protein concentration and the electrophoretic distribution; the absolute concentration of serum albumin being maintained relatively stable close to 3.2 g./100 ml. despite much greater changes in the absolute concentration of the serum globulins.

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Electrophoretic Studies of Ox Serum

2. THE SERA OF CATTLE INFECTED WITH THE VIRUS OF FOOT-AND-MOUTH DISEASE

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In view of the importance of a knowledge of the distribution of antibody activity in the sera of cattle infected with the virus of foot-and-mouth disease, a preliminary study was undertaken of the changes in the distribution of the serum proteins which might be revealed by electrophoretic analysis. The results of this study are presented here and serve as an introduction to a current investigation of the levels of antibody activity associated with definite physico-chemical fractions of whole serum.

MATERIALS AND METHODS

Cattle. The cattle employed were Devon steers from 18 to 30 months old and were 'normal' in the sense that none had suffered from, nor had been exposed to, foot-and-mouth disease infection. Details of the origin, history and diet of these cattle are given in the preceding paper. The cattle were housed in pairs in loose boxes without bedding until at least 3 weeks after infection with the virus of foot-and-mouth disease. Subsequently, they were kept in Dutch barns on the same diet but were bedded on oat straw.

The twenty-one cattle upon which these and other observations were made were infected by intradermal inoculation of the tongue. Table 1 lists the six different strains of the virus of foot-and-mouth disease which were employed. Primary lesions developed on the tongue of each animal within 18 hr. and secondary lesions appeared elsewhere in the mouth and on the feet during the next 5 days. In an animal affected with foot-and-mouth disease there is a marked loss of condition due to the systemic disturbance and to the reduced food consumption during the period when unhealed lesions are present in the mouth.

Preparation of sera. Except where otherwise stated, 100 ml. samples of blood were collected with aseptic pre-

cautions from the jugular vein and were defibrinated by shaking with glass beads in the collection bottle. The sera were separated by centrifugation and filtered through Seitz EK disks before dialysis for electrophoretic analysis. All analyses were made in the phosphate buffer of pH 7.6 and $I=0.18$ defined in the previous paper. Sera were prepared in this way from blood samples collected immediately before inoculation, 24 hr. later, and subsequently at intervals increasing from 2 to 7 days up to the fourth week of convalescence: a few animals were bled at less frequent intervals up to the sixth month. In each experiment with any one virus strain at least two cattle received an identical treatment and a separate series of serum samples was obtained from each animal.

Table 1. *Strains of virus and numbers of cattle employed*

Strain and type of virus	Origin	No. of cattle inoculated and bled in individual experiments
149, Waldmann C	Great Britain; cattle, 1934	2, 2, 2
39, Vallée O	Great Britain; cattle, 1928	2, 3
Ven. 1, Vallée O	Venezuela; cattle, 1950	4 (Table 2)
H. 2, Vallée O	Received from Holland through the courtesy of Dr H. S. Frenkel, 1948	2
Rho 1, S.A.T. 2	Northern Rhodesia; cattle, 1948	2
119, Vallée A	Great Britain; cattle, 1932	2

Electrophoretic analysis. Both the Hilger and Perkin-Elmer models of the Tiselius electrophoresis apparatus were employed, although the majority of analyses were made with the former apparatus using 10 ml. cells of the Longworth type. In view of the need to manipulate infective solutions, the cell and electrode assembly of the Hilger apparatus was modified to the completely closed form by the introduction of electrode vessels of the type shown in Fig. 1. After connexion by rubber sleeves to the electrophoresis cell, the electrode vessels, with electrodes removed, were filled to the level *A* with the buffer solution of the last dialysis exchange. The electrodes were then connected by the limbs *C* to the two 10 ml. syringes of the electrically driven compensator and filled completely, excluding all air bubbles, with a saturated solution of KCl. After the exterior of the electrode stems had been rinsed with buffer solution, the electrodes were lowered into the electrode vessels and the current leads *E* connected to the power supply. When the two taps on one side were opened and the male cone slightly raised, the KCl solution flowed slowly from the bulb *B* into the electrode vessel to submerge the coiled electrode wire. The male cone was then lowered and the upper tap closed. At this stage the level of the buffer solution had risen into the reservoir above the ground-glass joint and into the tube

below the bulb *D*. The lower tap was closed 1 hr. later at the conclusion of thermal equilibration. These vessels have proved to be extremely convenient, not only for the manipulation of infective solutions, but also for the application of the fractionation procedure recently described by Kekwick, Lyttleton, Brewer & Dreblow (1951). In the latter case the limbs *C* are sealed and the bulbs *D* omitted.

