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A poxvirus antigen associated with pathogenicity for rabbits

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SUMMARY

White pock variants of cowpox virus give papular lesions on intradermal inoculation of rabbits, without the necrosis and haemorrhage that are produced by wild type cowpox viruses. Rondle & Dumbell (1962) have shown that white pock variants of cowpox virus fail to produce a specific, precipitating antigen which they called 'd' substance. In this paper it is shown that 'd' is demonstrable in soluble antigen preparations of rabbitpox virus and of neurovirulent strains of vaccinia virus but not in soluble antigens of variola viruses. Two series of recombinant viruses prepared by Dumbell & Bedson (1964) from variola and cowpox and from variola and rabbitpox viruses were tested for the production of 'd' substance. These results were compared with the previously recorded effects of these recombinants when inoculated intradermally in rabbits. It is concluded that functional genes determining the production of 'd' and of rabbit skin pathogenicity are closely linked on the pox virus genome, but that there is insufficient evidence to say that the two functions are interdependent.

INTRODUCTION

The soluble antigen extracted from tissues infected with cowpox virus contains at least nine substances which precipitate with antibodies present in sera from rabbits hyper-immunized against cowpox virus. Rondle & Dumbell (1962) investigated these antigens and showed that one of them, 'd', was not demonstrable in soluble antigen from tissues infected with the Lister strain of vaccinia virus, though some production of 'd' could be inferred from the demonstration of small amounts of anti-'d' in anti-vaccinial sera. Substance 'd' was not demonstrable in soluble antigen from tissues infected with a white-pock variant of the Brighton strain of cowpox virus, nor could anti-'d' be demonstrated in antisera raised against this white variant. The absence of 'd' from white variants of cowpox virus was confirmed for five other strains of this virus.

White variants of cowpox produce non-haemorrhagic lesions on the chick chorioallantois (CAM) and non-necrotic papules in rabbit dermis (Downie & Haddock, 1952; van Tongeren, 1952). It is reasonable to ask whether the 'd' substance plays a role in the genesis of the haemorrhagic and necrotic lesions produced by wild type cowpox virus. Rondle and Dumbell attempted to show a direct effect of 'd' substance. In unpublished work they made a semi-purified

preparation containing 'd' substance; this was dialysed and lyophilized so that the total yield from four rabbits was dissolved in 0·2 ml which was inoculated intradermally in one site of each of two rabbits. A corresponding preparation of vaccinia was used as a control. At thirty hours after the inoculations there was nothing to see at the sites where vaccinia antigen had been inoculated, but the 'd' preparation had produced oedematous papules (0·5 cm in diameter) with a mild erythematous reaction but no trace of extravasation of red blood cells or of necrosis. This was an inconclusive result but it was judged that repetition would be unlikely to clarify the matter. An indirect approach to the same question would be to correlate the production of 'd' substance and of necrotic lesions by a series of recombinant viruses prepared from cowpox virus and another virus that gave neither 'd' nor necrotic lesions. This paper describes the result of such an attempt, using the two series of recombinants between cowpox and variola viruses and between rabbitpox and variola viruses which were prepared by Bedson & Dumbell (1964*a*, *b*).

MATERIALS AND METHODS

Viruses. The strains of vaccinia, rabbitpox, variola and cowpox viruses were from those listed by Bedson & Dumbell (1961). The additional strains of cowpox were those that had been used by Rondle & Dumbell (1962). The isolation and characterization of the recombinant viruses was described by Dumbell & Bedson (1964) and by Bedson & Dumbell (1964*a*, *b*). Soluble antigens and antisera were prepared as described by Rondle & Dumbell (1962). It was noted that not all cowpox antisera showed a good precipitation reaction with 'd'. Precipitation-in-gel tests were done on a semi-micro scale in 50 mm Petri dishes with an agar layer 1·5 mm thick in which wells were cut 5 mm in diameter and 7 mm between centres. The diffusion medium was 1% agar in 0·9% NaCl buffered at pH 7·4 with 0·02 M phosphate and with the addition of sodium azide to 0·01 M. Tests were read after diffusion for 18 h at 20 °C.

