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Na^+ TRANSPORT AND IMPEDANCE PROPERTIES OF THE ISOLATED FROG GASTRIC MUCOSA AT DIFFERENT O_2 TENSIONS

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SUMMARY

Na^+ was actively transported from the mucosal (secretory) to the serosal (nutritient) side of the isolated frog gastric mucosa at an O_2 tension of 300 mm Hg in the bathing solutions. At a high (700 mm Hg) and two lower (150 and 40 mm Hg) O_2 tensions no active transport of Na^+ was observed.

Although no elution of L-lactate into the bathing solutions was detected, the appearance of two impedance loci at O_2 tensions of 300 mm Hg or lower was interpreted as evidence that the frog gastric mucosa was in a somewhat hypoxic condition when actively transporting Na^+. This experimental finding may be of importance with regard to the evaluation of active transport of Na^+ in the isolated mammalian gastric mucosa, which was earlier found to have a considerable production of L-lactate, indicating tissue hypoxia.

Active transport of Na^+ in the hypoxic gastric mucosa is suggested to be due to an asymmetric distribution of the Na^+ pump, common to most cell membranes, in the acid-secreting cells.

INTRODUCTION

It has been established that there are two systems for active transport of ions in the isolated amphibian gastric mucosa, one for Cl^- (refs. 1,2) and one for H^+ (ref. 3). Transport of positive ions other than H^+ has been thought to depend, essentially, upon passive diffusion.

In mammals the situation has been reported to be different. Several authors[4–7] have demonstrated that active transport of Na^+ from the mucosal (secretory) to the serosal side takes place in the stomach wall and mucosa isolated from different mammalian species. The Na^+ transport contributes to the electrogenic properties of the mucosa in the same direction as the net transport of Cl^- (refs. 5, 6).

This difference in transport has been suggested to be a species difference[5,8]. It should be noted, however, that it is difficult to obtain satisfactory oxygenation in experiments with isolated mammalian tissues. DAVENPORT AND JENSEN CHAVRE[9] found that they had to raise the O_2 tension to a value as high as 3200 mm Hg in order to minimize lactate production and obtain maximal H^+ secretion in the isolated mouse stomach. Thus it would not seem improbable that in previous experiments

on Na^+ transport mucosae and stomach walls isolated from mammals have been in a somewhat hypoxic state.

As a consequence of this it was considered of interest to study the Na^+ transport in the isolated frog gastric mucosa at different low oxygen tensions. A preliminary report of some of the results has been reported elsewere[10].

METHODS

Frogs of the species *Rana temporaria* were kept in a tank containing tap water at a temperature of 6–10°. Each animal was given approximatively 125 mg of liver once or twice a week by manual force-feeding. No food was supplied during two days preceeding an experiment.

The time for isolation and mounting of the mucosa as a membrane between two perspex chambers as a rule did not exceed 6 min. The mucosal area was 1.8 cm^2. There was 20 ml of solution on each side which was changed 3–4 times during a 20-min period before the experiments were started. The serosal side solution had the following composition: 81.6 mM NaCl, 3.2 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.8 mM KH_2PO_4, 17.8 mM $NaHCO_3$, 3.0 mM sodium butyrate and 2.0 mM glucose. On the mucosal side a similar[11] but unbuffered solution was used. Histamine dihydrochloride was added on the serosal side to a concentration of 0.6 μM at the beginning of an experiment. At the same time continuous infusion of histamine at a rate which gave the same amount of histamine in one h was started. Both compartments were gassed with 5 % CO_2 *plus* different concentrations of O_2 from 95 % down to 5 %, the rest being N_2. All experiments were performed at 20 ± 0.1°.