At the conclusion of the analysis of an infective serum, the entire fluid contents of the electrophoresis assembly were pumped into a reservoir containing a solution of Na_2CO_3 at a final concentration of not less than 0.15 M (40 g./l. of washing soda). The assembly was filled completely with the same solution. The disinfection process was regarded as complete after a period of at least 24 hr. at room temperature. Disinfection by heat or acid treatment could not be employed in view of the construction of the electrophoresis cells. Serum samples were regarded as potentially infective if collected within 7 days of the day of inoculation.

Details of the general procedure for electrophoretic analysis and of the reproducibility of the distributions obtained are given in the previous paper.

EXPERIMENTAL RESULTS

In order to simplify the presentation of typical electrophoretic distributions, these are reduced to tables of differences in which each entry indicates the difference between the percentage concentrations of a given component in sera obtained from a single animal immediately before inoculation and at the stated time after inoculation. The total concentration of protein in each serum sample is taken as 100 %, and component concentrations are defined in terms of this percentage scale. A positive difference indicates that the concentration of the component at that time was greater than that observed in the normal serum of the same animal. Table 2 presents the electrophoretic data for the sera of a single group of four cattle which were bled at intervals up to the forty-second day following inoculation with the Ven. 1 strain of virus. The data for two other groups of two cattle are shown in Fig. 2. These data are typical of those obtained from the twenty-one individual series of ox sera which were analysed electrophoretically.

Details of the electrophoretic distributions and total protein concentrations of the normal sera of the present group of cattle are included in the previous paper. It is there shown that changes in the electrophoretic distribution of the normal serum of a single animal are such that differences exceeding 3 % in the percentage concentration of any component may be regarded as significant and abnormal. Accepting this level of significance the following general features are recognizable.

Serum albumin. Of the twenty-one series of analyses of sera from individual animals, three revealed an increase in the concentration of serum albumin of up to 6 % within the first 2 days following inoculation. Although the three cattle involved

Fig. 1. Closed electrode vessel for the manipulation of infective solutions. *A*, level of buffer solution before addition of saturated KCl sol. from bulb *B*; *C*, pressure-tubing connexion to one of the syringes of the electrically driven compensator; *D*, overflow bulb to receive buffer solution displaced by saturated KCl sol.; *E*, current lead sealed through the side arm and passing down through the capillary tube to the coiled electrode. Further details of this assembly are given in the text above.

Table 2. Changes in the electrophoretic distribution of individual ox sera following inoculation with the virus of foot-and-mouth disease (Virus Strain Ven. 1, type Vallée O.)