RESULTS

Initial screening of antisera selected an anti-cowpox serum which gave a clear band of precipitation in the line pattern with cowpox soluble antigen which was missing from the line pattern with vaccinia or white cowpox soluble antigens. An anti-vaccinia serum was selected which gave no evidence of anti-'d' reaction. A blocking test was devised which gave a single band of precipitation with soluble antigen from wild-type cowpox and no precipitation with soluble antigen from vaccinia Lister or from white cowpox soluble antigens. The blocking test was achieved by mixing two volumes of soluble antigen with one volume of the selected anti-vaccinia serum and filling the antigen wells with such mixtures. These were allowed to diffuse against a serum well filled with the selected anti-cowpox serum. Examples of the tests are shown in Plate 1. Adjacent antigen wells were filled with red and white cowpox soluble antigens mixed with the vaccinia serum. This served as a positive and negative control in each precipitation test. A single band, given by wild-type cowpox ran out into the well, filled with the white cowpox mixture. Antigen wells on the other side of the wild-type cowpox well were filled with the viruses under test. Reactions were judged positive if a single band of precipitation

was produced which showed a reaction of identity with the cowpox band (Plate 1 B). Antigens giving a precipitation reaction, separated from the cowpox by a negative well were retested on a fresh gel in a more appropriate position (Plate 1 A & B). Preliminary results suggested that 'd' showed a good correlation with the ability of the corresponding virus to produce necrotic lesions following intra-dermal inoculation of rabbits. It was decided to include soluble antigens prepared from strains of neurovaccinia as well as dermovaccinia. The results of all the tests are summarized in Table 1, in comparison with the results of rabbit inoculation obtained by Bedson & Dumbell (1964*a*, *b*) or by Fenner (1958). Some of the test viruses gave a definite band which was weaker than that given by wild-type cowpox antigens. This difference has been noted in Table 1.

No 'd' antigen was demonstrated in 23 of the 40 viruses examined; not one of these 23 viruses produced necrotic lesions following intradermal injection of 10^5 pock forming units (p.f.u.) of virus. Only one of 17 viruses which produced 'd' antigen did not produce a necrotic lesion in rabbit skin. The single exception (VC5) requires a brief comment. This virus failed to produce any lesion at all following the intradermal inoculation of 10^5 p.f.u.; further experiments showed that this virus was also unable to replicate in cell cultures of rabbit origin. In these circumstances the possession or absence of the capacity to make 'd' substance would be irrelevant.

DISCUSSION

The remarkable correlation between the presence of detectable 'd' substance in extracts of infected tissues and the ability of the corresponding virus to produce necrotic lesions in rabbit dermis warrants consideration of the hypothesis that 'd' substance may be directly concerned in the genesis of necrotic tissue in the rabbit.

In the spontaneously arising white variants of cowpox both of these properties have been lost. Pairwise crosses between different isolates of 'white' cowpox variants failed to show any revertants of wild phenotype (unpublished observations of Fenner and Greenland and of Dumbell and Fox-Hulme). The inference that a deletion mutation was involved was confirmed by Archard & Mackett (1979) who showed by analysis of the DNA extracted from cowpox virions that there was a deletion of approximately 30 kilobases from one end of the cowpox genome which was found in all of the white pock variants of cowpox Brighton which were tested. Such a substantial deletion, however, is sufficient to carry several, independent changes of properties and does not allow evidence from cowpox mutants to substantiate the hypothesis.

The dermovaccinia strains cannot be regarded as deletion mutants of neurovaccinia. Indeed some at least of the strains of neurovaccinia were derived from strains of dermovaccinia (Wokatsch, 1972). Also the genomes of representative strains of neurovaccinia such as HI and WR are comparable in length and general organization to dermal strains such as Lister (Mackett & Archard, 1979). It can also be seen from the work of Mackett and Archard that the DNA sequences deleted from the cowpox genome in the generation of the white mutants include sequences unique to cowpox and sequences shared with vaccinia, and rabbit pox viruses. Factors affecting 'd' and rabbit skin pathogenicity should be found within the shared sequences.

Table 1. *Production of 'd' substance and of haemorrhagic, necrotic skin lesions in the rabbit by certain cowpox, vaccinia and variola viruses, by white pock variants of cowpox and by recombinants of variola with either cowpox or rabbit pox virus*

		Viruses			
'd'	Rabbit skin reaction	Cowpox	Variola	Vaccinia	Recombinants
Absent	Papular	White pox variants of Bri., Lar., Rut., Car., Mau., Dai., Pri.	Har., Hin., But.	Lister, Connaught, Led 7N, HI W.	AR3, AR4, AR6, AR7, VC6, VC8, VC10, VC12, VC16.
Present	Necrotic, haemorrhagic	Haemorrhagic pock variants of Bri., Lar., Rut., Car., Mau., Dai., Pri.	—	Lev*, HI*, rabbitpox	AR1*†, AR2*† VC2, VC7, VC13, VC14
	No lesion	—	—	—	VC5

*'d' reaction weak positive.

† Rabbit skin reaction necrotic but not haemorrhagic.

Viruses are as defined in Materials and Methods. AR and VC denote recombinants between variola and rabbitpox or cowpox respectively.

