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Studies on the LS antigen of vaccinia virus

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The LS antigen of vaccinia virus was described first by Craigie & Wishart (1936*a*). It was a material which could be eluted from purified virus particles and occurred also in virus-free extracts of vaccinia-infected rabbit dermis (soluble antigen). The substance was not detectable in uninfected rabbit tissue. It was called LS because it possessed two different serological specificities. One specificity (L) was 'heat labile' and destroyed by heating soluble antigen to 56° C. for 45 min.; the other specificity (S) was 'heat stable' and not altered by this treatment. The two specificities were thought to be associated with a single substance because either specific anti-L antibody or specific anti-S antibody precipitated both L and S serological activities from soluble antigen. Specific anti-L antibody was prepared by inoculation of rabbits with partially purified LS and absorption of the resulting antisera with LS heated to destroy L. Specific anti-S antibody was prepared by inoculation of rabbits with heated LS (Craigie & Wishart, 1936*b*).

Craigie & Wishart (1938) stated that under certain conditions LS antigen might dissociate and occur in soluble antigen as separate L and S substances. Materials with the serological properties of S only were obtained from vaccinia-infected rabbit tissue by Smith (1932), Ch'en (1934) and Parker & Rivers (1937), but a material with the serological properties of L only was not described at this time. A substance which was electrophoretically and ultracentrifugally homogeneous and possessed the serological properties of LS antigen was isolated from vaccinia-infected rabbit dermis by Shedlovsky & Smadel (1942). Smadel & Shedlovsky (1942) suggested that by suitable treatment the purified LS antigen might be converted to various serological states which ranged from L-inactive, S-active to L-active, S-inactive.

More recently gel-diffusion studies have shown many distinct virus-specific serological entities in extracts of vaccinia-infected tissues. In immunodiffusion tests on such extracts Gispen (1955) found up to six line pattern components (l.p.c.), Rondle & Dumbell (1962) up to 9 l.p.c., and Westwood *et. al.* (1965) up to 17 l.p.c. Because of this complexity Westwood *et al.* suggested that the original concept of a single LS antigen was likely to have been an expression of the collective behaviour of a number of antigens. Joklik (1966) also stated that the LS antigen

was probably not a singular molecular species. Certainly Nicoli & Jolibois (1964) were unable to find an LS-specific substance in extracts of unspecified tissues infected with vaccinia virus. They detected seven virus-specific substances in the soluble antigen, found materials with properties originally ascribed to L and S, but separated these by fractionation on DEAE-cellulose. In addition, Cohen & Wilcox (1966) examined an LS preparation made from vaccinia-infected KB or L cells. The material which was obtained by precipitation of soluble antigen at pH 4·6 represented 6% by weight of the protein originally present and possessed 75% of the original complement-fixing activity. It gave two zones in polyacrylamide gel tests, 2 l.p.c. in immunoelectrophoresis tests, and 3 l.p.c. in immunodiffusion tests. The heat stability of the material was not reported. Other results were obtained by Marquardt, Holm & Lycke (1965*a*, *b*). They found eight virus-specific l.p.c. in immunodiffusion tests with extracts of vaccinia-infected rabbit dermis. Some of the l.p.c. (three or more) were designated as 'e–f complex'. The complex passed unchanged through a column of DEAE-cellulose, being eluted by 0·1–0·2 M sodium chloride. It resembled LS in that it precipitated at pH 4·8 and its serological activity was partly destroyed by trypsin, partly heat labile, and partly heat stable.

We studied LS antigen initially to relate it serologically to the 9 l.p.c. found by Rondle & Dumbell (1962) in immunodiffusion tests on extracts of vaccinia-infected rabbit tissue. We also attempted to isolate LS from vaccinia-infected sheep dermis and to study the properties of S-antigen. Our results tend to reconcile the conflicting views on LS and lead to interesting speculations on vaccinia virus replication in different hosts.

MATERIALS AND METHODS

Virus

The 'Lister' strain of vaccinia virus was used throughout this work.

Soluble antigen

Virus-free extracts of vaccinia-infected rabbit dermis and chick chorio-allantois were prepared as described by Rondle & Dumbell (1962). Samples of soluble antigen from vaccinia-infected sheep dermis were a gift from the Lister Institute, Elstree, through the good offices of Professor C. Kaplan.