The rate of H^+ secretion was measured continuously[12], the pH on the mucosal side being kept constant at 4.70 by infusion of a recorded volume of a 15 mM NaOH solution. The equilibrium open circuit electric potential difference was measured every 10 min via two matched calomel electrodes. Short-circuit current was applied to the mucosa from an external source via two Ag–AgCl electrodes, separated from the circulating solutions by two thick cellophane membranes. No correction was made for the potential drop between the calomel electrodes and the surfaces of the mucosa during short-circuiting. The d.c. resistance was determined from the equilibrium change of the open circuit potential when a fixed current (30 $\mu A/cm^2$) was passed through the mucosa in the same direction as the short-circuit current. In some experiments, a.c. impedance measurements according to a modified method described by FLEMSTRÖM[11] were performed. The phase angle and the impedance in the frequency range 20 Hz–50 kHz were determined by a vector impedance metre (Hewlett-Packard 4800 A) and recorded on an X-Y recorder. Impedance locus diagrams were drawn from the recordings.

In experiments with radioactive tracers, 100 μC of ^{24}Na was added to the solution on the mucosal or the serosal side and 20 μC of ^{22}Na on the other. 0.5 ml of sample was taken from each side every 60 min for analysis in a gammaspectrometer of good stability[13]. ^{24}Na was counted immediately in a high energy channel present. ^{22}Na was counted after two weeks when the ^{24}Na activity had become negligibly small. For the lowest counting rate the error of a single radioactivity determination was ± 4.35 %.

L-lactate was analysed enzymatically[14]. The reagents were obtained from

Boeringer and Soehne, GmbH, Ingelheim, Germany. The method permitted determination of L-lactate down to a concentration of 50 μM. The coefficient of variation was $\pm$ 1.2 % at 1 mM.

RESULTS

Stability conditions in a control material

ALONSO *et al.*[15] showed that short- and medium-chain fatty acids stimulated H^+ secretion by the isolated gastric mucosa of the bullfrog (*Rana catesbeiana*). FLEMSTRÖM[11] demonstrated that acetate, propionate and L-lactate in the presence of histamine increased the H^+ secretion in the frog (*Rana temporaria*) gastric mucosa *in vitro*. Butyrate has been used by HEINZ AND LINDENSTRUTH (personal communication) to obtain good long-term stability of H^+ secretion.

TABLE I

H^+ SECRETION AND SHORT-CIRCUIT CURRENT DURING THREE CONSECUTIVE HOURS AT THE SAME O_2 TENSION OF 700 mm Hg

The mean values during the last 30 min in each hour $\pm$ S.D. are given. The normalized figures were obtained by division of the results of each experimental hour by the mean results for the three consecutive hours in the same experiment. $n = 9$.

		Measured	*Normalized*
H^+ secretion	1st h	3.08 $\pm$ 1.00	1.01 $\pm$ 0.05
(μequiv/h per cm^2)	2nd h	3.12 $\pm$ 0.96	1.02 $\pm$ 0.01
	3rd h	3.01 $\pm$ 0.99	0.98 $\pm$ 0.04
Short-circuit current	1st h	1.98 $\pm$ 0.39	1.09 $\pm$ 0.13
(μequiv/h per cm^2)	2nd h	1.76 $\pm$ 0.41	0.97 $\pm$ 0.15
	3rd h	1.72 $\pm$ 0.33	0.95 $\pm$ 0.08

In the present study the solution of the serosal side contained glucose, butyrate and histamine. Table I shows the mean values for H^+ secretion and short-circuit current during three consecutive hours at the same high O_2 tension (700 mm Hg). The coefficient of variation was large. This was not due, however, to variation within each experiment but to differences between the individual mucosae, as can be seen from the normalized values. It is thus evident that the H^+ secretion rate and electric properties within each experiment were at a satisfactory steady state. The stability conditions were the same for the d.c. resistance and the electric potential difference. The differences between the individual mucosae made it advantageous to use each experiment as its own control, which was possible with the good stability conditions shown.

Na^+ transport at different O_2 tensions

The unidirectional fluxes of Na^+ were studied when the transmucosal electric potential was reduced to zero. The experiments were performed during three consecutive hours. During the first and third hour the O_2 tension was 700 mm Hg. These hours were used as controls. During the second hours the O_2 tension was reduced to different levels. The first samples for flux studies were taken 10 min after the

addition of tracers (^{22}Na and ^{24}Na). During the experiments with tracers the short-circuit current was not disconnected for potential and resistance measurement.

