Isolation of a Vaccinia-specific Hapten from Vaccinia-infected Sheep Dermis

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(Accepted 13 May 1968)

SUMMARY

Virus-free buffer extracts of vaccinia-infected sheep dermis were fractionated by elution from DEAE-cellulose with increasing concentrations of NaCl. The fraction eluted with 0.4 M-NaCl was serologically homogeneous when examined in precipitation-in-gel tests. Further fractionation on Sephadex G-200 gave a material which was also physically homogeneous when examined by both ultracentrifugal and electrophoretic techniques. A sedimentation coefficient of 3.47 S and a molecular weight of 31,000 were calculated from ultracentrifugal data. The isolated material contained 74.3% DNA, 9.4% protein estimated as bovine serum albumin and 14.9% carbohydrate estimated as glucose. The DNA contained the bases adenine, thymine, guanine and cytosine in the ratios 1.0:1.1:0.8:0.8. Paper chromatography of formic-acid hydrolysates of the material resolved seven substances reacting with ninhydrin. Qualitative colorimetric tests indicated, apart from deoxyribose, the presence of a hexose and a hexuronic acid.

The serological activity of the isolated material was heat-stable and identical with that of a previously described heat-stable extract of vaccinia-infected rabbit dermis. Treatment with enzymes indicated the presence of two different, serologically active sites. Failure to elicit an antibody response following injection into rabbits suggested that the isolated material was a hapten. Serum absorption studies showed serological identity with an antigen present on the surface of the vaccinia virus particle.

INTRODUCTION

Extracts of vaccinia-infected tissue have been shown to contain soluble substances which react with anti-vaccinial sera (Craigie, 1932). Many workers have attempted to determine the number of virus-specific substances involved in the reaction and most success has been achieved with precipitation-in-gel tests. Gispen (1955) showed the presence of five or six substances by the Oudin technique; Rondle & Dumbell (1962) demonstrated at least eight substances by an Ouchterlony technique and, by a similar method, Marquardt, Holm & Lycke (1965) demonstrated nine substances. More recently, Cohen & Wilcox (1966) resolved seven vaccinia-specific soluble substances by immuno-electrophoresis. Other studies, however, have claimed that as many as 17 substances may be involved in the reaction (Westwood et al. 1965; Rodriguez-Burgos et al. 1966).
Qualitative serological studies of virus-specific, soluble substances in extracts of vaccinia-infected tissue led to the description of LS antigen (Craigie & Wishart, 1936), heat-stable antigen (Smith, 1932; Ch'en, 1934; Parker & Rivers, 1937), and a protective antigen (Appleyard, 1960). The heat-stable antigen has been said to be a degradation product of LS (Smadel & Rivers, 1942) and the protective antigen could not be identified with antigens detected by precipitation-in-gel tests (Appleyard, Zwartouw & Westwood, 1964). Clearly, qualitative definition of virus-specific soluble substances in extracts of vaccinia-infected tissue is largely unresolved. Knowledge of the chemical and immunological nature of these substances would facilitate study of vaccinia virus replication.

This paper describes the fractionation of extracts of vaccinia-infected sheep dermis which resulted in the isolation and partial characterization of an immunologically and physically homogeneous virus-specific material. A preliminary account of this work has been given by Williamson & Rondle (1964).

METHODS

Extracts of vaccinia-infected tissue

The Lister Institute strain of vaccinia virus was used throughout this work. Extracts of infected sheep dermis were a gift from Dr C. Kaplan, Lister Institute of Preventive Medicine, Elstree, Hertfordshire. The infected sheep dermal pulp was prepared essentially as described for rabbit material by Parker & Rivers (1935). Before delivery of pulp extracts to this laboratory, most of the virus had been removed by centrifugation at 10,000 g for 3 hr.

Extracts of infected chick chorioallantois and rabbit dermis served as reference material and were prepared by classical methods modified as described by Rondle & Dumbell (1962).

