In quinoline itself, metabolic hydroxylation also occurs in the 2- and 8-positions (Knox, 1946; Scheunemann, 1923), but the extent to which hydroxylation occurs in the various positions has still to be worked out. In 2-quinolone, the 6-position appears to be the most readily hydroxylated in the rabbit, since paper chromatography of the urine shows only one glucuronide. In 4-quinolone, the 3-position appears to be most readily hydroxylated, but hydroxylation in the 6-position also takes place to a small extent. It is interesting to note that in many of the quinoline antimalarials, for example quinine, the 6-position is usually blocked by a methoxyl group. Cinchonine, which has no 6-methoxyl group, has less than 0·2 of the antimalarial activity of quinine (cf. Sexton, 1953).

It is also interesting to compare the position of metabolic hydroxylation of the quinolones with the orientation of chemical substitution in these compounds. The 2- and 4-quinolones are nitrated with mixed sulphuric and nitric acids mainly in the 6-position; in the absence of sulphuric acid, 4-quinolone is nitrated in the 3-position (Schofield, 1950). Thus, the positions of metabolic hydroxylation of these compounds are similar to those found in nitrations.

SUMMARY

1. The glucuronide obtained by feeding carbostyril (2-quinolone) to rabbits has been proved to be 2-quinolonyl-6-glucosiduronic acid, since it yields 2:6-dihydroxyquinoline on hydrolysis.

2. The crystalline glucuronide (m.p. 208°) obtained in small yield from the urine of rabbits receiving 4-quinolone has been proved to be 4-quinolonyl-6-glucosiduronic acid, since after feeding with 4:6-dihydroxyquinoline the same glucuronide can be isolated.

3. The main glucuronide isolated after feeding with 4-quinolone appears to be 4-quinolonyl-3-glucosiduronic acid, since it yields 3:4-dihydroxyquinoline on hydrolysis.

4. The positions of metabolic hydroxylation of the 2- and 4-quinolones are the same as those found when these compounds are nitrated.

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Electrophoretic Studies of Ox Serum

1. THE SERA OF NORMAL CATTLE

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During the course of investigations of the sera of cattle which had been infected with the virus of foot-and-mouth disease or of vesicular stomatitis, a large number of normal sera were analysed electrophoretically. In view of the widespread interest in the proteins of ox serum and the fragmentary nature of published electrophoretic data, it was considered that the present observations from a uniform group of normal cattle should be published in some detail.

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 or to vesicular stomatitis infection. They were representative of the store cattle of the Devon breed from the southern and south-western counties of England. Post-mortem examination of 173 cattle of similar origin used in other experiments during the period of the present work showed that 24 had lesions of tuberculosis, 133 had biliary cirrhosis as a result of liver fluke infestation and one had actinobacillosis infection of the tongue. The ages of the cattle were estimated from the stage of eruption of the incisor teeth.

The cattle were obtained in various southern counties and were kept at a dealer's farm for at least 1 or 2 weeks before delivery to the Institute. At this farm the cattle were yard fed during the winter and on pasture for the rest of the year. At the Institute the cattle were housed in pairs in loose boxes without bedding and the daily ration per animal was approximately 8 lb. chopped hay, 2 lb. crushed oats, 2 lb. bran, 10 lb. sliced roots and 2 lb. linseed cake.

Preparation of sera. The first bleedings were made within 1–3 days of the arrival of the cattle. Except where stated otherwise, 100 ml. samples of blood were collected, with aseptic precautions, 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. When larger volumes of blood were required glass butter churns of 5 l. capacity were employed for collection and defibrination. The total protein concentration of serum samples was determined on duplicate samples by the Kjeldahl procedure using a protein/nitrogen ratio of 6·25. Although ox serum contains about 35 mg./100 ml. of non-protein nitrogen, no allowance was made for this in the determination of the much higher level, about 1000 mg./100 ml., of protein nitrogen.

Electrophoretic analysis. Preliminary analyses were conducted in a range of buffer systems in order to define appropriate conditions for subsequent work. The buffer systems employed were:

(i) Veronal buffer, pH 8·6, $I = 0{\cdot}10$, 0·1 N sodium diethyl barbiturate and 0·02 N diethylbarbituric acid.

(ii) Phosphate buffer, pH 7·6, $I = 0{\cdot}18$, 0·058 M-Na_2HPO_4 and 0·0087 M-KH_2PO_4.

