lytic process. In view of this contingency, it was decided to investigate the agglutinability of suspensions of lysed cells prepared from chicken erythrocytes which had been rendered inagglutinable by treatment with virus. Receptor-free cells were prepared by the following method: One ml of adequately washed and packed erythrocytes was added to 9 ml of undiluted PR8 allantoic fluid (Salk titer, 2560). The virus-cell suspension was placed at 37°C for 6 hours, and was shaken gently every 30 minutes. The cells were then washed twice with 0.9% NaCl and packed to the original volume, one ml. The amount of hemolysis and change in color which occurred during the incubation period were negligible and did not exceed that observed in a similarly incubated 10% suspension of normal cells in saline. Receptor-free cells prepared in the above manner were not agglutinated by the virus. When used in Salk titrations, the pellets obtained in tubes containing virus were as well-formed as those in either the treated cell-saline or the normal cell-saline control tubes.

Virus-treated erythrocytes were lysed with saponin according to the method described previously. The resulting suspensions were indistinguishable grossly and microscopically from suspensions of lysed normal cells. They were not, however, agglutinable by influenza virus. Table I summarizes the results of one of three identical experiments in this connection.

Comment and conclusions. From these experiments it would seem that saponin has no effect on the receptors responsible for the agglutination of chicken erythrocytes by influenza virus. Internal receptors, if such exist, are not exposed during the lytic process. The

[6] Berger, F. M., *Brit. J. Exp. Path.*, 1943, **24**, 252.

TABLE I.
Agglutinability of Normal and Receptor-Free Erythrocytes Before and After Saponin Lysis.

	Virus dilutions							
	10	100	200	400	800	1600	3200	Saline
Normal, lysed	++*	++	++	++	++	+R	RR	PP
Receptor-free, lysed	PP	PP	PP	PP	PP	PP	PP	PP
Normal, not lysed	++	++	++	++	++	++	PR	PP
Receptor-free, not lysed	PP	PP	PP	PP	PP	PP	PP	PP

* Duplicate tubes.
\+ Complete agglutination.
R—Ring, partial agglutination.
P—Pellet, no agglutination.

agglutinability of saponin-lysed cells by the virus is, in all probability, due to unaltered surface receptors and not to any substance associated with the nucleus. This interpretation is valid, however, only if it can be assumed that virus particles do not penetrate to the interior of the erythrocytes during treatment with the virus. That such a process could occur seems unlikely in view of present knowledge concerning the interaction between red cell and virus.

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The Tinctoral Demonstration of a Glycoprotein in Whipple's Disease. (17388)

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The etiology and pathogenesis of Whipple's Disease (lipodystrophy intestinalis), a clinical syndrome similar to sprue, has been a subject of speculation since its original description.[1] As its synonym implies, it is believed by most observers to represent an obscure disturbance of fat metabolism.

Black-Schaffer, Hendrix and Handler[2] reported a study of 4 cases which led them to the following conclusion: The disease, in contrast to sprue, may be readily recognized, anatomically, by non-lipid macrophagocytosis in the lamina propria of the small intestine and occasionally the proximal colon, lipogranulomatosis of the mesenteric lymph nodes, absence of significant evidence of chylous obstruction; and clinically, poor fat, glucose and probably protein absorption and the absence of macrocytic anemia.

The characteristic intestinal lesion is a crowding of the lamina propria by macrophages containing an isotopic refractile substance which Whipple found unstainable with osmic acid. This observation has been repeatedly overlooked in the literature, almost all authors assuming a lipid nature for this curious substance. The study of Black-Schaffer, Hendrix and Handler confirmed Whipple's observation. In 3 cases* the phagocytosed material did not stain with Sudan IV, and 2† were likewise negative with Nile blue sulphate as well as osmic acid. Chemical analysis of the intestinal mucosa of two cases revealed no increase, over normal controls, of the lipid content.

The characteristic enlarged, cystic, fat-filled mesenteric lymph nodes (lipogranulomatosis) are so prominent that they have dominated the

[1] Whipple, G. H., *Johns Hopkins Hosp. Bull.*, 1907, **18**, 382.

[2] Black-Schaffer, B., Hendrix, J. P., Handler, P., *Am. J. Path.*, 1948, **24**, 677.

* No suitable material was available from one case.

† No suitable material was available from 2 cases.