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THE ABSORPTION OF CHLORIDE IONS FROM THE RETICULO-RUMEN SAC

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Chloride ion can move against its concentration gradient to the blood from the contents of the reticulo-rumen of sheep (Parthasarathy, 1952; Parthasarathy & Phillipson, 1953; Sperber, Hydén & Eckman, 1953) and from the rumen pouch of the goat (Sperber & Hydén, 1952). The object of the work presented here is to explain this transport in terms of the forces moving the ion.

An ion which diffuses freely through a membrane should move down its electrochemical gradient. The electrochemical potential difference in volts of an ion between two solutions denoted by subscripts 1 and 2, $(\mu_1-\mu_2)/F$, is given by the following expression

$$\frac{\mu_1-\mu_2}{F}=\frac{RT}{F}\ln\frac{c_1}{c_2}+\frac{RT}{F}\ln\frac{f_1}{f_2}+z(V_1-V_2),$$

where c is the concentration of the ion, f is its activity coefficient, z the ion charge and V the potential. The separate evaluation of the potential difference and activity coefficient cannot be justified thermodynamically, as measurements can be made which lead to only certain combinations of these two quantities (e.g. Harned & Owen, 1950). Nevertheless, the measurement of the potential between two aqueous phases by strong KCl bridges making contact with reversible electrodes, with correction, if necessary, for differences in the activity coefficients, has given results of considerable significance in studies of permeability, particularly of frog's skin and of nerve fibres. The potential difference between the blood and the contents of the reticulo-rumen sac of unanaesthetized sheep with normal rumen contents was found to be of a size and direction which would account for the movements of chloride against its concentration gradient. Acute experiments were then undertaken to discover whether it would account for the direction of chloride movement in detail. A preliminary account of these experiments has been given (Dobson & Phillipson, 1954).

METHODS

Electrical measurements on conscious sheep

Sheep were fitted with a permanent ebonite rumen cannula at least a month before the experiment. Measurements were made with the animal lightly restrained on a low wooden stand. The potential differences were measured every 1 or 2 min to $\pm 0{\cdot}5$ mV, using a Cambridge pH meter with floating earth. Saturated calomel electrodes were used, dipping into saturated KCl at room temperature in a water-bath. Saturated KCl–agar bridges led from the saturated KCl to the animal. These bridges were made by filling polythene tubing with hot solution containing 40% KCl and 3% agar. A bridge in tubing of 0·5 mm bore was used to make contact with the blood by inserting it through a wide bore hypodermic needle into the jugular vein. Contact to the rumen contents was made by two bridges in polythene tubing of 2 mm bore. These were inserted into the rumen contents through glass tubes in a bung in the rumen cannula. The tip of each bridge was protected by a polythene sheath at the end of each glass tube.

Acute experiments

Surgery. The surgery for these experiments has been described in detail (Masson & Phillipson, 1951). The rumen of a small sheep was cannulated at least a month previously. After fasting during the night, the reticulo-rumen sac contents were washed out and replaced with sufficient 150 mM-NaCl. The sheep was then anaesthetized with pentobarbital and the sac isolated from the rest of the gut by a ligature round the omasal neck, and another round the oesophagus. Before the abdomen was closed a small bridge was introduced into a small rumen vein draining the right side of the posterior part of the ventral sac, and passed into the main posterior rumen vein.

Absorption experiment. The saline in the sac was washed out with tap water at 39° C to reduce to a minimum the chloride remaining. The experimental solution was then introduced and, after approximately half an hour, was removed and the rumen drained. The residue of the experimental solution was washed out by introducing immediately 3 l. of warm tap water which was then removed, and the rumen drained. As soon as possible the next experimental solution was poured in. The volume of solutions and water introduced into and removed from the rumen was measured in cylinders with a S.E. of $\pm 2{\cdot}5$ ml. and corrected to 39° C.