(iii) Veronal/saline buffer, pH 7·6, $I = 0{\cdot}10$, 0·025 N diethylbarbituric acid and 0·017 N sodium diethylbarbiturate in 0·083 N-NaCl.

(iv) Phosphate/saline buffer, pH 7·5, $I = 0{\cdot}18$, 0·0087 M-Na_2HPO_4 and 0·0013 M-KH_2PO_4 in 0·15 N-NaCl.

Of these buffer systems, the phosphate and veronal/saline systems at pH 7·6 were superior in the extent of separation of the β- and γ-globulin components. The more convenient phosphate system was adopted finally, since the difference between the ascending and descending distributions was less.

Prior to electrophoretic analysis, each sample of serum was dialysed for 48 hr. at 4° against four changes of the buffer solution; the volume of buffer solution at each change being ten times that of the serum. The phosphate buffer system employed is defined above. Immediately before electrophoretic analysis the dialysed sera were diluted three-fold in the buffer solution of the final dialysis exchange. Thus analysis was conducted at a protein concentration of 1·8–2·6 g./100 ml.; the exact concentration being determined refractometrically. This adoption of a standard dilution of the dialysate simplified the later manipulation of infective sera and, as shown by the data of Table 1, introduced a systematic error which was negligible for the purposes of this study. Koenig, Perrings & Hogness (1946) have investigated the influence of the ionic strength of the buffer system on the electrophoretic distribution of the components of ox serum and have summarized earlier studies of the influence of both ionic strength and protein concentration.

Both the Hilger and Perkin-Elmer models of the Tiselius electrophoresis apparatus were employed, although the majority of analyses were made in the former apparatus using 10 ml. cells of the Longsworth type. The cell and electrode assembly of the Hilger apparatus were modified to the completely closed form; the boundary position being adjusted by two syringes driven in opposition. The arrangement of the electrode vessels was designed for the convenient manipulation of infective sera and is described in detail in the paper immediately following.

A voltage gradient of 6 v/cm. was maintained in both types of cell, and migration was allowed to continue for 3 hr. in the Hilger apparatus and for 2 hr. in the Perkin-Elmer apparatus. Photographic records of the ascending and descending boundaries were made at 30 min. intervals and those obtained during the last 30 min. of migration were analysed planimetrically. The photographic record was obtained as a dark line on a light background, and in all cases the lower contour of this record was traced at a $\times 8$ enlargement. The dominant albumin peak was separated by a construction based on the assumption of symmetry, and the globulins and the anomalies were separated by the construction of minimum ordinates. Although ox γ-globulin comprises at least three electrophoretic components, it was not always profitable to attempt a quantitative subdivision of the peak area. The δ-anomaly was separated readily as

Table 1. *The influence of protein concentration on the electrophoretic distribution of normal ox serum*

Animal	Protein concentration in electrophoresis cell (g./100 ml.)	Albumin (%)	α-Globulin (%)	β-Globulin (%)	γ_1-Globulin (%)	γ_2-Globulin (%)
BL 15	2·02	45·6	18·2	7·7	12·0	16·5
	1·02	46·9	16·7	8·6	10·5	17·3
BL 20	3·08	48·2	17·4	8·6	11·9	13·9
	2·02	47·3	17·3	8·8	12·3	14·3
	0·97	48·9	16·0	7·9	11·7	15·5
BL 21	3·02	51·1	16·3	8·4	8·9	15·3
	1·96	47·7	15·4	9·9	10·5	16·5
	0·95	50·6	14·3	8·6	9·1	17·4

Table 2. *Typical mobility data for the components of normal ox serum*

(Analyses made in a phosphate buffer of pH 7·6, ionic strength 0·18 and conductivity 0·00476 Ω^{-1} cm.$^{-1}$ at 1°.)

