Electrophoretic Studies of Ox Serum

3. THE SERA OF CATTLE INFECTED WITH THE VIRUS OF VESICULAR STOMATITIS

By C. J. BRADISH and J. B. BROOKSBY
Research Institute (Animal Virus Diseases), Pirbright, Surrey

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Previous electrophoretic studies of ox sera have shown that, during the course of infection with the virus of foot-and-mouth disease, systematic changes occur in the proportion of the β- and γ-globulin components. The present paper records the results obtained when the opportunity arose to undertake a comparative study of the sera of four cattle infected with the virus of vesicular stomatitis.

There is no record of the occurrence in Great Britain of the specific disease of vesicular stomatitis, and it is appropriate to summarize its main features. In vesicular stomatitis, as in foot-and-mouth disease, there is pyrexia of a few days' duration, with the coincident appearance of vesicular lesions on the epithelium of the mouth. Lesions on the feet are less frequent than in foot-and-mouth disease. The development of immunity to vesicular stomatitis has not been studied in detail in cattle. Animals are immune for at least 1 month to reinfection with the homologous type of virus inoculated intradermally into the tongue.

MATERIALS AND METHODS

The procedures employed for the preparation and analysis of serum samples are similar to those described in the two papers immediately preceding this, and only details of certain essential differences will be given here.

Description of cattle and preparation of sera. The cattle used were Devon steers about 2 years of age. Their history and origin are given in the first paper on p. 329. The animals were housed in pairs in loose boxes and received a diet of cattle cake and oats with unlimited hay.

The vesicular stomatitis viruses employed were the stock strains in use at this Institute; Ind.C of the Indiana type and NJ.M of the New Jersey type. Individual samples of 100 ml. of blood were collected for normal serum and the cattle were then inoculated intradermally into the tongue with the virus suspension. Local lesions were apparent within 24–30 hr. but no secondary lesions were observed except for a mild, non-vesicular eruption on the fore feet of one animal infected with virus strain Ind.C. Further collections of 100 ml. of blood were made on the first or second day following inoculation and subsequently at intervals of from 2 to 5 days for the first 2 weeks. Later bleedings were made at less frequent intervals as shown in the tables.

Electrophoretic analysis. Before electrophoretic analysis the serum samples were dialysed against a phosphate buffer solution of pH 7·6, $I = 0{\cdot}18$ and finally diluted threefold in this buffer solution. Analyses were made in the Perkin-Elmer model of the Tiselius apparatus using 3-section cells of 2 ml. capacity. Some initial difficulty was experienced in the use of the standard top-section of the Perkin-Elmer cell owing to the ejection of buffer solution which occurred when the ground-glass gate was closed at the conclusion of the period of equilibration. This had to be avoided when potentially infective materials were to be examined. The difficulty was overcome by the introduction of a hydrostatic bridge, which consisted of an inverted U-tube, 3 cm. in length, formed from capillary tubing of 1·5 mm. diameter and 1 mm. bore. This U-tube was filled with the buffer solution from a Pasteur pipette and then inverted to rest across the top-section of the electrophoresis cell with its open ends dipping into the buffer solution contained by the two limbs of the cell. Hydrostatic equilibrium was then established by the flow of the buffer solution through the inverted U-tube. At the conclusion of equilibration the U-tube was removed carefully with a forceps and disinfected by boiling. This made it unnecessary to raise the ground-glass gate.

RESULTS

In Tables 1 and 2 are summarized the electrophoretic distributions for the serum samples obtained at the indicated times from individual animals. In these tables only changes from the 'normal' distribution are given. Positive differences indicate that the percentage of that component at that time was greater than the percentage found in the normal serum of the same animal. In the second inoculation series the differences are calculated with respect to the serum collected immediately before the second inoculation and at the conclusion of the first series. The percentage scale is based on a total serum protein concentration of 100 %.