To allow corrections to be made for the residues left in the rumen, the emptying of the rumen and the washing were carried out in a standard way, and all operations were timed. The residue of the experimental solution, about 50 ml., was calculated by determining the acetate content of the tap water with which the rumen was washed. Acetate was chosen as a marker because it was found that no detectable quantity of this ion entered a volume of tap water introduced for a short time into an empty rumen previously washed out with tap water. This was not true for sodium, potassium and chloride. The residues of the wash solutions were assumed to be the same as the residues from the experimental solutions, as these were drained in the same manner. Loss of water from tap water residues would be greater than from the experimental solution residues during the short time between draining the rumen and introducing the next solution, as the experimental solutions were approximately isotonic with plasma. It has been assumed that the loss of water while the wash solution was in the rumen, approximately 40 ml. in 3 min, continued linearly up to the time the experimental solution was introduced. This assumption permitted a calculation to be made of the volume of residue at the instant the experimental solution was introduced into the sac, thus allowing the initial volume for an absorption period to be estimated. The error in calculating the water changes during an experimental period was probably not greater than ± 20 ml.

Chloride entered the wash solution to the extent of 0·5 m-equiv in 3 min. This entry was assumed to continue linearly until the experimental solution was introduced. This small correction for entry plus the amount of chloride left behind in the wash residue were added to the amount introduced in the experimental solution. When the net chloride movement was small the application of these small corrections to the change of chloride in the rumen allowed more confidence to

be placed on the results. Leakage of chloride into the rumen from the bridges was found to be negligible.

It was found that the errors in chloride flux measurement lay mainly in the method of analysis and in measurement of the volumes of experimental solution introduced and recovered from the rumen. As fractional changes in volume and concentration during an absorption period were small, the errors were estimated from the expression

$$d\Delta = \sqrt{[2(V^2\,dc^2 + c^2\,dV^2)]},$$

where $d\Delta$ is the S.E. of the change in chloride; V is the mean volume, with S.E. dV; c is the concentration, with S.E. dc.

The electrochemical potential difference was calculated from the following values:

(1) The concentration contribution was calculated from the mean of the calculated initial concentration and the measured final concentration in the rumen, together with the plasma chloride from a sample from the jugular vein taken halfway through the absorption period. As it was not practical to collect plasma samples anaerobically, 4% was subtracted from the measured concentration to allow for chloride shift on exposure to air.

(2) The activity coefficient ratio was assumed to be unity, as the tonicity, and presumably the ionic strength of the solutions used, was close to that of the plasma.

(3) The potential between two bridges in the rumen contents and a bridge in the venous blood leaving the rumen was measured. When the reading of the two electrodes differed, the two readings were averaged. The mean value over the absorption period was taken as the potential contribution to the electro-chemical potential difference. To obtain steady readings it was necessary to keep the sac contents stirred by pressing rhythmically on the sheep's abdomen.

Analysis. Chloride was determined by electrometric titration with $AgNO_3$ in 50% (w/v) acetic acid (Sanderson, 1952). Analysis of experimental solutions was made using a differential technique (Keys, 1931) which gave a standard error of duplicate estimations of $\pm 0{\cdot}012$ mM for concentrations up to 50 mM. Protein interference was negligible. Plasma chloride was found by titrating suitably diluted samples (S.E. $<1\%$). The wash solutions were titrated directly with a similar accuracy. Acetate was determined as steam volatile fatty acid with a S.E. of $\pm 0{\cdot}6$ mM. For 1·5 l. of 150 mM acetate, the total S.E. in acetate uptake is $\pm 1{\cdot}4$ m-equiv.

Conventions. A net flux is given a positive sign if its direction is from rumen contents to plasma. The electrochemical potential difference and its components are measured with respect to the plasma. This means that the sign of the electrochemical potential will be the same as the sign of the flux for an ion which moves down its electrochemical potential difference.