Time following inoculation of normal animal (days)	Steer BB79				Steer BB80				Steer BB81				Steer BB82			
	Globulins			Albumin	Globulins			Albumin	Globulins			Albumin	Globulins			Albumin
	α	β	γ		α	β	γ		α	β	γ		α	β	γ	
0*	42.7	15.8	8.9	32.6	41.1	12.2	9.2	37.5	46.7	15.6	8.1	29.6	47.6	14.4	8.5	29.5
Changes from normal distribution:																
1	+1.8	-2.0	+0.5	-0.3	+0.9	+0.6	0	-1.5	+0.5	-0.7	+0.4	-0.2	-4.6	+1.3	+0.8	+2.5
3	-2.0	+0.6	+0.1	+1.3	+0.7	+1.8	0	-2.5	-3.4	+2.2	+1.4	-0.2	-1.4	+0.4	+0.7	+0.3
5	-2.1	+3.1	+2.4	-3.4	-1.1	+2.7	0	-1.6	-3.4	+1.1	+4.5	-0.2	-5.1	+1.8	+4.5	-1.2
7	+2.2	-1.9	+2.6	-2.9	-1.1	+3.1	+5.9	-7.9	-2.0	-1.7	+4.5	-0.8	-3.0	+0.2	+5.5	-2.7
10	+1.5	-1.7	-0.6	+0.8	-2.9	+1.9	+4.1	-3.1	-3.1	-2.1	+2.9	+2.3	-4.5	+0.7	+4.4	-0.6
13	-0.9	-0.2	-0.4	+1.5	-1.4	-0.7	-1.8	+3.9	-2.8	+0.1	+3.8	-1.1	-3.5	-0.7	+0.3	+3.9
17	-1.1	-0.9	0	+2.0	-3.0	-0.4	-1.7	+5.1	-6.3	+4.1	+1.3	+9.1	-2.4	-1.9	+0.6	+3.7
20	+2.7	-3.1	-1.6	+2.0	-3.1	-0.6	-1.6	+5.3	-6.1	-5.5	0	+11.6	-3.3	-0.7	+0.2	+3.8
26	-4.4	+0.4	+0.6	+3.4	-2.3	-0.9	-1.9	+5.1	-6.7	-6.8	+0.5	+13.0	-5.0	+0.2	+0.3	+4.5
35	-0.5	-2.8	-0.2	+3.5	-4.1	-0.9	-0.4	+5.4	-10.1	-3.1	+0.6	+12.6	-5.9	+0.6	+0.7	+4.6
42	-1.6	-2.2	+0.9	+2.9	-5.7	-0.5	-0.7	+6.9	-10.5	-3.3	+0.8	+13.0	-2.8	-1.0	+0.6	+3.2

* Normal distribution.

were all members of the group of six cattle inoculated with virus strain 149, the data are insufficient to conclude that a strain difference exists in this respect. Apart from this sharp initial rise, the serum albumin concentration fell during the month following inoculation to levels down to 12% below the normal value. This fall was observed in eighteen of the twenty-one series.

α -Globulin. Four of the twenty-one series of sera showed a rise of up to 4% in the concentration of α -globulin between the third and seventh days. In eighteen series the concentration fell steadily after the first week to values down to 9% below the normal level.

β -Globulin. In twelve series the concentration of β -globulin rose between the fifth and eleventh days to values up to 8% above the normal level. Following this rise the normal level was regained within 7 days.

γ -Globulin. This component fell during the first week following inoculation but subsequently rose, in nineteen of the twenty-one series, to values up to 17% above the normal level between the second and the fourth week. Of the two series which failed to reveal a significant rise in the concentration of γ -globulin before the 23rd day, one was essentially typical but of low γ -globulin increment. The remaining series was atypical in that the concentration of γ -globulin showed no rise during the first 23 days, although four analyses made between the 59th and 188th days indicated that by then the level of γ -globulin had risen by 4%.

The maximum changes in the concentration of β - and γ -globulin during the first month following inoculation for a group of twenty cattle are represented in magnitude and time of occurrence in the histogram of Fig. 3. Data for the single atypical series are excluded from this figure since no maximum was observed during this initial period. Fig. 4 illustrates the typical development of the electrophoretic pattern during the course of the disease.

Of the twenty-one series of ox sera studied, fifteen were carried beyond the first month following inoculation. One of these was the atypical series described above. In five of the remaining series, the γ -globulin concentration began to fall from the maximum level during the fourth week and regained the normal or initial level between the sixth and twelfth week. In nine series the γ -globulin concentration was maintained at the maximum level until the close of the experiment in the sixth week, or in one case, in the twelfth week. Thus the extent to which the initial normal electrophoretic distribution is regained is variable, even within groups of cattle receiving inoculations of the same strain of virus. No distinction between the six strains of virus employed can be made on the present evidence.

Fig. 2. Changes in the electrophoretic distribution of individual ox sera during the course of foot-and-mouth disease: steers AV 16 and AV 17 inoculated with virus strain 149; steers AW 4 and AW 5 inoculated with virus strain Rho 1.

It must be noted that because of the representation of concentration on a relative percentage scale, a change in the percentage concentration of any one component is compensated by an equal and opposite change in the percentage concentration of the remaining components. The present data suggest that the changes in the concentrations of the β - and γ -globulins are of primary importance and that changes in the concentrations of albumin and α -globulin are largely compensatory. Further studies of the antibody activity of defined fractions are required to clarify this point.