		Mobility (cm.^{2} v^{-1} sec.$^{-1}$ × 10^{-5})						
		Maximum ordinates of component peaks				Minimum ordinates between peaks		
Animal	Limb	Albumin	α-Globulin	β-Globulin	γ-Globulin	αβ	βγ	γδ
BB 34	Ascending	5·97	4·54	3·57	2·91	4·13	3·02	0·63
	Descending	5·70	4·14	3·17	1·59	3·32	2·74	—
BB 79	Ascending	6·05	4·71	3·54	2·59	4·05	3·08	0·92
	Descending	5·72	4·05	3·23	2·49	3·41	2·95	—
BB 82	Ascending	6·08	4·76	3·73	2·75	4·18	3·35	0·83
	Descending	5·57	4·07	3·28	1·79	3·57	2·77	—

a single peak representing about 15 % of the total area of the ascending pattern. The ε-anomaly represented about 4 % of the total area of the descending pattern and it was necessary occasionally, when no true minimum occurred between the γ-globulin peak and the anomaly, to estimate the position of the dividing ordinate. In all cases two ascending and two descending patterns were analysed and the mean distribution was adopted. Analyses were discarded if the percentage of either albumin or γ-globulin indicated by the two limbs of the U-tube differed by more than 6 %. (Total serum protein taken as 100 %.)

Determination of electrophoretic mobility. Electrophoretic mobilities were calculated from the slope of the best-fitting linear relationship between time and displacement; four exposures taken at 30 min. intervals were employed for this purpose. Buffer conductivities were determined on a bridge of the type described by Shedlovsky (1930) with cells following the design of Jones & Bollinger (1931). The Baldwin null-detector (Baldwin Instrument Co. Ltd.) was found to be particularly convenient and accurate in this arrangement.

Typical mobility data for the components of normal ox serum are presented in Table 2. In view of the complexity of an analysis of migration in a system of many components, the mobilities of the minimum ordinates used in construction are quoted as well as those for the peak maxima. The mobilities of these minimum ordinates, taken in pairs, define the range of mobility values accepted for each component. Apparent variations in the mobility of γ-globulin arise from the multiplicity of this component; more consistent values are obtained when the three minor components are resolved and analysed separately.

EXPERIMENTAL RESULTS

Accuracy of analysis. An analysis of the differences between the distributions indicated by the two limbs of the U-tube in thirty-nine analyses of normal ox sera showed that albumin was greater by 1·8 ± 2·6 % in the descending pattern and that γ-globulin was greater by 0·4 ± 1·7 % in the ascending pattern. Duplicate analyses in the Hilger apparatus indicated that the percentage of each component in the mean distribution was reproducible to within ± 1 %. The percentage scale employed here and throughout the paper is based on the total concentration of serum protein as 100 %.

Electrophoretic distribution. The electrophoretic data for the sera of fifty-one cattle are summarized in Table 3 and presented as a distribution histogram in Fig. 1. Each vertical column of the histogram represents the number of analyses in which the percentage of the appropriate component was within 0·5 % of the indicated value; the lower limit being included in the group. Typical electrophoretic diagrams are shown in Fig. 2. In some distributions the major components may be subdivided further, particularly the complex γ-globulin, and representative data are included in Tables 1 and 6. The separation of the components of γ-globulin is discussed

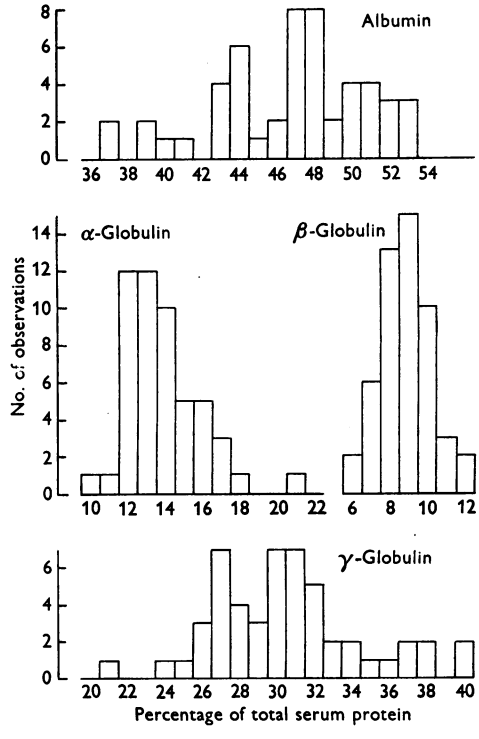

Fig. 1. Histogram of distribution of serum proteins in fifty-one normal ox sera.