RESULTS

Electrical potential difference across reticulo-rumen epithelium

Stability of potential reading. Handling the bridges or dipping a loop into water had no effect on the potential difference measured. If an end artifact was suspected the removal of the end centimetre of the bridge laid bare a fresh surface of KCl–agar with which to renew contact. This was done frequently at first, until it was clear that no appreciable change in potential resulted from this operation (Fig. 1). Touching the meter did not affect the readings except under very damp conditions in the sheep pens. This effect was eliminated by standing the meter and observer on polythene sheeting. The potentials measured showed no change, transient or otherwise, on moving the rumen bridge through the contents, or when the sheep bleated or fidgeted.

Potential differences within the reticulo-rumen contents. The potential difference was measured between a bridge with its tip fixed a few centimetres

inside the rumen cannula and another with its tip at a distance from the cannula which could be varied. This potential difference was less than ± 0·5 mV from within the lumen of the ebonite cannula (in the dorsal sac of the rumen), and across the contents of dorsal and ventral sacs. Whenever the movable bridge tip touched the rumen wall it became positive, with a fluctuating potential difference of up to 14 mV.

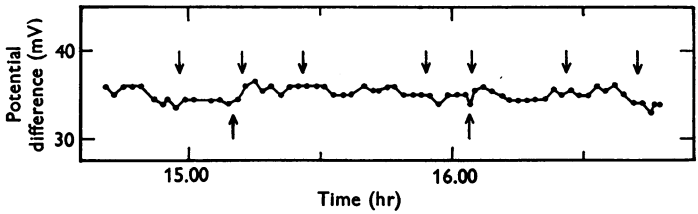

Fig. 1. Potential of jugular bridge with respect to rumen bridge. The arrows above the record indicate where the rumen bridge tip was cut off to renew the surface of KCl. The arrows below the record show where the jugular bridge tip was renewed.

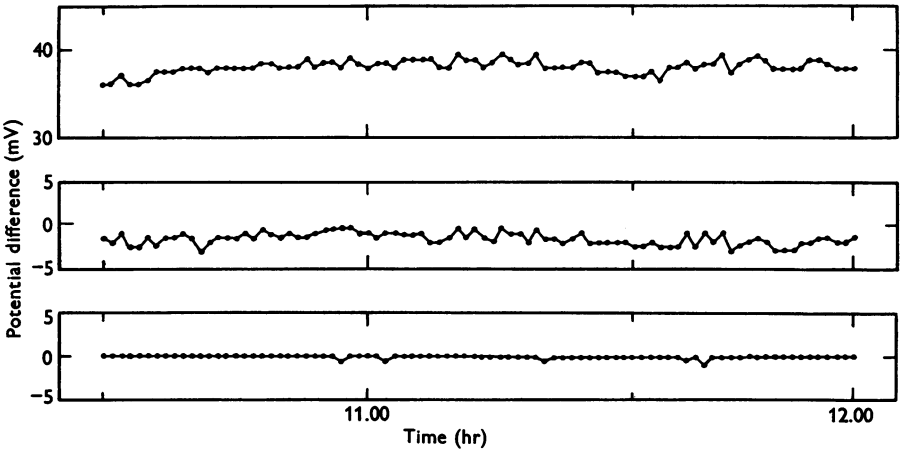

Fig. 2. Top record: potential of jugular bridge with respect to rumen bridge 1; middle record: potential of reticulum bridge with respect to rumen bridge 1; bottom record: potential of rumen bridge 2 with respect to rumen bridge 1. The reticulum bridge was inserted through a reticulum cannula. The lower records were obtained by difference which probably accounts for the small differences appearing between the two rumen electrodes.

The potential difference between the reticulum and rumen was measured in one sheep which had been successfully fitted with a polythene cannula in its reticulum. Similar results were obtained with other sheep when the reticulum bridge tied to a recording balloon was introduced by way of the rumen cannula. Small potential differences of from 0 to 4·5 mV with the rumen positive were measured (Fig. 2). It was concluded that potential gradients across the reticulo-rumen contents were small compared to the potentials between the contents and the blood.