Bedson & Dumbell (1964 *a*, *b*) found that the biological markers they used segregated independently and readily among the recombinant viruses. Thus the close association between 'd' and rabbit skin pathogenicity among these same recombinants implies a close physical linkage on the genome of functional genes involved in the two observed characters; it does not necessarily argue for identity of the characters.

Haemorrhagic pocks on the CAM also, are associated with 'd' among the cowpox and vaccinia viruses. It is more difficult to consider pock character of the recombinants because they were originally selected on the basis of a white pock. Those cowpox-variola recombinants which had 'd' substance (VC 2, 5, 7, 13, 14) all gave intermediate pocks, described by Bedson & Dumbell (1964*b*) as having some degree of central haemorrhage, while those with no detectable 'd' (VC 6, 8, 10, 12, 16) gave uniformly white pocks. The two variola-rabbitpox recombinants (AR 1, 2) which had 'd' gave very small white pocks without any haemorrhage, but these two viruses also gave rabbit skin lesions which were necrotic but not haemorrhagic (Bedson & Dumbell, 1964*a*).

It is to be concluded that a gene involved in the production of 'd' is located on the pox virus genome close to genes involved in the genesis of haemorrhage and necrosis in rabbit skin and in CAM, but it cannot be said, on present evidence that the two functions are interdependent. Further study of the nature and function of 'd' will be required.

The 'd' substance as detected in soluble antigen preparations may be only a haptenic molecule and not a complete antigen. In support of this we advance some preliminary observations. The rapid rate of diffusion of 'd' showed that it was a smaller molecule than the majority of precipitating substances in the soluble antigens but no attempt was made to measure its molecular weight. The precipitating qualities of 'd' were unaffected by heating for one hour at 55 °C and it was still present in extracts that had been digested with trypsin or with ficin. The combination of these observations suggests that 'd' in the soluble antigens was not a protein and that it was unlikely to be a complete antigen. Baxby & Rondle (1968) showed that 'd' was an early antigen in that it was produced in cell cultures infected with cowpox virus in the presence of inhibitors of DNA synthesis. Tagaya suggested, in a personal communication, that 'd' might correspond to an early surface antigen that appeared in cells infected with wild cowpox viruses but not with their white variants and which was also absent from cells infected with the Lister strain of vaccinia (Amano, Ueda & Tagaya, 1979). These authors, however, found the early surface antigen in other strains of dermovaccinia and in the Harvey strain of variola, and we would, for these reasons hesitate to identify their early surface antigen with 'd'.