Table 3. *Summary of the analysis of fifty-one normal ox sera*

Analysis of serum		Mean and standard deviation	Range of variation	Standard deviation / Mean
Electrophoretic distribution of components (total serum protein taken as 100%)	Albumin	46·6±4·1	36·6–52·9	0·087
	α-Globulin	14·0±2·1	9·7–21·4	0·151
	β-Globulin	8·9±1·4	5·7–11·7	0·154
	γ-Globulin	30·5±4·0	21·0–39·8	0·130
Total serum protein concentration (30 analyses) in g./100 ml.		6·97±0·53	6·10–8·10	0·076

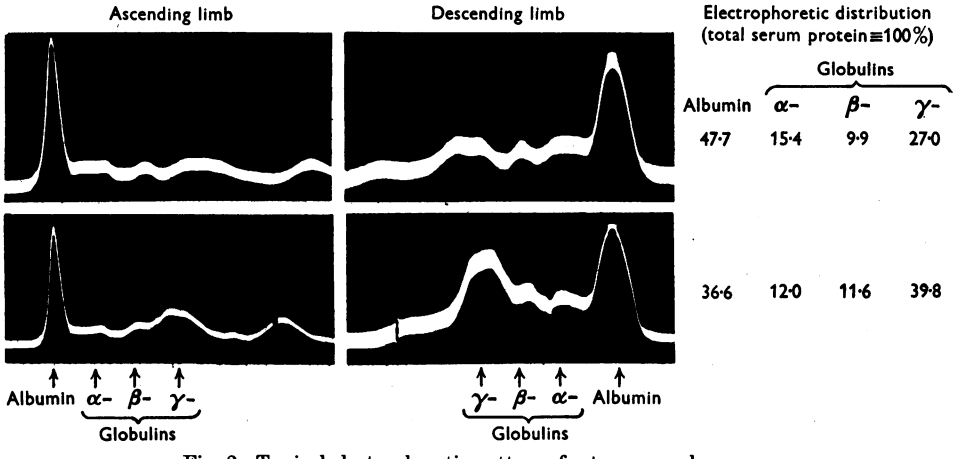

Fig. 2. Typical electrophoretic patterns for two normal ox sera.

more fully in the next paper. The range of variation of these electrophoretic distributions is such that the greatest differences occur within the group of globulins; the apparently narrow distributions of the α- and β-globulins in the histogram are due to the relatively low proportion of these globulins and the choice of group width.

Correlation between electrophoretic distribution and serum protein concentration. In view of the possibility that differences between individual distributions of serum proteins may be related to observed factors in the history or treatment of the cattle, the available data were examined for significant correlations. Within the present narrow range of values, the age of the animal and the season in which it was received and bled were unrelated to the distribution of its serum proteins. It was found, however, that the percentage of albumin in the distribution, A, as determined electrophoretically, was related to the total concentration of serum protein (C g./100 ml.) in such a way that changes in the absolute concentration of serum albumin [(A. C/100) g./100 ml.] were slight compared with the 4 to 5 times greater change in the absolute concentrations of the globulins. Thus the concentration of serum albumin tended to remain relatively steady at about 3·2 g./100 ml. Table 4 summarizes the analysis of the data for thirty electrophoretic distributions and total protein concentrations. The correlation was calculated in terms of the reciprocal of C in view of the form of the expression indicated above in brackets. The significant correlation between the percentage of albumin or γ-globulin and the total serum-protein concentration implies that an increment, say, of 1 g./100 ml. in the total protein concentration would be partitioned as about 0·8 g./100 ml. of total globulins and only 0·2 g./100 ml. of serum albumin. The absence of any significant correlation between the total serum protein concentration and the percentages of α- and β-globulins reflects the relatively limited accuracy with which components of this low concentration may be determined electrophoretically. The histogram of the total serum-protein concentration for this group of cattle is shown in Fig. 3. This indicates a mean concentration of 6·97 g./100 ml. with a

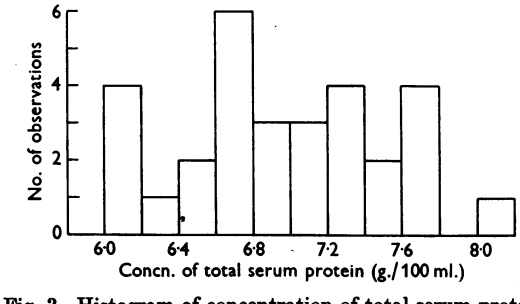

Fig. 3. Histogram of concentration of total serum protein in thirty normal ox sera.