Preparation of LS from rabbit soluble antigen

Seven preparations of LS were made from extracts of vaccinia-infected rabbit dermis by a method which differed little from that described by Shedlovsky & Smadel (1942). In the largest experiment soluble antigen (341 ml.) from sixteen rabbits was filtered through a Seitz EK filter, concentrated by pervaporation in 'Visking' dialysis tubing to 41 ml. and dialysed 16 hr. at 4° C. against 0·9% saline. Pervaporation was done in a cold room at 4° C. by suspending a dialysis sac filled with soluble antigen in a fan-induced draught. The dialysis sac containing the concentrated soluble antigen was agitated constantly for 6·5 hr. at 4° C. in

1 l. of 0·06 M sodium acetate–acetic acid buffer, pH 4·6. The contents of the sac were then centrifuged for 10 min. at 3000 rev./min. and the supernatant fluid discarded. The precipitate was washed twice with acetate buffer, pH 4·5, suspended in 20 ml. of 0·05 M phosphate–phosphate buffer pH 6·3 and dialysed against this buffer for 16 hr. at 4° C. Soluble and insoluble materials were separated by centrifugation for 10 min. at 3000 rev./min. The insoluble precipitate was extracted twice with phosphate buffer, pH 6·3, and the clarified extracts added to the bulk of the soluble material. The soluble material was then dialysed against water until free from salt and dried from the frozen state. The material obtained corresponded to 'Fraction B' of Shedlovsky & Smadel (1942), and was considered to be LS. In the experiment described the 23 mg. of LS obtained represented a yield of 2 % in terms of the indiffusible material present in the starting material.

Preparation of S from sheep soluble antigen

An attempt was made to prepare from sheep soluble antigen a material analogous to the S substance prepared by Parker & Rivers (1937) from rabbit soluble antigen. Sheep soluble antigen was dialysed against water until free from salt and dried from the frozen state. Some of the material (5 g.) was dissolved in 50 ml. 0·004 M phosphate–citrate buffer, pH 7·0, and heated at 98° C. for 5 min. The precipitate was discarded and the supernatant fractionally precipitated by additions of saturated ammonium sulphate. The material precipitating between 25 % and 50 % saturation with ammonium sulphate was preserved, dissolved in water, and obtained free from ammonium sulphate and dissolved in 0·01 M phosphate–phosphate buffer, pH 7·5, by use of a column of Sephadex G_{25} (Pharmacia Ltd., Uppsala, Sweden). The fractional precipitation with ammonium sulphate was repeated. To the dissolved precipitate nine volumes of ethanol were added at 4° C. The precipitate was taken up in 0·01 M phosphate buffer pH 7·5, residual ethanol removed on a column of Sephadex G_{25} and 5 ml. of M sodium acetate–acetic acid buffer, pH 4·5, added to the 35 ml. of solution obtained. As measured with a glass electrode the solution was pH 4·55. After standing overnight at 4° C. the precipitate was collected and dissolved in 0·01 M phosphate buffer, pH 7·2. Insoluble material was discarded and the solution adjusted to pH 7·8 with 0·1 N sodium hydroxide. The solution was heated to the apparent boiling point for 3 min. but no precipitate formed. Acetic acid (N) was then added to pH 6·0 and the solution again heated to the apparent boiling point for 3 min. Insoluble material was removed by centrifugation for 10 min. at 3000 rev./min., the supernatant dialysed against distilled water until free from salt and dried from the frozen state. The weight of material obtained (6·6 mg.) represented a yield of 0·013 %. In this paper the material obtained is referred to as S(PR).

Preparation of L and S from sheep soluble antigen

Five attempts were made to prepare LS from vaccinia-infected sheep dermis by the acid precipitation technique of Shedlovsky & Smadel (1942). Each attempt was made on a fresh sample of sheep soluble antigen and each attempt was unsuccessful. The precipitate obtained had no heat-labile serologically active com-

ponents and precipitated poorly with antisera. Further attempts were made to fractionate sheep soluble antigen by using DEAE-cellulose (Kodak Ltd., Kirby Trading Estate, Liverpool). Soluble antigen was dialysed to equilibrium against 0·05 M phosphate buffer pH 7·4 and absorbed on a column of DEAE-cellulose. Fractionation was then attempted by flushing the column with buffer solutions containing stepwise increases in NaCl concentration. The results are described in full by Williamson (1963). With antisera prepared as described below the eluate at 0·137 M-NaCl reacted serologically as a specific L substance and the eluate at 4 M-NaCl reacted serologically as a specific S substance. Further work on the latter material gave a physically and serologically homogeneous S substance (Williamson & Rondle, 1964).

Disk electrophoresis

This technique measures the electrophoretic mobility of materials in columns of polyacrylamide gel and was carried out as described by Ornstein & Davies (1961).

Analytical ultracentrifugation

Through the courtesy of Dr W. Taylor, Department of Chemical Pathology, The United Liverpool Hospitals, samples of the S preparation were examined in a Spinco Model E ultracentrifuge by Dr A. Neill Wright of the Thornton Research Centre.

