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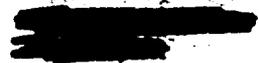
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Concerning the existence of soluble and virus-linked fractions of complement-fixing antigen and hemagglutinin of vaccinia virus.

by R. Wigand and G. Nielsen.

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Archiv f. d. ges. Virusforschung 10, 215-225 (1960). Partial translation.

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Studies of the serological properties of vaccinia virus led to a question concerning the extent to which the virus is separable quantitatively, by fractional centrifugation, from its soluble complement-fixing antigen (S-antigen); a problem that is applicable also to a specific complement fixation reaction (CFR) with S- and virus-linked (V-) antigen. We were also interested in the manner in which S- and V-antigen are formed during the chronological course of cell infection, as part of our studies of the reproduction of vaccinia virus in HeLa cell cultures (1). In this connection we investigated the behavior of vaccinia virus hemagglutinin at high-speed centrifugation. Although it was common knowledge since the studies of Burnet and Stone (2) that it is separable from virus particles, in contrast to the hemagglutinin of myxoviruses, we did not know whether virus particles free of hemagglutinin could be obtained by repeated centrifugation. This paper describes the behavior of complement-fixing antigen (CF-antigen) and of hemagglutinin upon high-speed centrifugation of virus material from rabbit skin and HeLa cultures. Chronological production of S- and V-antigen and of hemagglutinin in the HeLa cell will be published elsewhere (1). We further report on some experiences with CFR utilizing different antigens of vaccinia virus.

#### Discussion

Results of centrifugation tests confirm the existence of a virus-linked and a soluble CF-antigen. The fact that they possess different specificity follows from studies by Mayr and others (9) of a variable course in formation of S- and V-antibodies. It was shown, moreover, that repeated (up to 9-fold) centrifugation fails to produce virus suspensions free of S-antigen, even though the S-antigen content can be reduced to small values. It is not clear whether subsequently liberated S-antigen comes from the surface or the interior of virus particles, or from foreign substances. In addition, V-antigen was found to retain the same CF-activity after repeated sedimentation, even when storage had liberated additional S-activity.

According to investigations by Chu (12) and Mayr (13), there can be no doubt about the serological disparity of S-antigen and hemagglutinin. It is true that both activities behave similarly in supernatants of rabbit skin upon repeated centrifugation (cf. Table 1). However, while the complement-fixing activity in the sediments remained constant upon repeated centrifugation, a steady decrease in hemagglutinin content was noted during treatment, but especially upon storage of the suspension. We conclude

that there is no hemagglutinin which is securely linked to the virus particle (in contrast to complement-fixing V-antigen). Rather, the hemagglutinin is liberated by the virus particle or by accompanying substances, as in the case of S-antigen.

With respect to the behavior of hemagglutinin upon high-speed centrifugation of vaccinia virus from rabbit skin or chorioallantois (Table 5), the studies of Chu (12) and Tagaya (14) showed reduction in the HA titer, which occurred more rapidly in the supernatant and sediment than in our investigations. These findings are easily reconciled with our hypothesis. Upon centrifugation of ectromelia and vaccinia viruses from chorioallantoic membranes, Mayr (13) found rapid reduction of HA titer in supernatants, whereas the decrease was only slight in the sediment. He inferred the existence of a hemagglutinin fraction bound to the virus particle. Mayr's results with respect to sediment deviate only slightly from ours (Table 5), making his interpretation less forceful (we did not subject the virus to frequent centrifugation and storage).

Certain differences in the properties of hemagglutinin found in the supernatant and sediment, as described by Gillen and others (15) and recently by Youngner and Rubinstein (16), may be explained by dissimilarities in the reaction milieu in which the tests were carried out. We therefore endorse the concept expressed by several other authors (17,14,13) that vaccinia virus hemagglutinin is a homogeneous substance. This assumption is supported by recent data of other authors. Hemagglutinating and infective activities of vaccinia virus were separated quantitatively by columnar chromatography (18). Passage through ascites tumor cells produced virus strains that not only failed to show HA, but no longer produced HA-inhibiting antibodies upon immunization (19). These properties would resist explanation if there were two hemagglutinins with different qualities, one of them securely bound to the virus particles.

The behavior of vaccinia virus from HeLa cultures resembles that from rabbit skin upon centrifugation. As shown above, the liquid phase contained almost exclusively S-antigen, the cell phase a major share of the latter. Due to the low activity of HeLa antigens, we limited ourselves to two-fold centrifugation and dispensed with storage tests.

Virus centrifuged after extensive removal of S-antigen gave variable ratios of infectivity:CFR for HeLa cultures; the mean ( $5 \cdot 10^5$ ) was considerably lower than in the case of virus from rabbit skin (2 to  $5 \cdot 10^7$ ) (cf. Tables 1 and 2). The significance of this difference will be discussed elsewhere (1). The ratio of infectivity:HA was within the same order of magnitude; however, this fact has no great importance due to the liberation of hemagglutinin during preparation.

Based on our experience with various CF antigens of vaccinia virus (cf. Table 4), we consider antigens of rabbit skin most suited for practical use. They permit ready production of S- and V-antigen in great quantities and adequate purity. S-antigen with a high titer is prepared by two-fold centrifugation at high speed; the low residual infectivity

cannot be expected to cause serological V-activity. Although this antigen certainly contains far more impurities than that produced by Mayr and others (9) from chorioallantoic membranes by purification, it should also serve practical needs. V-antigen is obtained by repeated centrifugation of virus particles at high speeds; treatment is preceded by a final spin. Anticomplementary activity and reaction with standard rabbit sera are so low that they fail to set in at working dilutions. Thus Craigie and Wishart (20) noted neither reaction with standard sera nor anticomplementary activity in similarly prepared antigens, presumably due to high dilutions. Antigens from HeLa cultures can be stored in a sterile state without freezing, but their relatively weak action and the anticomplementary activity in the liquid phase (5) constitute disadvantages. Antigens from chorioallantoic membranes are not suitable if work is to be done with rabbit sera. The tested antigens of vaccinia virus may be stored without loss of activity at refrigerator temperatures (3 months) if sterile, or frozen for at least half a year.

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