Table 4. *Analysis of correlation between electrophoretic distribution and total protein concentration for thirty normal ox sera*

Component	Mean electrophoretic distribution (%)	Mean absolute concentration (g./100 ml.)	r	P	K	k (g./100 ml.)	k as percentage of mean absolute concentration
Albumin	45·9	3·20	+0·501	0·004	+173	0·21	6·6
α-Globulin	14·0	0·98	−0·119	0·53	Correlation not significant	0·27*	17*
β-Globulin	8·8	0·61	−0·219	0·15			
γ-Globulin	31·3	2·18	−0·376	0·04	−144	0·52	24
Total protein	100	6·97	—	—	0	1·00	14·3

r, the correlation coefficient between the percentage of the indicated component and the reciprocal of the total concentration of serum protein.

P, the significance of the correlation. The value of $P=0{\cdot}04$ for γ-globulin indicates that the observed correlation coefficient would be exceeded 4 times in 100 similar trials if the paired values were unrelated.

K, the regression coefficient for the dependence of the percentage of the component upon the reciprocal of the total protein concentration.

k, the average increment in the absolute concentration of the component arising from an increment of 1 g./100 ml. in the total protein concentration.

* Combined value for α- and β-globulins required to give correct value of k for total serum protein.

Table 5. *The influence of volume bled at one time on the electrophoretic distribution of normal ox serum*

Animal	Volume bled prior to collection of 100 ml. sample for analysis (l.)	Albumin (%)	α-Globulin (%)	β-Globulin (%)	γ-Globulin (%)
BB 32	0·0	42·6	15·4	10·7	31·3
	2·1	43·2	16·4	9·5	30·9
	4·2	43·1	16·2	9·7	31·0
BB 33	0·0	41·7	14·0	9·7	34·6
	2·1	40·1	15·5	9·8	34·6
	4·2	40·4	15·4	9·9	34·3

Table 6. *Variations in the electrophoretic distributions of sera obtained over periods of one month from individual normal cattle*

(The values quoted are the means and the standard deviations; the extreme range of variation is shown in brackets.)

Animal	No. of bleedings and analyses during one month	Albumin (%)	Total γ-globulin (%)	γ_1-Globulin (%)	γ_2-Globulin (%)
BD 2	5	48·1±1·0 (3·1)	30·4±1·4 (3·4)	12·6±0·7 (2·6)	17·8±0·9 (2·5)
BD 3	5	47·2±0·5 (1·4)	29·8±1·0 (2·7)	12·2±0·5 (1·4)	17·6±0·6 (1·6)
BF 65	7	46·1±2·9 (9·7)	30·9±1·4 (3·8)	—	—
BF 66	7	47·8±1·5 (4·7)	23·9±1·5 (4·7)	10·6±0·4 (1·3)	13·3±1·6 (4·3)

standard deviation of 0·53 g./100 ml. These data are summarized in Table 3 and are consistent with those published by Wehmeyer (1950*a*) for older cattle.

Influence of volume bled at one time. In some cases when blood was required for other experiments, as much as 5 l. were collected from each animal and the possible influence of this difference in procedure on the electrophoretic distribution of the serum proteins was studied. Analyses were made of the serum from the first 100 ml. of blood and of the serum from subsequent 100 ml. samples collected separately after 2·1 and 4·2 l. had been taken. The distributions presented in Table 5 indicate that the removal of up to 4 l. of blood is without significant influence on the distribution of the serum proteins.

Variations in distribution over periods of 1 month for individual cattle. In parallel experiments on the influence of infection with the virus of foot-and-mouth disease on the composition of ox serum, a knowledge was required of the variation in composition which might occur when a normal animal was housed under standard conditions for periods up to 1 month. Four normal cattle were accordingly kept for one month and bled for sera at intervals of from 4 to 7 days. Electrophoretic analysis of these sera showed greater changes than those indicated by Table 5 for the sera obtained by repeated bleedings on 1 day. As anticipated, the greatest departures from the period mean occurred in the predominant albumin and γ-globulin components. The variations in these components during the month of observation are given in Table 6. These data probably indicate the variability of individual sera rather than the level of experimental error, although both factors must influence the result. It is justifiable to con-

clude that a systematic change in any component which exceeds 3% (total serum protein taken as 100%) may be regarded as significant and abnormal. This criterion will be applied in the following two papers.