Potential differences between different sites in the blood. In acute experiments when the potential between the blood leaving the rumen and rumen contents was measured, another connexion to the blood was made by inserting a bridge into the jugular vein. A small potential difference was often found between the bridges in the circulation. Individual readings of jugular with respect to rumen bridge during twenty-nine absorption periods on eight sheep ranged from −6 to +6 mV. The mean potentials during each absorption period ranged from −6 to +3·2 mV. Except for one experiment the mean potentials varied from −3·6 to +3·2 mV. The average of all mean potentials during the absorption periods was −1·1 mV. In experiments on two sheep a third bridge was inserted into the saphenous vein and passed up into the posterior vena cava. The range of individual potential difference of the jugular with respect to saphenous bridge was from −3 to +3 mV. The average potential difference during ten absorptions ranged from −1·9 to +1·6 mV, with an average of −0·5 mV. The calculated mean potential difference of the saphenous bridge with respect to the rumen bridge ranged from −1·8 mV to +2·4 mV with an average of 0·2 mV. It was concluded that the potential difference between bridges at different sites in the circulation was small compared with the potential drop between the circulation and the contents of the reticulo-rumen sac.

Potential difference between reticulo-rumen contents and blood in conscious animals. Measurements were made on sheep fed on a diet of hay, or hay and meals, standing with as little interference as practicable. In every case the jugular bridge was positive with respect to rumen contents. The range found was 8–49·5 mV. The potential measured showed small regular fluctuations superimposed on steady potentials. The regular fluctuations are action potentials of the contraction of the rumen (Dobson, 1956). Apart from the action potentials, the more steady component showed sometimes distinct trends upwards or downwards, while at others it would remain stationary for several hours (Figs. 1–3). The trends had no obvious relationship to the eating, drinking or ruminating of the sheep. Some of the more steady records were obtained the first time the sheep was introduced into the stand, although the animal was nervous and difficult to pacify. The range of potential of each recording period is shown in Table 1, together with a representative selected potential. The latter represents the potential during the first period of 15 min or more in each recording when the potential remained fairly constant. Only one period was taken from each morning or afternoon recording to give potentials fairly independent of each other. The mean potential over the recording period was close to the potential chosen.

The mean of these selected results is 31·2 mV, S.D. ±6·8. The contribution to the electrochemical potential difference for a univalent ion of a potential difference of this size is equivalent to that of a three times concentration ratio.

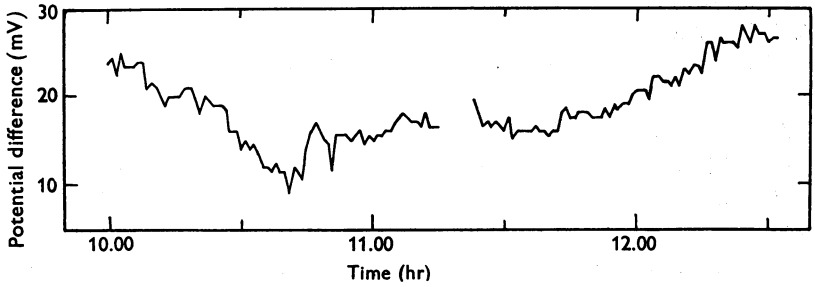

Fig. 3. Potential of jugular bridge with respect to rumen bridge. The line is drawn to join observations taken at 1 min intervals.