Ultraviolet absorption spectra

These were determined using a Hilger 'Uvispek' spectrophotometer.

The antisera used

The anti-vaccinia sera used in this paper were prepared with rabbit-grown materials as described by Rondle & Dumbell (1962). The specific anti-S serum was one of the sera prepared by Harris, Harrison & Rondle (1963) by repeated intravenous injection of rabbits with virus-free extracts of vaccinia-soluble antigen prepared from infected chick chorioallantois. Before injection the soluble antigen was heated to 60° C. for 45 min. in a sealed ampoule submerged in a water bath. A 'semi-specific' anti-L serum was prepared from an anti-vaccinia serum. Samples of serum were taken and mixed with different amounts of S(PR). The mixtures were tested for their ability to react with S(PR) in gel-diffusion experiments. The mixture which contained the least amount of S(PR) but which failed to react with S(PR) was regarded as a 'semi-specific' anti-L serum. From the results of Rondle & Dumbell (1962) such a mixture must have contained many different anti-vaccinia antibodies, but it was considered to contain no anti-S.

Gel diffusion tests

The final concentrations of materials in the gel used were: agar 1%; 0·05 *I* phosphate buffer, pH 7·2; merthiolate (a gift from Eli Lilly and Co.) 1/10,000. On microscope slides a gel thickness of 1 mm. was employed and wells were cut in isometric patterns 4 mm. in diam. with centres 7 mm. apart. In Petri dishes a gel thickness of 3 mm. was used and wells were 9 mm. diam. with centres 14 mm. apart.

Antisera were used undiluted. Unless stated otherwise the LS and S(PR) preparations were used at a concentration of 0·5 mg./ml., the S preparation at 5 mg./ml.

RESULTS

Physical examination of materials

Two of the rabbit LS preparations tested gave one band when subjected to electrophoresis in polyacrylamide gel. The distance moved by this band divided by the distance moved by the bromophenol blue marker band (relative distance or R_d value) was 0·95. The other rabbit LS preparations gave an intense band at $R_d = 0{\cdot}95$ and a second very weak band at $R_d = 0{\cdot}56$.

The sheep S preparation gave a single band at $R_d = 0{\cdot}92$ when tested, but the preparations of sheep S(PR) and sheep L both gave two bands. The position of these bands did not correspond with each other or with the single band given by sheep S, or with the one or two bands given by rabbit LS.

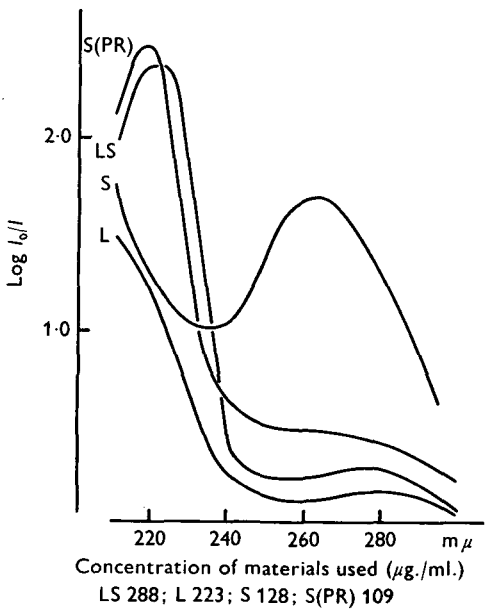

Fig. 1. Ultraviolet absorption spectra of LS, L, S, S(PR).

It was possible to test only the sheep S preparation in an analytical ultracentrifuge. As stated by Williamson & Rondle (1964) it gave a single sharp peak and from the data a molecular weight of approximately 31,000 was calculated.

These results show that the sheep S(PR) and L preparations were physically heterogeneous; they suggest that most of the rabbit LS preparations contained a major and a minor component; they support the view that the sheep S preparation was homogeneous and electrophoretically different from the rabbit LS and sheep S(PR) preparations.

Ultraviolet-light absorption curves for the various preparations are shown in Fig. 1. They indicate that the rabbit LS, sheep S(PR), and sheep L preparations

contain protein but little nucleic acid while sheep S contains much nucleic acid. Calculated from a standard curve of commercial calf thymus nucleic acid, S(PR) contains less than 6% and LS and L less than 2% nucleic acid, while S contains approximately 70% of it. Chemical studies have shown that the nucleic acid of sheep S is DNA (Williamson & Rondle, 1964).

Gel diffusion study of materials

Results obtained with those preparations of rabbit LS which gave only one band in polyacrylamide electrophoresis will be given first.