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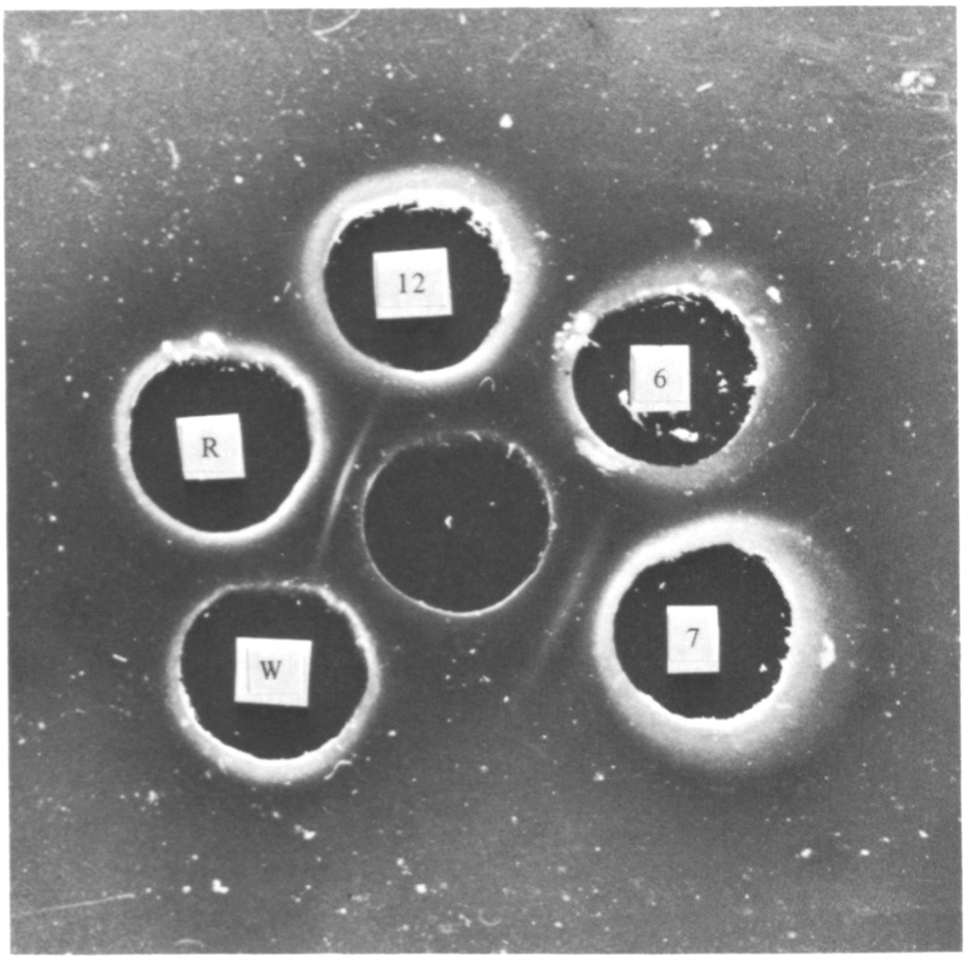
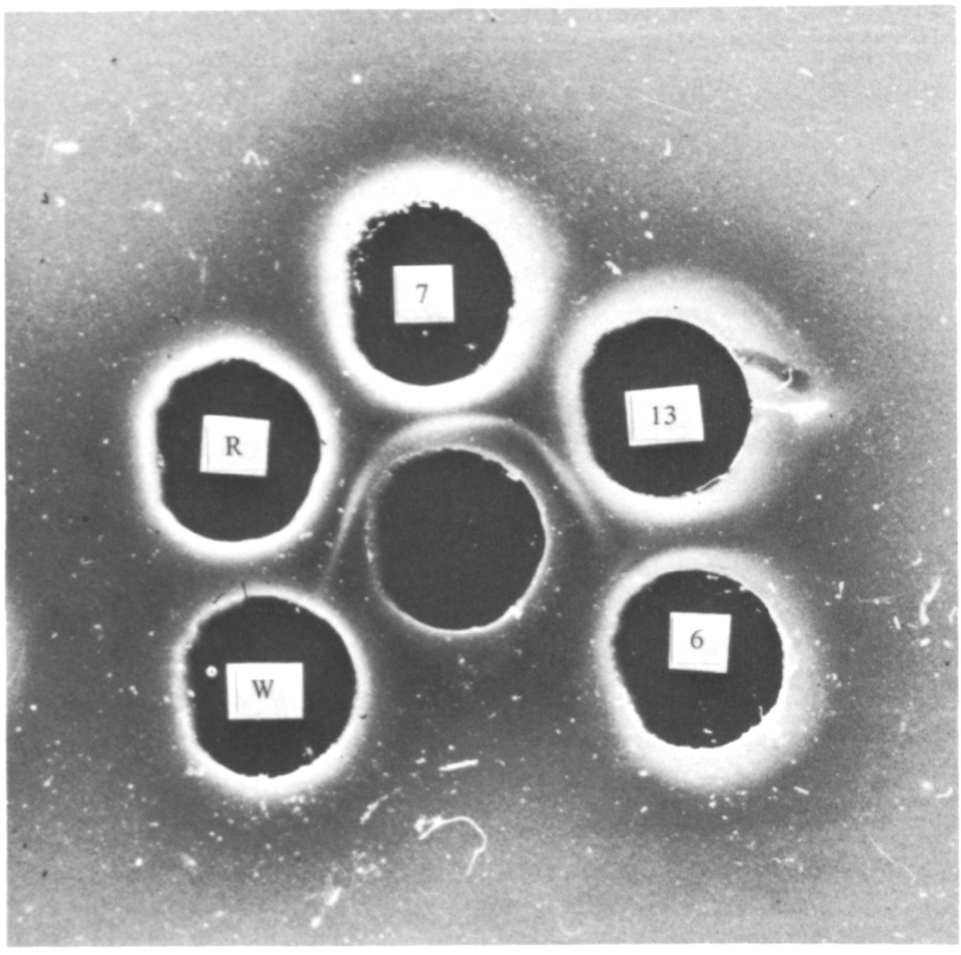
EXPLANATION OF PLATE 1

Precipitation of cowpox antiserum by soluble antigens of various pox viruses blocked with vaccinia antiserum

The inner well of each test contained hyperimmune rabbit serum raised against cowpox virus. Each outer well contained one part of vaccinia antiserum and two parts of soluble antigen prepared from extracts of CAM infected with a particular pox virus, namely wild type cowpox (R); cowpox white variant (w); viruses VC6, VC7, VC12, VC13 (6, 7, 12 and 13 respectively), each being a recombinant of variola and cowpox.

Plate 1 A shows a single band of precipitation given by wild cowpox and by VC7. Plate 1 B repeats the test of VC7, showing additionally the reaction of identity with the band given by cowpox and by extension, with the band given by VC13. VC6 is confirmed as negative.





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