Preliminary fractionation of vaccinia-infected sheep dermis

The almost virus-free material was dialysed against distilled water until free from salt, water-soluble and water-insoluble materials separated by centrifugation and each fraction dried from the frozen state. The results of precipitation-in-gel studies (see below) suggested investigation of the water-soluble material. Portions of this material were dissolved to a concentration of 5% (w/v) in 0.04 M-phosphate buffer, pH 7.4, and the solution centrifuged at 100,000 g for 3 hr. Following separate recovery of the supernatant fluid and deposit, a portion of the supernatant was dialysed against distilled water until free from salt and dried from the frozen state. The weight of non-diffusible material obtained enabled solid recoveries to be calculated in subsequent experiments. The bulk of the clarified extract was preserved for further fractionation. The precipitate was washed twice with buffer, dialysed until free from salt and dried from the frozen state.

Chromatography on DEAE-cellulose.

A bulk fractionation was performed using 160 g. DEAE-cellulose packed to a height of 62 cm. in a glass column 7.4 cm. diameter. The cellulose was equilibrated to 0.004 M-phosphate buffer, pH 7.4 and 425 ml. clarified extract containing 11.0 g. non-diffusible material, which had been dialysed against the equilibrating buffer, was
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applied to the column. Elution was effected by step-wise addition to the column of 0.004 M-phosphate buffer, pH 7.4, 0.04 M-phosphate buffer, pH 7.4, followed by 0.137 M-NaCl and then 0.175 M- to 0.375 M-NaCl in steps of 0.025 M. Final elution was with 0.5 M-NaCl. All NaCl solutions were buffered with 0.004 M-phosphate buffer, pH 7.4. In this experiment, maintained at 4° throughout, 17.5 l. of effluent were collected in 12 ml. fractions.

Gel filtration

Experiments were done at 4° on a column of Sephadex G-200 with the gel packed to a height of 21 cm. in a column 2 cm. diameter. The results given were obtained when 5 ml. of a solution in 0.15 M-NaCl containing 10 mg. non-diffusible material per ml. were applied to the column, eluted at 12 ml./hr with 0.15 M-NaCl and collected in 5 ml. fractions.

Physical examination of fractions

Column effluents were monitored automatically for light absorption at 2538 Å by a L.K.B. ‘Uvicord’ apparatus linked to a Cambridge Model DE potentiometric recorder. Fractions of interest were examined further in a Hilger ‘Uvispek’ spectrophotometer: this apparatus was used to measure all light absorptions given in this paper. Conductivities were measured at 4° with a Mullard conductivity bridge and appropriate micro-cell. Hydrogen-ion concentrations were measured by a Pye pH meter using Ingold-type combined reference calomel and glass electrode.

Fractions were examined by disc electrophoresis in polyacrylamide gel (Ornstein & Davis, 1962). Analytical ultracentrifugation experiments were done in a Beckman-Spinco Model E ultracentrifuge. Sedimentation data used for molecular weight determination were obtained from an experiment with the material under test at a concentration of 8.80 mg./ml. in 0.04 M-phosphate buffer, pH 7.4. Centrifugation was at 56,720 rev./min. with a rotor temperature of 25° and using schlieren optics with a bar angle of 60°.

Chemical analysis

DNA was determined by the diphenylamine reaction of Dische (1930) using purified calf-thymus DNA as a reference standard. The ratios of purine and pyrimidine bases present were determined by the techniques described by Wyatt & Cohen (1953).

Protein was determined by the quantitative biuret-phenol reaction described by Sutherland et al. (1949); a standard curve was obtained from a series of concentrations of bovine serum albumin. Amino acids were investigated by two-dimensional paper chromatography using as opposing solvents butan-1-ol + acetic acid + water (4:1:5, by vol.) and water-saturated phenol.

Total carbohydrate was assessed quantitatively as glucose by the anthrone reaction modified as described by Morris (1948). Hexoses and some other classes of sugar were detected by the cysteine-sulphuric acid reactions of Dische, Shettes & Osnos (1949). The occurrence of pentose was investigated by the cysteine-sulphuric acid reaction of Dische (1949), hexuronic acid by a carbazole reaction (Dische, 1947) and deoxyribose by the Stumpf modification of the Dische reaction (Stumpf, 1947). Quantitative analytical data are expressed on the basis of dry weight.
**Precipitation-in-gel tests**

The double diffusion technique of Ouchterlony was mainly used with reagent concentrations and spacing and dimensions of the reagent cups as given by Rondle & Dumbell (1962). The distances between and sizes of reagent cups were sometimes reduced proportionately so that experiments could be made on microscope slides coated with a layer of buffered agar 1 to 2 mm. thick (micromethod).