Freshly prepared solutions (30 μg./ml. to 1 mg./ml.) of fresh preparations of LS from vaccinia-infected rabbit dermis frequently gave two lines in a gel diffusion test when examined against anti-vaccinia rabbit sera (AVS). The phenomenon is shown in Pl. 1, fig. 1, which shows also that one l.p.c. of LS disappeared on conversion of LS to LS(H) by heating LS at 56° C. for 45 min. and that the l.p.c. which was stable to heat treatment was serologically identical with sheep S(PR).

This result could have been obtained from a mixture possessing discrete L and S serological specificities. This is unlikely, however, since, as shown in Pl. 1, fig. 2, mixing rabbit LS with a specific anti-S serum resulted in complete loss of reaction with an anti-vaccinia serum which was known to contain anti-L. The specificity of the anti-S serum is demonstrated in Pl. 1, fig. 3, which shows that the anti-S serum gave only one line of precipitation when tested against vaccinia-soluble antigen prepared from infected rabbit dermis. The soluble antigen (V) used gave a complex line pattern when tested against an anti-vaccinia serum. The experiment shown in Pl. 2, fig. 11, shows additionally that the anti-S serum did not react with a preparation of a sheep L substance (see below).

A further indication that the two specificities were closely associated was that they could be absorbed on DEAE-cellulose equilibrated at pH 7·4 with phosphate–phosphate buffer $I = 0{\cdot}05$ and eluted together as a single protein peak by 0·2 M-NaCl.

Solutions made from preparations of rabbit LS stored for some time at 4° or −20° C. or stored solutions of LS preparations usually gave one line of precipitation when tested against anti-vaccinia sera. The reason for this behaviour is not understood. It was not due to a qualitative loss of one serological specificity since, as shown in Pl. 1, fig. 4, results similar to those shown in Pl. 1, fig. 1, could still be obtained with such solutions save that the LS–AVS reaction appeared to show only one line of precipitation which exhibited 'spur-formation' at the junction of the LS(H)–AVS reaction. The same figure shows, however, that the one line of precipitation formed in the LS–AVS reaction exhibited spur-formation with the reaction observed between a 'semi-specific' anti-L serum and the LS preparation under test. The inability of the 'semi-specific' anti-L serum to react with sheep S(PR) is shown clearly in Pl. 1, fig. 5. These results could have been obtained if the stored solution of rabbit LS used for test contained some degraded material which was L-inactive, S-active. Such a material could have diffused through the LS-('anti-L') precipitate and reacted with anti-S antibody diffusing from the well containing anti-vaccinia serum.

In some experiments more than two lines of precipitation were detected when

these preparations of rabbit LS were tested at 10 mg./ml. against an anti-vaccinia serum. The preparations of rabbit LS which gave two bands in polyacrylamide gel electrophoresis were invariably heterogeneous when tested in this way even when examined at 1 mg./ml. Plate 1, fig. 6, shows that by study at a number of concentrations up to five lines of precipitation could be produced by the heterogeneous preparation LS 7. However, by suitable adjustment of the concentration of reagents the result shown in Pl. 2, fig. 7, was obtained. Comparison of the result with theoretical diagrams for preparations containing either discrete L and S substances or a single LS substance (Pl. 2, fig. 8*a*, *b*) suggests that a single LS substance was present in LS 7. Similar results were obtained with four of the five patently heterogeneous rabbit LS preparations. The atypical preparation behaved as though it contained discrete L and S substances.

Attempts were made to fractionate the heterogeneous rabbit LS preparations on DEAE-Sephadex (Pharmacia Ltd., Uppsala, Sweden). These were unsuccessful as useful resolution of components was not achieved.

Serological examination of the two materials obtained from vaccinia-infected sheep dermis showed that both substances were related to the LS material obtained from vaccinia-infected rabbit dermis. (Pl. 2, fig. 9). Plate 2, fig. 10, shows further that the serological activity of one material (sheep S) was stable to heating at 56° C. for 45 min. and that the serological activity of the other material (sheep L) was destroyed by such treatment. The figure shows also that the L and S substances isolated from vaccinia-infected sheep dermis were not related serologically to each other since the lines of precipitation formed in the S–AVS reaction and the L–AVS reaction cross each other. The discrete serological identity of L and S preparations isolated from vaccinia-infected sheep dermis is shown again in Pl. 2, fig. 11. Here also the lines of precipitation formed in an S–AVS reaction cross each other. Moreover in this figure the anti-S serum is shown to react with the S preparation and not the L preparation.