TABLE 1. Potentials of jugular vein with respect to rumen contents in conscious sheep. The basis of selection of representative potentials is discussed in the text

	Morning			Afternoon		
Sheep no.	Range (mV)	Selected (mV)	Recording time (min)	Range (mV)	Selected (mV)	Recording time (min)
1040	—	—	—	33–36·5	35	125
	—	—	—	21–38·5	34	66
	18–34	25	156	—	—	—
	25–35	34	167	19–27	25	176
	32·5–40	38	82	—	—	—
	9–25	22	80	25–30	27	61
	23–32	27	108	—	—	—
	—	—	—	22–28	26	140
1048	—	—	—	29·5–34	30	32
	9–28	16	152	—	—	—
	20·5–31	26	69	—	—	—
449	—	—	—	38–43	41	60
	—	—	—	34–38	35	26
	35·5–39·5	38	157	—	—	—
0	26–35	30	40	31·5–35	32	20
351	29–34	31	16	—	—	—
1050	—	—	—	30–34	32	60
32	29·5–31	30	24	—	—	—
	—	—	—	47·5–49·5	49	20
30	35–38	36	17	—	—	—
Mabel*	—	—	—	28·5–33	31	60

* Mabel was a red poll non-lactating heifer.

Thus the concentration of chloride ion in the reticulo-rumen sac at equilibrium should be about one-third of the plasma concentration if the ion moves by free diffusion through the epithelium. As this is near the level found in acute experiments by Parthasarathy & Phillipson (1953) it seemed promising to measure simultaneously the flux and electrochemical gradient of chloride during acute experiments.

Chloride flux measurements

Acute experiments were performed on three sheep, each with five experimental solutions placed in the isolated reticulo-rumen sac for about half an hour. The composition of the solutions is given in Table 2. Concentrations of

chloride were chosen to give fluxes on either side of the expected equilibrium point. With the second and third preparations the potassium concentration was varied over a fivefold concentration range about the expected equilibrium concentration in the rumen—approximately 12 mM. The bicarbonate concentration was chosen to give a pH of 7·2 at 39° C. Masson & Phillipson (1951) showed that the pH of solutions of the salts of fatty acids placed in the reticulo-rumen tends towards pH 7·4, so that the pH of the solutions used in this study is unlikely to alter by much. The tonicity of the solutions used was chosen to give minimal water fluxes. In the first experiment the tonicity of 390 m-osmolar produced movements of water into the sac (Table 3), and so it was adjusted to 347 and 360 m-osmolar in the second and third preparations

TABLE 2. Composition of experimental solutions, made up in tap water and equilibrated with 95%O_2–5% CO_2 at 39° C

		Concentration of ions (mM)				
Expt.	Soln.	Na^+	K^+	Cl^-	Ac^-	HCO_3^-
34	1	174·7	15	15	166	8·7
	2	174·7	15	70	111	8·7
	3	174·7	15	25	156	8·7
	4	174·7	15	50	131	8·7
	5	174·7	15	40	141	8·7
36	1	158·7	15	35	130	8·7
	2	153·7	20	25	140	8·7
	3	148·7	25	40	125	8·7
	4	168·7	5	50	115	8·7
	5	163·7	10	15	150	8·7
38	1	160	20	20	151·3	8·7
	2	170	10	30	141·3	8·7
	3	170	10	20	158·3	1·7
	4	160	20	30	148·3	1·7
	5	175	5	15	156·3	8·7
Tap water	—	0·8	0·06	0·8	—	—

TABLE 3. Ion concentration changes, and amounts absorbed from solutions placed in the isolated reticulo-rumen sac

		Chloride concn. (mM)			Acetate concn. (mM)		Uptake from sac		
Expt.	Period	Initial	Final	Plasma*	Initial	Final	Water (ml.)	Chloride (m-mole)	Acetate (m-mole)
34	1	16·2	17·02	106	167	152	−30	−2·08	26
	2	69·4	65·67	105	110	101	−90	+1·59	8
	3	26·3	26·23	104	152	144	−65	−1·60	6
	4	50·2	48·70	103	130	120	−36	+1·17	14
	5	40·6	39·74	102	141	132	−45	−0·10	11
36	1	35·4	33·98	96	126	116	+ 5	+2·96	22
	2	25·0	24·72	94	137	129	− 5	+0·40	15
	3	39·5	37·75	96	123	118	+44	+5·21	15
	4	49·0	48·00	96	112	107	+18	+2·82	10
	5	15·3	15·49	96	144	138	−15	−0·54	8
38	1	20·2	22·78	97	144	125	+16	−3·88	32
	2	30·1	31·31	94	134	118	−10	−2·34	25
	3	20·4	21·69	93	150	133	− 1	−2·24	28
	4	30·0	30·01	93	141	125	−17	−0·56	24
	5	15·4	17·69	92	148	132	+ 4	−3·72	27