**Hyperimmune antivaccinial rabbit sera**

Rabbits convalescent from infection with vaccinia were given 3 or 4 intravenous injections of partially purified vaccinia virus. The injections contained approximately $10^6$ pk.f.u. of virus each and were given at 5-day intervals. The virus was prepared from infected rabbit skin to avoid the possible production of interspecies antibody. Rabbits were bled 5 or 6 days after the final injection.

**Preparation of purified vaccinia virus**

Purified vaccinia virus was obtained from buffer extracts of vaccinia-infected chorioallantoic membranes by differential centrifugation, fluorocarbon extraction and centrifugation through 36% sucrose as described by Joklik (1962a). Electron microscopic examination showed such preparations to be free from cell debris.

**RESULTS**

**Preliminary fractionation**

The non-diffusible material obtained after dialysis of the extracts of vaccinia-infected sheep dermis consisted of 84% water-soluble and 16% water-insoluble material. Each material was examined by precipitation-in-gel tests against a hyperimmune antivaccinial serum and the line patterns obtained were compared with those given by extracts of vaccinia-infected rabbit dermis and chick embryo chorioallantois with the same antiserum. The water-soluble extracts of sheep dermis contained materials giving 7 or 8 of the 8 or 9 lines given by the crude rabbit or chick extracts. The water-insoluble extract of sheep dermis reacted poorly in precipitation-in-gel tests and was not investigated further.

Ultracentrifugation of the water-soluble extracts of infected sheep dermis resulted in a supernatant fluid which contained 64% and a precipitate which contained 36% of the material treated. Precipitation-in-gel tests showed that the supernatant was identical in its vaccinia-specific serological composition with the starting material. The washed precipitates did not react in such tests. Hence, these procedures removed non-specific material giving an approximate twofold purification of vaccinia-specific serological activity.

**Chromatography on DEAE-cellulose**

Figure 1 shows the changes in light absorption of the column effluent at 260 and 280 nm. in relation to the changes in ionic composition of the column effluent which were caused to occur at different elution volumes. With the exception of effluents obtained with buffered $0.35\ M$- and $0.375\ M$-NaCl each change in salt concentration resulted in the elution from the column of materials absorbing u.v. light.
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The material eluted with 4.0 M- NaCl differed from other fractions in that the light absorption at 260 nm was greater than that at 280 μm.

The column void volume was approximately 650 ml. Elution volumes of 1.0 to 1.5 1. were required to elute completely each light-absorbing peak and hence the fractions obtained with buffered 0.35 and 0.375 M- NaCl were collected to volumes of 1.5 1. The large volume required to elute each light-absorbing peak possibly reflects a partial solubility of each material eluted in the respective eluent. Fractions corresponding to each light-absorbing peak were combined, dialysed against water until free from salt and dried from the frozen state. The effluents obtained with buffered 0.35 and 0.375 M- NaCl were treated similarly. The total amount of material recovered was 60% of that applied to the column.

Fig. 1. Elution profile of buffer extracts of vaccinia-infected sheep dermis recovered from DEAE-cellulose column equilibrated with 0.004 M-phosphate buffer, pH 7.4. The column was then eluted with 0.04 M-phosphate buffer, pH 7.4 at the point indicated followed by step-wise elution with 0.137 M- to 4.0 M- NaCl at a constant pH of 7.4. ———, E at 280 nm.; ----, E at 260 nm.; and ———, molarity of NaCl in the eluent.

The fractionation yielded 13 materials and portions of each material were dissolved in 0.04 M-phosphate buffer, pH 7.4, to a concentration of 10 mg./ml. The serological activity of each solution was compared with that of the preliminary purified sheep extract by precipitation-in-gel tests using a hyperimmune antivaccinal rabbit serum (Table 1). To construct the table, numbers were assigned to the 7-line pattern components seen in precipitation-in-gel experiments when the preliminary purified extract was tested against this antiserum. The line pattern components were numbered consecutively 1 to 8, omitting 6, progressing from the line pattern component nearest the antigen well toward the serum well. This arbitrary standard was used as a reference for all other materials tested. The column fractions tended to exhibit different ranges of line pattern components, although materials reacting as any one line pattern component were present in more than one column fraction. The column fraction obtained by elution with 4.0 M- NaCl, however, reacted to give a single line of precipitation, identified with line pattern components 5 and 7 of the standard system, and this material was selected for further study.
Physical examination of the 4.0 M fraction by polyacrylamide disc electrophoresis revealed two components when the gels were stained with Amido Black. Both components migrated at a speed similar to rabbit serum albumin: the slower component migrated 97% of the distance migrated by the other component. Analytical ultracentrifugation showed a single peak asymmetrical at the trailing edge.