DISCUSSION

The physical criteria which govern the choice of buffer solution for electrophoretic analysis have been presented by many authors (Longsworth & MacInnes, 1939; Longsworth, 1942). It is evident, however, that these criteria relate to factors which limit the application of many buffer solutions only in the most critical physical studies. Thus, for most electrophoretic studies of unfractionated and complex protein systems, the choice of the buffer solution must be determined by the quality of the electrophoretic separation obtained. This is demonstrated by the recommendation by Longsworth (1942) of a veronal buffer for the analysis of human plasma and serum and a phosphate buffer for the analysis of horse plasma.

The phosphate buffer of pH 7·6 employed in the present study of ox serum was similar to that employed by Svensson (1941) who noted the high proportion of a complex γ-globulin and the low level of β-globulin. Janssen (1951), using a phosphate/saline buffer for the analysis of ox serum, commented on the poor separation of the γ-globulin from the boundary anomalies. This was confirmed in the present study, although the phosphate/saline buffer was superior to the phosphate buffer in the separation of albumin and the α-globulins. Many investigators, particularly in the United States (Deutsch & Goodloe, 1945; Cann, Brown & Kirkwood, 1949), have employed various veronal buffer systems for the analysis of ox serum and plasma and have obtained electrophoretic distributions which differ considerably from those obtained in phosphate buffers. Their electrophoretic analyses in veronal buffers at pH 8·6 provided good separation of albumin and the α-globulins but failed to resolve satisfactorily the slower globulins.

The variability apparent in the published electrophoretic data for ox serum, particularly in the proportion of β- and γ-globulins, probably arises from the diversity of methods of analysis and precludes any consideration of differences which may arise from other causes. Although few investigators have had occasion to undertake the electrophoretic analysis of serum samples from large and well-defined groups of normal cattle, the majority of available analyses in phosphate buffers are consistent with the distribution histogram of Fig. 1. It is clear from this histogram that even within a uniform group of cattle, the variations from individual to individual are such that a large number of analyses is necessary if the results of different investigators are to be compared or if properties which are characteristic of the animal and its history are to be related.

The present electrophoretic data for steers of a defined age demonstrate the tendency for the absolute concentration of serum albumin to change relatively little despite much greater changes in the total concentration of the globulins. The observation implies that changes in the total concentration of the serum proteins are determined largely by changes in the absolute concentration of the 'immune' globulins, in particular of the γ-globulins, and that the serum albumin, for cattle of 18–30 months, is maintained at a level close to 3·2 g./100 ml. It is of interest to note that the correlation between age and the concentration of γ-globulin, which has been established for the ox (Wehmeyer, 1950*b*) and many other species, is not inconsistent with the present observation of a relatively stable level of serum albumin.

The frequent observation of an increase in γ-globulin as an accompaniment to the development of immunity suggests that under certain conditions, and for a particular disease, the result of a single electrophoretic analysis of a possibly convalescent serum could be used as an indication of previous infection. It is apparent, however, that the range of variation in the normal percentage of γ-globulin is such that it would be necessary to demonstrate a level of γ-globulin in excess of 38% before a sample of ox serum could be classified as abnormal. If this identification were to fail only once in twenty cases, it would be necessary for the level of γ-globulin in individual convalescent sera to be regularly 13% greater than the level of γ-globulin in the corresponding normal sera. Thus, on account of the variation between individual animals, it is essential in any critical investigation, where changes of this magnitude may not occur, to undertake the electrophoretic analysis of sera derived from regular bleedings of single animals during the period before infection and until immunity has developed.

SUMMARY

1. The sera of fifty-one normal Devon steers from 18 to 30 months old were analysed electrophoretically in a phosphate buffer of pH 7·6 and ionic strength 0·18. The mean electrophoretic distribution was found to be albumin, 46·6; α-globulin, 14·0; β-globulin, 8·9 and γ-globulin, 30·5%. The γ-globulin comprises at least three minor components. The frequency distributions of these data are presented.

2. The data for thirty of these steers indicated an absolute concentration of serum protein of 7·0 ± 0·5 g./100 ml. A significant correlation was found to exist between individual values for the serum

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 precautions 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 $I = 0{\cdot}18$ 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