An attempt was made to relate rabbit LS to the poxvirus l.p.c. described by Rondle & Dumbell (1962). Plate 2, fig. 12, shows that an anti-cowpox serum (ACS) reacted with a rabbit LS preparation to give one sharp line of precipitation. The rabbit LS preparation used gave only one band in polyacrylamide gel electrophoresis. The line of precipitation formed was contiguous with one of the l.p.c. given by this serum when tested against vaccinia soluble antigen; it was not formed when cowpox soluble antigen replaced vaccinia. This l.p.c. was called 'f' by Rondle & Dumbell (1962). Further work showed that anti-cowpox sera varied markedly in anti-L and anti-S antibodies but that either antibody would give an 'f' line with vaccinia soluble antigen which could not be obtained using cowpox soluble antigen. This suggests that the classical L and S specificities are absent from cowpox soluble antigen as usually prepared although one specificity at least can be released from cowpox-infected rabbit dermis by treatment with trypsin (Rondle & Dumbell, 1962).

The L and S specificities of LS were found to correspond respectively to l.p.c. 3 and 5 of the standard vaccinia system described by Williamson (1963) and Baxby & Rondle (1968).

Quantitative serological studies

The smallest amounts of rabbit LS, sheep S, and sheep S(PR) that would give visible precipitates when tested against different anti-vaccinia sera were determined using gel-diffusion tests on microscope slides. Two materials, rabbit LS and sheep S(PR), were titrated at 1/1000, 1/2000, 1/3000 and so on; the other material, sheep S, was titrated at 1/50, 1/100, 1/150 and so on. The results are given in Table 1. It is evident that S(PR) was the most active material and hence the ratios of 'smallest reacting dose of LS or S/smallest reacting dose of S(PR)' were calculated for each antiserum and included in the Table. The agreement shown by the several ratios with the exception of S/S(PR) for serum 96 supports the contention that the same serologically reactive site (S) was titrated in each case. The ratios also show that the physically heterogeneous S(PR) was at least 20 times more active serologically than the physically homogeneous S preparation. This result is considered more fully in the Discussion but it must be stated here that the serological activity of solutions of crude preparations of sheep S was increased three- to fourfold when they were heated for 3 min. at 100° C. at pH 8·0 followed by the same heat-treatment at pH 6·0.

Table 1. *Quantitative serological reactivity of LS, S(PR) and S*

	Reciprocal of end-point titre			Ratio of titres	
Serum	LS	S(PR)	S	LS/S(PR)	S/S(PR)
93	10,000	16,000	7,500	1·6	21·4
96	12,000	21,000	6,000	1·7	35·0
97	10,000	16,000	7,500	1·6	21·4
99	10,000	16,000	7,500	1·6	21·4
104	10,000	14,000	6,000	1·4	23·4

DISCUSSION

The results given in this paper show that the preparation of LS antigen by classical methods from vaccinia-infected rabbit dermis yielded usually a heterogeneous material containing three or more virus-specific serologically reactive components. This is in agreement with recent work by Marquardt, Holm & Lycke (1965*a*) and Cohen & Wilcox (1966). For reasons not understood, occasional preparations were less complex both physically and serologically.

The isolation and study of the less complex materials followed by careful serological examination of the more heterogeneous substances supported the view of Craigie & Wishart (1936*a*, *b*) that extracts of vaccinia-infected rabbit dermis contain *inter alia* a single substance (LS) which possesses both a heat-labile (L) and a heat-stable (S) serologically reactive site. Under some conditions in the present experiments the supposed single substance gave two lines of precipitation when tested against suitable antisera in gel-diffusion tests. The two lines of precipitation were not due to separate L and S substances contaminating the LS material since treatment of the material with a specific anti-S serum abolished both

the L and S reactions. That a single substance could give more than one line of precipitation when tested by gel-diffusion techniques has been demonstrated or postulated previously by Jennings (1954), Wilson & Pringle (1954, 1955), Kaminski (1955) and McDuffie, Kabat, Allen & Williams (1958).

Craigie & Wishart (1938) suggested that LS antigen from vaccinia-infected rabbit dermis might, under some conditions, dissociate into discrete L and S substances. This may have occurred with one of the seven preparations studied. Alternatively the earlier view could have been confused by the presence of the additional but then unsuspected antigens now shown to occur frequently in 'LS' preparations. Results with the other six materials suggested that stored preparations of LS or stored solutions of LS decomposed and behaved serologically as a mixture of LS and L inactive, S active substances. This finding is in accord with the views of Smadel & Shedlovsky (1942). Clear evidence for an LS antigen was obtained only from freshly prepared fractions of virus-infected tissue and such a substance was not very stable.