Table 1. Serological activity (precipitation-in-gel) of fractions obtained from extracts of vaccinia-infected sheep dermis after chromatography on DEAE-cellulose

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Eluent</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.004 M-phosphate buffer</td>
<td>+</td>
<td>+</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>2</td>
<td>0.04 M-phosphate buffer</td>
<td>+</td>
<td>+</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>3</td>
<td>0.137 M-NaCl</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>.</td>
<td>.</td>
<td>.</td>
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</tr>
<tr>
<td>4-10</td>
<td>0.175 M- to 0.325 M-NaCl</td>
<td>.</td>
<td>.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>0.35 M-NaCl</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>+</td>
<td>+</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>12</td>
<td>0.375 M-NaCl</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>+</td>
<td>+</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>13</td>
<td>4.0 M-NaCl</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>+</td>
<td>+</td>
<td>.</td>
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</tr>
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</table>

Separation of the two components was achieved by filtration of the 4.0 M fraction through Sephadex G-200 (Fig. 2). Spectrophotometric examination of the column effluent showed that the applied material eluted as two partially resolved peaks, each with an absorption maximum at 260 nm. However, the \( E_{210}/E_{260} \) ratio was much higher for the second peak than the first. Elution volumes showed that the material associated with the first peak was eluted immediately after the void volume. For further study the column effluent was divided into fractions I, II and III (Fig. 2) to allow for incomplete separation of the peaks. The fractions were dialysed against distilled water until free from salt and dried from the frozen state. Total solid re-
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coveries were about 70% of the material applied to the column of which 65% was recovered in fraction I, 25% in fraction II and 10% in fraction III.

Each fraction from Sephadex G-200 gave the same single line of precipitation with hyperimmune antivaccinial rabbit serum in immunoprecipitation experiments. However, fraction I was active at a concentration of 0.75 mg/ml and fractions II and III at 1.5 and 2.5 mg/ml respectively when dilutions of each fraction were examined for their ability to form lines of precipitation against the same antivaccinial serum in micro precipitation-in-gel tests. A single electrophoretic component was detected in fraction I by polyacrylamide disc electrophoresis, whereas two components were detected in fractions II and III. Serologically and electrophoretically homogeneous material was isolated in the first Sephadex G-200 fraction.

Physical and chemical properties of the isolated material

Physical homogeneity of the isolated material was confirmed by ultracentrifugal analysis. A single symmetrical peak was obtained with a sedimentation coefficient of 3.47 S. Data obtained during these experiments were used for an assessment of molecular weight by the sedimentation equilibrium technique of Archibald (1947). The partial specific volume, determined pyknometrically, was 0.57. These experiments gave a mean value for the molecular weight of the isolated material of 31,120 (S.D. ± 1320).

<table>
<thead>
<tr>
<th>No. of determinations</th>
<th>Mole/100 mole estimated bases</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Adenine</td>
</tr>
<tr>
<td>5</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>(±0.6%)</td>
</tr>
</tbody>
</table>

The material was hydrolysed and analysed by the method of Wyatt & Cohen (1953).

The u.v. absorption spectrum of the isolated material gave a maximum at 258 nm. and a minimum at 232 nm. with an \( E_{258} : E_{280} \) ratio of 1.8, suggesting that the material contained nucleic acid. Qualitative colorimetric tests (Stumpf, 1947) indicated that the nucleic acid was DNA since deoxyribose was readily detected, whereas tests for ribose were negative. The value of the extinction at 260 nm. indicated a DNA content of about 70%.; further examination (Dische, 1930) showed that the isolated material contained 74.3% DNA. The base composition of the DNA associated with the isolated material was determined (Table 2). Identical results were obtained on material hydrolysed for 30 to 60 min. Control experiments using highly purified calf-thymus DNA gave results in very close agreement with data previously reported.