Of the two pairs of animals, one pair (Table 1, steers BD 83 and 84) received an inoculation of the Ind.C virus followed on the fifty-third day by an inoculation of the NJ.M virus: both inoculations produced a typical reaction. The second pair of animals (Table 2, steers BD 95 and 96) received a first inoculation of the NJ.M virus followed on the thirty-first day by a second inoculation of the same strain of virus: in this case the second inoculation produced no reaction.

Table 1. *Changes in the electrophoretic distribution of individual ox sera following inoculation with the virus of vesicular stomatitis*

Time following inoculation (days)	Steer BD 83				Steer BD 84			
		Globulins				Globulins		
	Albumin	α	β	γ	Albumin	α	β	γ
First inoculation sequence: *Ind.C* virus								
0*	49·6	9·7	8·4	32·3	47·4	13·7	10·2	28·7
Changes from normal distribution:								
1	−4·8	+4·2	+0·9	−0·3	−3·5	+0·2	+1·7	+1·6
2	−8·8	+7·3	+2·5	−1·0	−3·4	+0·4	+1·2	+1·8
4	−4·7	+4·7	+1·8	−1·8	−3·0	+2·4	+2·7	−1·1
7	−7·0	+6·0	+3·7	−2·7	−4·6	+1·9	+1·5	+1·2
11	−4·3	+2·1	−0·9	+3·1	—	—	—	—
13	−3·2	+3·3	−2·0	+1·9	+1·3	−2·3	−0·6	+1·6
17	−6·0	+2·4	−0·4	+4·0	−0·2	−0·5	−1·8	+2·5
39	−4·4	+3·4	+2·0	−1·0	−3·9	−1·6	−2·6	+8·1
53	−6·6	+5·2	−0·1	+1·5	−3·3	−0·1	+1·1	+2·3
Second inoculation sequence: *NJ.M* virus (Initial bleeding and inoculation on fifty-third day of first sequence)								
0†	43·0	14·9	8·3	33·8	44·1	13·6	11·3	31·0
Changes from initial distribution:								
2	−4·6	+0·2	+2·3	+2·1	+1·4	+0·6	−0·6	−1·4
3	−1·7	−1·0	+0·7	+2·0	−0·6	+0·3	+0·8	−0·5
5	+1·6	−1·4	+0·3	−0·5	+0·2	+1·5	+0·3	−2·0
8	—	—	—	—	+2·4	−0·2	−0·7	−1·5
10	−0·5	−3·0	+0·7	+2·8	+1·0	+1·1	−0·7	−1·4
15	−1·5	−4·0	−0·1	+5·6	+4·8	−1·6	−2·0	−1·2
22	−1·4	−0·8	−0·5	+2·7	+3·0	+0·4	−2·3	−1·1

* Normal distribution. † Initial distribution.

Table 2. *Changes in the electrophoretic distribution of individual ox sera following inoculation with the virus of vesicular stomatitis*

Time following inoculation (days)	Steer BD 95				Steer BD 96			
		Globulins				Globulins		
	Albumin	α	β	γ	Albumin	α	β	γ
First inoculation sequence: *NJ.M* virus								
0*	48·8	13·7	9·5	28·0	44·7	13·0	8·5	33·8
Changes from normal distribution:								
1	+1·0	−0·5	−0·7	+0·2	−1·0	0	−0·5	+1·5
4	−3·0	−0·2	−0·5	+3·7	—	—	—	—
6	−4·9	+1·7	−0·1	+3·3	−3·6	+0·8	+0·4	+2·4
11	+1·2	−5·6	−1·2	+5·6	+2·7	−2·0	−0·6	−0·1
14	−4·1	−2·9	−2·5	+9·5	+1·0	−0·6	−0·5	+0·1
21	−4·7	−0·6	−1·1	+6·4	+0·8	−2·0	−1·6	+2·8
31	−9·6	+0·9	−1·1	+9·8	−1·3	−0·6	−1·3	+3·2
Second inoculation sequence: *NJ.M* virus (Initial bleeding and inoculation on thirty-first day of first sequence)								
0†	39·2	14·6	8·4	37·8	43·4	12·4	7·2	37·0
Changes from initial distribution:								
2	−1·2	−0·2	+1·3	+0·1	−2·3	+0·7	+1·7	−0·1
3	+1·8	−1·8	+0·2	−0·2	−1·0	−0·5	+1·9	−0·4
5	+1·8	−2·6	−0·3	+1·1	—	—	—	—
8	+1·6	−2·3	−0·7	+1·4	−0·2	+0·6	+0·9	−1·3
15	+0·1	−1·9	−0·7	+2·5	−2·8	+1·3	+0·9	+0·6
22	+1·3	−0·7	−1·5	+0·9	+1·9	+1·1	+1·8	−4·8