*Corrected for Cl^- shift.

respectively. The resulting reduction in water flux minimized the possibility of solvent flow through pores influencing the chloride equilibrium (Ussing & Andersen, 1956).

TABLE 4. Components of the electrochemical potential difference of chloride with respect to plasma and chloride fluxes with respect to rumen contents

Expt.	Period	E_{Cl}* (mV)	zV (mV)	$E_{Cl}^* + zV$ (mV)	Chloride flux (μequiv/min)	95% half confidence interval ($\pm\mu$equiv/min)
34	1	−49·6	44·0	− 5·6	− 63	4
	2	−11·8	28·1	16·3	48	15
	3	−36·3	31·0	− 5·3	− 48	6
	4	−19·7	32·3	12·6	35	11
	5	−25·1	36·0	10·9	− 3	9
36	1	−27·2	36·5	9·3	91	8
	2	−35·9	37·4	1·5	13	6
	3	−24·6	35·8	11·2	163	9
	4	−18·3	25·4	7·1	86	11
	5	−49·1	33·1	−16·0	− 17	4
38	1	−40·4	34·2	− 6·2	−121	5
	2	−30·2	26·6	− 3·6	− 74	7
	3	−39·9	30·2	− 9·7	− 70	5
	4	−30·4	28·3	− 2·1	− 18	7
	5	−46·1	30·5	−15·6	−118	4

*$E_{Cl} = -62 \log c_{Pl}/c_{sac}$.

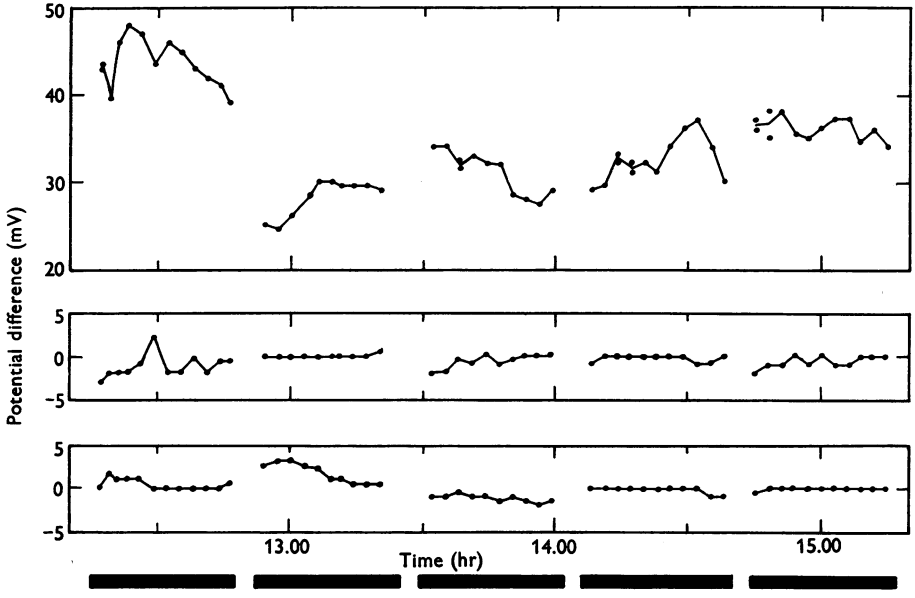

Fig. 4. Potential record of Expt. 34. Top record: potential of rumen artery bridge with respect to rumen bridges 1 and 2. Line joins mean of the two readings where they differ. Middle record: potential of jugular bridge with respect to rumen artery bridge. Bottom record: potential of jugular bridge with respect to saphenous vein bridge. The solid bars represent the length of the absorption periods.