Investigations of the structural configuration of the DNA by treatment with heat or 1% formaldehyde gave equivocal results. In preliminary studies, Williamson & Rondle (1964) failed to detect an increase in absorption at 260 nm. after heating to 100° for 10 min. followed by rapid cooling. This suggested that the DNA was single-stranded. More recent experiments using solvents of higher ionic strength have shown that heating was followed by an increase in \( E_{260} \) of about 10%. However, treatment of the unheated material with 1% formaldehyde at 37° for 60 min. gave an increase in \( E_{260} \) of 16%. Concomitant increases in absorption at wavelengths below 240 nm. obscure the significance of these results.

The isolated material contained 9.4% protein estimated as bovine serum albumin.
Hydrolysates obtained with 0.5 or 6 N-HCl at 100° for different times failed to react with ninhydrin. Paper chromatography of the hydrolysed material used for base-ratio estimations, however, indicated seven different ninhydrin positive spots. Four spots had \( R_f \) values which corresponded most closely to those of ornithine, alanine, tryptophan and leucine or one of its isomers. The isolated material also contained 14.9% carbohydrate estimated as glucose. Since calf-thymus DNA failed to react in this test it was concluded that the reaction was due to carbohydrate other than deoxyribose. Attempts to identify the sugars present by paper chromatography of acid hydrolysates of the material were unsuccessful. Qualitative colorimetric tests, however, showed the presence of a hexose and a hexuronic acid. Interference in these tests by the deoxyribose present prevented further identification of these sugars.

Serological studies

Heating at 60° for 60 min. or at 100° for 3 min. did not impair the ability of the isolated material to precipitate when tested against antivaccinial serum in precipitation-in-gel tests. The line pattern component observed in these experiments was identical with that given by the same antivaccinial serum and a preparation of the heat-stable S antigen of vaccinia soluble antigen prepared as described by Parker & Rivers (1937).

The chemical nature of the immunological determinant groups was investigated initially by the effect of specific enzymes on the ability of the isolated material to precipitate with antivaccinial serum in immunoprecipitation tests. Enzymes were dissolved to a final concentration of 0.5% in separate solutions of the isolated material at 5 mg./ml. and incubated for 2 hr at 37°. Treatment with deoxyribonuclease yielded material which gave two lines of precipitation each giving a reaction of identity with the single line of precipitation of the untreated material. Pepsin and trypsin were without detectable effect but the product of treatment with \( \alpha \)-chymotrypsin had a reaction of only partial identity with the untreated control. Incubation with \( \alpha \)-chymotrypsin also produced a 50% reduction in complement-fixation titre with hyperimmune antivaccinial serum the control giving a 50% end-point at 3 \( \mu g./ml \). Treatment with 0.02 M-sodium periodate for up to 24 hr was without effect on either immunoprecipitation or complement-fixation reactions.

Attempts to prepare antisera containing vaccinia-specific precipitins by injection of the isolated material into rabbits either intravenously, or intradermally with adjuvant (Hayward & Augustin, 1957), were unsuccessful. Antisera prepared by the latter method, however, did contain precipitins to host material. Further precipitation-in-gel tests established that the host-specific antigen was not associated with the virus-specific material. It was concluded that the isolated material was a vaccinia-specific hapten containing separate host material in a concentration too low to be detected by the physicochemical techniques employed.

In the absence of a specific antiserum to the isolated virus-specific material further serological studies were made using standard hyperimmune antivaccinial rabbit serum absorbed with purified vaccinia virus preparations. Absorptions were carried out at 37° for 2 hr and then at 4° overnight; virus was removed by centrifugation at 10,000 \( g \) for 1 hr. The complement-fixation titre of the absorbed serum against the isolated material was 1/30 compared with a titre of 1/180 for the unabsorbed control. The actual endpoint of the titration with the absorbed serum could not be determined due to anti-complementary activity in serum concentrations below 1/30.
DISCUSSION

The extract of vaccinia-infected sheep dermis used in this study showed a serological complexity in immunoprecipitation tests comparable to that described for other tissues infected with vaccinia virus (Rondle & Dumbell, 1962; Marquardt et al. 1965; Cohen & Wilcox, 1966). At least partial resolution of this complex is provided by fractionation on DEAE-cellulose. Elution by successive applications of solutions of NaCl to the cellulose may have been responsible for the presence of serologically identical materials in different fractions (Levin, 1958). Alternatively, these results might suggest the presence of identical antigenic determinant groups on different carrier molecules.