Despite repeated trial it was not possible to isolate a single LS substance from extracts of vaccinia-infected sheep dermis. It was possible, however, to use the technique described by Parker & Rivers (1937) and obtain a material S(PR) which was heat-stable and serologically indistinguishable from the S specificity of LS. It was also possible to fractionate the sheep material on DEAE-cellulose. This fractionation yielded two chemically and physically different substances, one possessing the serological specificity of L, the other that of S. No serological relationship could be demonstrated between the two materials; indeed, experimental results indicated that they were serologically distinct. The isolation from vaccinia-infected tissue of a material exhibiting L activity only has not been reported previously, although Smadel, Hoagland & Shedlovsky (1943) claimed to convert a purified LS preparation to an L active, S inactive substance by treatment with chymotrypsin. Since the sheep L preparation was shown to be physically heterogeneous, chemical studies of the material were not attempted and no comment can be made on the possibility that the sheep L substance could have arisen by degradation of a hypothetical LS substance present initially in extracts of vaccinia-infected sheep dermis. Comment can, however, be made in respect of the S substance isolated from vaccinia-infected sheep dermis by absorption–elution chromatography on DEAE-cellulose. This material was subjected to further treatments and isolated as a physically homogeneous substance of mol.wt. *ca.* 31,000 which contained *ca.* 75 % DNA (Williamson & Rondle, 1964). When tested against suitable antisera it gave an S reaction up to a dilution of 1 in 750. The LS preparations from vaccinia-infected rabbit dermis used for this paper contained less than 5 % nucleic acid but reacted as S containing materials up to dilutions of 1/10,000 when tested against those anti-vaccinia sera used for the titration of the sheep S preparation. Smadel & Shedlovsky (1942) quoted their purified preparation of rabbit LS as mol.wt. 250,000. The mol.wts., nucleic acid contents and serological activities of sheep S and rabbit LS are not compatible with the view that the homogeneous sheep S might have arisen by degradation of a hypothetical LS substance present initially in vaccinia-infected sheep extracts and similar in

chemical composition to the LS present in vaccinia-infected rabbit dermis. It would appear that although the serological specificities L and S both occur in extracts of vaccinia-infected rabbit and sheep dermis, these specificities are not necessarily associated with identical 'carrier' molecules. It may be speculated that in different host tissues vaccinia virus is not synthesized by precisely the same biochemical pathways. At least these findings suggest that caution should be exercised when comparing results obtained on the synthesis of any one virus in different host tissues.

The final point of discussion is the quantitative difference in serological reactivity between sheep S(PR) and sheep S. The physically heterogeneous S(PR) was prepared by vigorous techniques. It contained little nucleic acid and was at least 20 times as active serologically as the physically homogeneous S substance prepared by much milder procedures. If it is assumed that the 75% DNA in S was serologically inactive then it follows that the 'serologically active weight' of S was only one quarter of its actual weight. Since S(PR) contained not more than 6% nucleic acid the serologically active weight of this substance represented not less than 94% of the actual weight. Comparison of serologically active weights shows that in this respect S(PR) was only 5 or 6 times more serologically active than S. It is tempting to speculate that a difference in serological activity of this magnitude might have been due to a steric hindrance effect of the DNA moiety upon the serologically active moiety of S. The speculation is supported by the observation that vigorous heat-treatment of S resulted in an increase in serological activity. The heat-treatment might have degraded the nucleic acid and made available more serologically reactive material on S. The result also suggests that S(PR) might have been a degraded form of S.

SUMMARY

A material (LS) analogous to that described by Craigie & Wishart (1936*a*) has been detected in extracts of vaccinia-infected rabbit dermis. Gel-diffusion tests on this material support the view that it is a single substance possessing two distinct serologically reactive sites. The tests also show that under some conditions rabbit LS may give two lines of precipitation against suitable antisera and that this is not due usually to the dissociation of LS into discrete L and S substances.

A study of vaccinia-infected sheep dermis failed to give evidence of a single LS substance. Instead two chemically different substances were isolated, one having the serological specificity of L, the other that of S. The physical, chemical and quantitative serological properties of sheep S were such that it could not have arisen by degradation of an LS material similar to that found in vaccinia-infected rabbit dermis. On this evidence it is suggested that vaccinia virus is not necessarily synthesized in the same way in different host tissues.

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EXPLANATION OF PLATES

The terms LS, L, S, and S(PR) refer to those antigens or haptens so designated in the text; LS heated at 56° C. for 45 min. is represented as LS(H). Anti-vaccinia sera are represented as AVS, the specific anti-S serum as aS, and the anti-vaccinia serum mixed with S(PR) to abolish anti S activity as aL.

Plate 1

Fig. 1. The LS-AVS reaction shows two lines of precipitation. One of the lines of precipitation is contiguous with both the line present in the LS(H)–AVS reaction and the line present in the S(PR)–AVS reaction. The other line of precipitation is absent from these reactions.