It was shown in the previous paper that there is a tendency for the absolute concentration of albumin in normal serum to remain relatively stable despite much greater changes in the absolute concentration of γ -globulin. If the same general principle were applicable to ox serum during the month following inoculation, then an increase of, say, 10% in the level of γ -globulin would be accompanied by an increase of about 1 g./100 ml. in the total protein concentration. Data for the protein concentrations in fourteen series of sera have not

shown significant changes in excess of the normal variation. It thus appears likely that the increment observed in the γ -globulin component is largely compensated by a fall in the absolute protein concentrations of the remaining components. This problem was not pursued in detail in view of an apparent dependence of the protein concentration of individual sera upon factors other than the disease history of the animal. The electrophoretic distribution, expressed on a relative percentage scale, was largely independent of these additional factors and therefore was not reduced to an absolute scale of protein concentration.

Complexity of the γ -globulin component. Although it was not possible in all analyses to separate electrophoretically the γ_1 - and γ_2 -globulin components, sufficient data are available to permit consideration of their individual changes during the course of infection and convalescence. It was shown in the previous paper that repeated determinations of the percentage concentration of the γ_1 - and γ_2 -globulins in normal ox serum are reproducible to $\pm 1.5\%$ and that changes exceeding 3% may be regarded as

Fig. 3. Histogram of magnitude and time of occurrence of maximum changes in the electrophoretic distributions of twenty individual ox sera during the course of foot-and-mouth disease.

Fig. 4. Typical development of the electrophoretic pattern of the serum of an individual steer during the course of foot-and-mouth disease: steer BD 2 inoculated with virus strain H. 2.

Table 3. *Distribution of the γ_1 - and γ_2 -globulin components in relation to the increase in total γ -globulin.*

Time following inoculation of normal animal (days)	% of total protein			% of total protein		
	γ_1	γ_2	Increase in total γ -globulin	γ_1	γ_2	Increase in total γ -globulin
Virus strain 39, type Vallée 0						
	(Steer BB 34)			(Steer BB 38)		
0	30.3		0	36.4		0
2	11.5	17.5	-1.3	14.9	19.9	-1.6
4	11.7	19.8	+1.2	17.1	18.7	-0.6
7	9.7	19.4	-1.2	14.6	18.9	-2.9
11	10.8	20.5	+1.0	15.0	21.8	+0.4
14	11.9	23.6	+5.2	17.4	21.6	+2.6
21	12.9	26.6	+9.2	14.7	25.7	+4.0
28	13.9	27.0	+10.6	14.0	26.5	+4.1
36	11.2	29.4	+10.3	14.5	22.8	+0.9
42	12.0	25.4	+7.1	14.4	23.4	+1.4
Virus strain H.2, type Vallée 0						
	(Steer BD 2)			(Steer BD 3)		
0	12.2	19.3	0	13.0	17.6	0
1	12.9	19.2	+0.6	12.2	18.3	-0.1
3	—	—	—	12.0	19.0	+0.4
8	—	—	—	11.7	19.3	+0.4
18	15.3	26.4	+10.2	11.1	21.0	+1.5
24	15.4	33.1	+17.0	—	—	—

Table 4. *Average mobility data for the components of ten samples of 10- to 40-day convalescent ox serum*(Analyses in a phosphate buffer of pH 7.6, ionic strength 0.18 and conductivity $0.00476\Omega^{-1}\text{ cm}^{-1}$ at 1° .)

Electrophoretic mobility in units of $\text{cm}^2\text{ v}^{-1}\text{ sec}^{-1} \times 10^{-5}$, all ± 0.2 units									
Limb	Maximum ordinates of component peaks					Minimum ordinates between peaks			
	Albumin	α -Globulin	β -Globulin	γ_1 -Globulin	γ_2 -Globulin	$\alpha\beta$	$\beta\gamma$	$\gamma_1\gamma_2$	$\gamma\delta$
Ascending	5.85	4.71	3.55	2.72	1.98, 1.10*	4.09	3.19	2.29	0.73
Descending	5.48	4.16	3.11	2.44†	1.50†	3.49	2.76	1.96†	—

* An approximate figure.