The serologically homogeneous material separated by elution from DEAE-cellulose with 4.0 M-NaCl was isolated as a physically homogeneous preparation by further gel filtration. In these experiments, elution of the virus-specific material immediately after the void volume should indicate a molecular weight in excess of 200,000 (Flodin, 1962). This value was not compatible with that subsequently determined from ultracentrifugal data. This apparent discrepancy can be reconciled if sterical or other properties of the isolated material provided resistance to diffusion into the porous gel granules. Support for this hypothesis is provided both by high dilution of the applied sample during filtration and persistent failure to achieve complete recovery. Such limitations do not apply to the result calculated from ultracentrifugal data, and we believe the value of 31,000 determined by this technique is valid.

Chemical analysis of the virus-specific material identified nearly 98% of the total material present as DNA, protein and carbohydrate. Similar material was isolated from three separate preparations of vaccinia-infected sheep tissue; the composition was not changed by second application to DEAE-cellulose and it again eluted with 4.0 M-NaCl (Williamson, 1963). These results and the established physical homogeneity argue strongly that both protein and carbohydrate are specifically associated with the nucleic acid. The experimentially determined values for the base composition of the DNA in this material differ from those reported for DNA isolated from vaccinia virus (Wyatt & Cohen, 1953; Pfau & McCrea, 1962) and from sheep tissue (Chargaff, 1955).

The serological activity of the isolated material appears to be associated with two distinct antigenic sites. Deoxyribonuclease did not affect precipitation with hyperimmune antivaccinial serum in immunoprecipitation tests but after exposure to this enzyme the antigenic determinants diffused separately. Changes in behaviour in immunoprecipitation tests and partial reduction of complement-fixation titre following treatment with a-chymotrypsin indicate that one antigenic site is associated with the protein component. Although apparently insensitive to periodate, other properties suggest that the carbohydrate may determine the second antigenic specificity. The serological activity was heat-stable and identical with that of a heat-stable extract of vaccinia-infected rabbit dermis described by Parker & Rivers (1937). Chemical studies of their extract showed that it also contained both protein and carbohydrate (Smadel, Lavin & Dubos, 1940). Further studies of virus-specific materials isolated from tissues of different hosts infected with vaccinia virus will be described in more detail elsewhere (Rondle & Williamson, 1968).

Specific antibodies to the isolated material were removed from hyperimmune
antivaccinial serum by absorption with purified vaccinia virus. Examination by electron microscopy showed that the virus preparations used for serum absorption contained mature virus forms. It must be concluded that material serologically identical with the virus-specific substance isolated from vaccinia-infected sheep tissue is present on the surface of the vaccinia virus particle. Evidence has been presented that the isolated material is specifically associated with DNA. Structural studies of vaccinia virus particles indicate that the DNA of the virus genome is contained within enclosed cores under the outer coat of the virus particle (Easterbrook, 1966). Studies using radioactively labelled rabbit pox virus have shown that, although the virus genome is not digested by exposure of the virus to deoxyribonuclease, small amounts of DNA are released from the surface of the virus particle. However, it was concluded in this study that these small amounts of DNA were non-specifically adsorbed (Joklik, 1962b). It would appear, on the basis of these observations and the results described in the present study, that the DNA of vaccinia virus may not be exclusively associated with the virus core.

We are indebted to Dr W. Taylor, Department of Chemical Pathology, United Liverpool Hospitals, for preliminary ultracentrifugal studies, to Dr M. Summerell, Searle Research Laboratories, High Wycombe, for determination of the sedimentation coefficient and to Dr A. Neill Wright, Thornton Research Centre, Wirral, Cheshire, for computation of the molecular weight. Part of the work reported here was done in the Department of Bacteriology, University of Liverpool, when C. J. M. Rondle was on the teaching staff and J. D. Williamson was a research fellow.

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(Received 22 March 1968)