Fig. 2. Dilution of LS with an equal quantity of preimmunization serum (LS/2) did not affect the reaction with AVS and two lines of precipitation were formed. All serological reactivity of LS was abolished, however, on admixture with an equal volume of aS (LS/aS).

Fig. 3. Soluble antigen from vaccinia-infected sheep dermis (V) gave a complex line pattern when tested against AVS. It gave only one line of precipitation when tested against aS.

Fig. 4. A stored solution of LS gave only one line of precipitation when tested against AVS. This line however shows spur formation at the junction of both the LS(H)–AVS reaction and the LS–aL reaction. This result which suggests that stored LS contains some free S is discussed in the text.

Fig. 5. Although S(PR) gives one line of precipitation when tested against AVS it fails to react with aL.

Fig. 6. The result obtained when the heterologous preparation LS 7 was tested against AVS depended upon the concentration of LS 7 used. LS 7 was at an initial concentration of 5 mg./ml. Dilutions of 1/2, 1/4, etc., were made with saline.

Plate 2

Fig. 7. By suitable selection of the concentration of LS 7 (here 0·8 mg./ml.) and by using LS(H) and specific aS results were obtained which suggested that LS 7 contained a single LS substance.

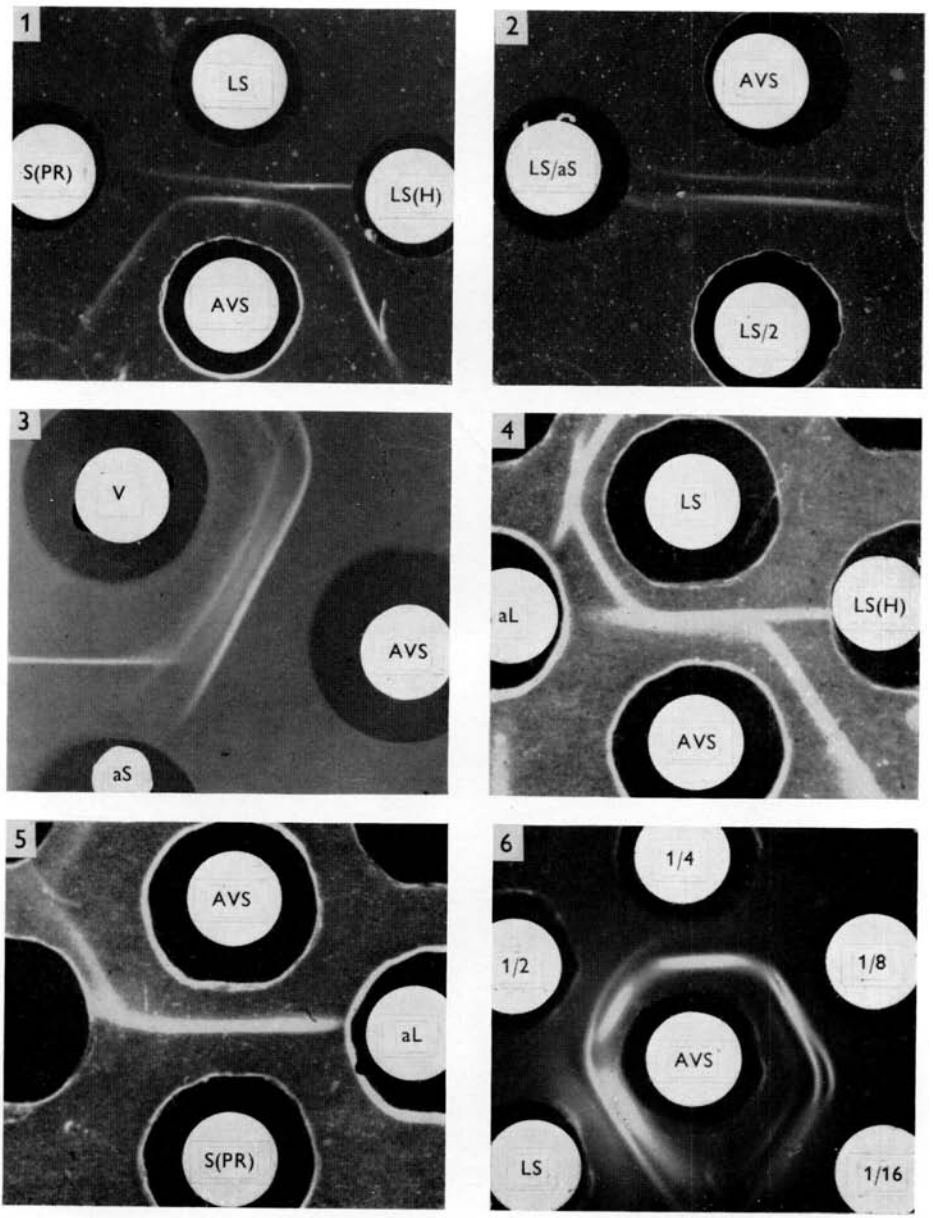
Fig. 8. (*a*) Theoretical result if no LS present, but L and S substances existed independently. (*b*) Theoretical result expected in the experiment shown in Fig. 7 if *inter alia* a single LS substance were present in LS 7.