The changes in concentration of chloride and acetate, and the changes in amount of water, chloride and acetate, for the three acute experiments are given in Table 3. The contributions of the electrical potential and concentration ratio to the electrochemical potential are given in Table 4. It is not possible to calculate an error to associate with the mean potentials. The potential record of the first acute experiment (Fig. 4) is given to show how the individual potential readings could vary during the course of an absorption period. In this record the rumen blood bridge was inserted into a rumen artery. In the other experiments a rumen vein was used.

DISCUSSION

The acute experiments on the sheep were designed to allow a comparison between the net chloride movement between the rumen contents and blood and the driving forces involved. One component of the driving force, the electrical potential, was within the range encountered in unanaesthetized sheep with normal rumen contents. Thus the range of mean potentials found for the fifteen absorption periods was 22–44 mV.

In one experimental period the flux of chloride failed to reach a value significantly different from zero (Expt. 34, period 5). Of the fourteen remaining periods, six gave movements against the chloride concentration ratio with concentrations in the rumen from 25 to 69 mM. These observations agree with those found by Parthasarathy & Phillipson (1953) using similar animal preparations. They also agree with the changes found in the rumen pouch in a conscious goat, where the chloride concentration dropped from 86 to 25 mM in 22 hr with a large volume decrease (Sperber & Hydén, 1952). Uptake of chloride from the normal rumen contents of an unanaesthetized sheep, with the concentration dropping from 60 to 25 mM, is also reported (Sperber *et al.* 1953). As the electrical potential found in the acute experiments is similar to that found in the conscious animal, this correspondence between results in both types of preparation could mean that we are dealing with conditions in the acute preparation essentially similar to those in the intact animal.

When the electrical potential difference between the reticulo-rumen contents and blood is taken into account the chloride ion is moving down the electrochemical potential difference. As significant chloride fluxes were found in the expected directions with electrochemical potential differences as small as ± 2 mV, any additional gradient to which chloride is subjected must be small compared to the concentration and electrical contributions. It is concluded, therefore, that chloride diffuses freely across the epithelium of the reticulo-rumen sac. This statement requires some qualification, as the results obtained give no information on the possibility that the fluxes of chloride in both directions interact. To explore this possibility would naturally require the measurement of these component fluxes. In frog skin the component

fluxes of chloride do not interact (Koefoed-Johnson, Levi & Ussing, 1952), but component fluxes of phosphate (Mitchell, 1953) and of potassium (Hodgkin & Keynes, 1955) are known to interact at cellular membranes.

It seems unprofitable to define any sort of permeability constant, in the sense of flux per unit driving force. For instance, in Expt. 36 the flux per unit driving force during the first four periods is 10, 9, 15 and 12 μequiv/min/mV, where as in the fifth period it drops to 1 μequiv/min/mV. This means that caution is necessary in comparing results of one experimental period with another for chloride in this kind of acute experiment.

In all experimental periods an uptake of acetate was found, consistent with previously published results (e.g. Masson & Phillipson, 1951).

The small potential differences between the reticulum and rumen contents may be due in part to the difference in composition of the contents of these organs (Gray, Pilgrim & Weller, 1954). The potentials between different sites in the blood are too large to be explained by differences of composition. Their origin remains obscure.

SUMMARY

1. Potential gradients within the reticulo-rumen contents and within the blood are small.

2. A potential makes the blood about 30 mV positive with respect to reticulo-rumen contents both in conscious sheep with normal rumen contents and in anaesthetized sheep with artificial solutions in the rumen.

3. Chloride absorption from the reticulo-rumen sac takes place down its electrochemical gradient.

4. It is concluded that the movement of chloride against its concentration gradient from the rumen contents into the plasma occurs because of the potential difference between these two phases.

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