* Normal distribution. † Initial distribution.

In keeping with the previous two papers, systematic changes in the percentage concentration of any component which exceed 3% are regarded as significant. On this basis, the following features are apparent in Tables 1 and 2.

First inoculation series

Serum albumin. The concentration of serum albumin fell rapidly in three of the four series to values down to 9% below the normal level. The series for steer BD 96 (Table 2) showed no regular change.

α-Globulin. A significant change occurred only in the series for steer BD 83 (Table 1).

β-Globulin. No significant change occurred in any series, although the data of Table 1 suggest that a slight transient rise may have occurred in the first week following inoculation.

γ-Globulin. An increase in the concentration of γ-globulin occurred in each first inoculation series. Maximum increases of from 3 to 10% above the normal level were observed between the fourteenth and fortieth days following inoculation.

Second inoculation series

Of the four second inoculation series, only that for steer BD 83 (Table 1) showed a significant increase in the concentration of γ-globulin. In this series a rise to 6% above the pre-inoculation level was observed in the third week. In two of the remaining three series (Steers BD 84 and 96) there was a tendency for the electrophoretic distribution of the convalescent serum to return to that of the normal serum. The present data are insufficient to distinguish between the second inoculation series in which the virus employed was (Table 2) or was not (Table 1) of the same type as that of the first inoculation.

DISCUSSION

The changes observed electrophoretically in the sera of cattle infected with the virus of foot-and-mouth disease or of vesicular stomatitis appear to differ in degree only. In the former case, as discussed in the previous paper, a transient increase in the concentration of β-globulin by about 4% was observed on about the seventh day after inoculation. This was followed by a rise in the concentration of γ-globulin to about 10% above the normal level during the third or fourth week. The increment of γ-globulin probably arose in the γ_2-globulin component of lower mobility. In the present study of vesicular stomatitis a significant rise in the concentration of β-globulin was not observed. An increase of from 3 to 10% was observed in γ-globulin between the third and the sixth weeks, but this was not identified as arising in the γ_2-globulin component. The apparent reduction in both the magnitude and the regularity of the electrophoretic changes in the present case may have prevented the recognition of these features. The relationship between the electrophoretic changes and the nature of the disease cannot be discussed on the basis of the present evidence.

Many aspects of this work are discussed more fully in the previous two papers.

SUMMARY

1. The sera of four cattle were analysed electrophoretically during the course of infection with the Ind.C and NJ.M strains of the virus of vesicular stomatitis. An increase in γ-globulin of from 3 to 10% above the normal level was observed between the third and sixth weeks following inoculation.

2. A second inoculation of two cattle with the same type of virus (strain NJ.M) given 31 days after the first failed to induce any significant change in the electrophoretic distributions of sera collected within the following 22 days. Of two animals which received a second inoculation of a different type of virus (strain NJ.M) 53 days after the first (strain Ind.C), the serum of only one showed a significant rise in the proportion of γ-globulin during the following 22 days.

3. The present data suggest that the electrophoretic changes in ox serum associated with the development of vesicular stomatitis are less pronounced and less regular than those associated with foot-and-mouth disease.

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