† The γ -globulin components are less well separated in the descending limb. These three mean values represent only five of the ten samples analysed. The remaining five samples indicated only a complex γ -globulin component having a mean 'peak' mobility of $1.83\text{ cm}^2\text{ v}^{-1}\text{ sec}^{-1} \times 10^{-5}$.

significant. Table 3 shows the distribution of these components in the series of sera for four animals. The observed increase in the total γ -globulin is shown for comparison in the same tables. In each case the sum of the concentrations of the γ -components is equivalent to the concentration of total γ -globulin. It is apparent that the concentration of γ_1 -globulin remains throughout at a constant level while the concentration of γ_2 -globulin reflects the increment in the total γ -globulin. Thus it may be concluded that the γ -globulin increment arises almost completely in the γ_2 -globulin component.

In many analyses, particularly of immune serum, it was possible to recognize three components of γ -globulin. This is illustrated by Fig. 4. In the few cases where electrophoretic separation was possible, the component of higher mobility was identified by mobility as the γ_1 -component. The two components of lower mobility together constitute what has been

termed the γ_2 -globulin. The mobility data for all of the components studied are given in Table 4. In normal ox serum, containing, say, 30% of total γ -globulin, the three minor components contribute about 12, 13 and 5%, respectively. Present evidence suggests that the increment in γ -globulin discussed above may be confined to the third component of lowest mobility, but an unequivocal identification of this degree of refinement is beyond the scope of this investigation.

DISCUSSION

At the time that these observations were being made the determination of antibody activity in ox serum required a neutralization test in cattle. Although earlier determinations of antibody activity in ox serum were made in this way (Brooksby, 1949) the activity of a large number of samples could not be determined by the same

method in view of the expense and other factors involved in extensive experiments employing cattle. Recent work at this Institute (Skinner, 1951; Skinner, Henderson & Brooksby, 1952) has shown that it is possible to use the unweaned mouse to demonstrate antibody activity in ox serum. The use of this animal eliminates many of the restrictions inherent in the use of cattle and will now permit the ready determination of the antibody activity of defined physico-chemical fractions of ox serum. A study of this kind is essential to the full interpretation of the present electrophoretic data.

In view of the confusion which can arise in the nomenclature of electrophoretic components, the present paper includes not only the usual mobility data for the observed components but also the approximate range of mobility values which the method of analysis accepts for each. If the electrophoretic data are to be the only identification, it follows that any components which appear in convalescent sera within the range of mobility values defined by the analysis of normal sera as, say, γ -globulin must be regarded as subdivisions of this major component. Thus the components observed in the sera of cattle convalescent from foot-and-mouth disease have been called the γ_1 - and γ_2 -globulins in order of decreasing mobility. In many analyses the γ_2 -globulin has been shown to consist of at least two components but these have not been named separately in this paper. It is unlikely that these γ -components are homogeneous species and investigations of greater refinement will almost certainly reveal greater complexity. Although earlier electrophoretic studies of ox convalescent sera by Janssen (1951) have suggested that a rise may occur in a γ_2 -globulin component, the lack of specific data prevents any comparison with those reported here. Other electrophoretic data for ox serum in foot-and-mouth disease research are confined to Wehmeyer's (1948, 1950) studies of vaccinated cattle.

The data of this and the previous paper demonstrate that the range of variation of the electro-

phoretic distributions of the sera obtained from different normal cattle is of about the same magnitude as the maximum change observed for the sera of individual animals following infection with the virus of foot-and-mouth disease. For this reason it was essential to undertake the electrophoretic analysis of sera collected at regular intervals from individual animals since only thus could these changes be recognized.

SUMMARY

1. The sera of twenty-one cattle were analysed electrophoretically during the course of infection with the virus of foot-and-mouth disease. Six strains of virus were employed and a regular development of the electrophoretic distribution was observed in all but one of the 21 series of sera.

2. In the serum of the 'average typical animal' the β -globulin rose from the normal level of 10 to about 14% on the seventh day following inoculation. The normal level of β -globulin was regained during the following week. The γ -globulin component increased from the normal level of 30 to about 40% during the third or fourth week and maintained this value with little change for several weeks.

3. The γ -globulin component in ox serum comprises at least three minor components which have been characterized by their mobility ranges. The observed increment in γ -globulin arose in the components of lowest mobility.

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