Fig. 9. The L preparation obtained from vaccinia-infected sheep dermis is serologically identical with part of the LS–AVS reaction. The S preparation is also related to the LS–AVS reaction. As expected from the result shown in Plate 1, fig. 1, the S preparation is serologically identical with S(PR).

Fig. 10. This result shows that the S preparation was serologically stable to heating for 45 min. at 56° C. (HS). The L preparation lost its serological activity on conversion to HL by this heat-treatment. The crossing of the lines of precipitate formed in the L–AVS and the S–AVS reactions shows that the L and S preparations are serologically distinct.

Fig. 11. The specific antiserum aS reacts with sheep S but not sheep L. Both S and L, however, react with AVS to give single lines of precipitation. These single lines cross each other and indicate again that the L and S preparations are serologically distinct.

Fig. 12. Anticowpox serum (ACS) reacted with LS to give one l.p.c. This l.p.c. was given by soluble antigen from vaccinia-infected rabbit dermis (V) but not by soluble antigen from cowpox-infected rabbit dermis (CR).



C. J. M. RONDLE AND J. D. WILLIAMSON

(*Facing p.* 426)

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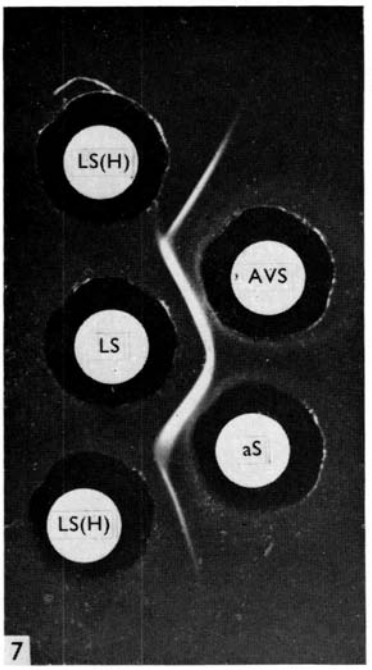

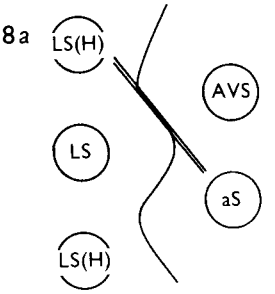

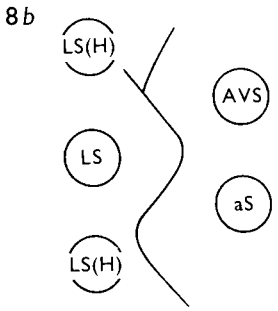

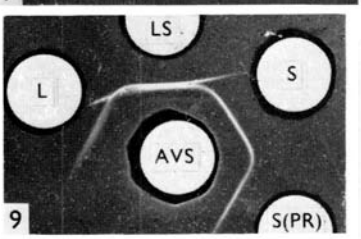

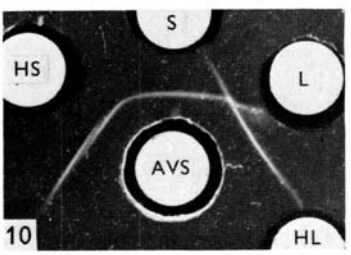

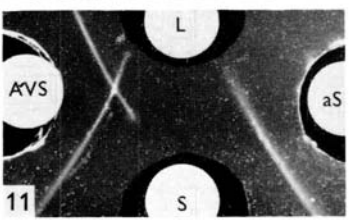

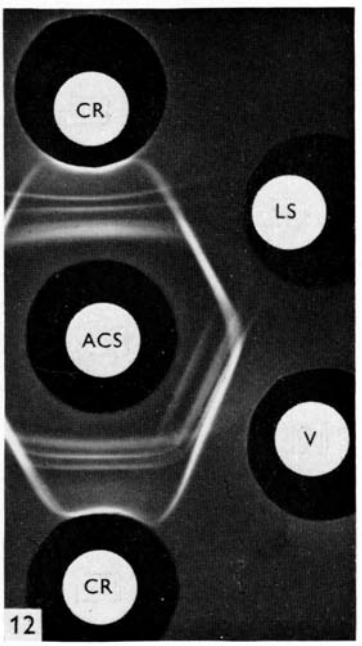

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