The results are given in Table II. At an O_2 tension of 300 mm Hg the flux from the mucosal to the serosal side was significantly greater ($0.01 > P > 0.02$) than the flux in the opposite direction. At an O_2 tension of 700 mm Hg there was no significant difference between the two unidirectional fluxes. Nor was there any difference when the O_2 tension was reduced to 150 or 40 mm Hg. The net Na^+ flux at 300 mm Hg was, on the average, 20 % of the short-circuit current in the same experiments. The Na^+ fluxes at an O_2 tension of 700 mm Hg were not significantly different if they were measured before or after a period at an O_2 tension lower than 700 mm Hg. These values are therefore combined in the table. In three experiments significant differences between the unidirectional Na^+ fluxes were not found during two consecutive hours at an O_2 tension of 700 mm Hg.

TABLE II

Na^+ FLUXES AT DIFFERENT O_2 TENSIONS (pO_2) IN THE BATHING SOLUTIONS

The flux ratio is calculated as the ratio between the flux from the mucosal (m) to the serosal (s) side and that in the opposite direction in each experiment.

pO_2 (mm Hg)	Na^+ flux (μequiv/h per cm^2 ± S.E.)			flux ratio ± S.E.	n
	$m \rightarrow s$	$s \rightarrow m$	net flux $m \rightarrow s$		
700	0.38 ± 0.03	0.31 ± 0.02	0.06 ± 0.03	1.21 ± 0.09	24
300	0.61 ± 0.08	0.29 ± 0.04	0.32 ± 0.07	2.32 ± 0.24	6
150	0.32 ± 0.05	0.30 ± 0.04	0.02 ± 0.03	1.07 ± 0.10	5
40	0.28	0.23	0.05	1.20	2

The flux ratio equation of TEORELL[16] and USSING[17] is often used for differentiation between active transport and passive diffusion of ions. In the absence of electrochemical potential gradients and a bulk flow of water the ratio will be 1.00 for an ion that moves by passive diffusion alone. The equation was originally derived for ion fluxes in an uncharged membrane. Biological membranes such as the gastric mucosa may have fixed charges. As pointed out by TEORELL (see ref. 18, pp. 320 and 346), the flux rates will change with the charge of the membrane. The flux ratio, however, will be independent of this factor.

During the short-circuiting procedure in this study no correction was made for the electric potential drop between the calomel electrodes and the surfaces of the mucosa. For this reason, when the electrode potential was reduced to zero the transmucosal potential was not completely reduced to zero. This was preferable to an attempted complete reduction of the transmucosal potential with the aid of an estimated value of the varying mucosal d.c. resistance, as a possible short-circuit overshoot would have induced a passive net flux of Na^+ from the mucosal to the serosal side. It can be calculated from the flux ratio equation that the maximal error so induced would have given a ratio 10 % to low.

The flux ratio at an O_2 tension of 300 mm Hg was 2.32, however. This is thus interpreted as an indication of active transport of Na^+ from the mucosal to the serosal side of the isolated frog gastric mucosa at this O_2 tension.

H^+ secretion and electric parameters at differents O_2 tensions

The O_2 tensions were varied in the same way as during the Na^+ flux experiments. The results are given in Table III.

At an O_2 tension of 300 mm Hg the H^+ secretion was smaller than in the preceding control hour ($P < 0.01$). No significant changes occurred in the short-circuit current, the d.c. resistance or the electric potential difference. The H^+ secretion in the second control hour, after exposure to an O_2 tension of 300 mm Hg, was smaller than in the first control hour ($P < 0.01$). There were no significant changes in short-circuit current, d. c. resistance or electric potential difference between the first and second control hours.

TABLE III

H^+ SECRETION, SHORT-CIRCUIT CURRENT, D.C. RESISTANCE AND ELECTRIC POTENTIAL DIFFERENCE IN EXPERIMENTS PERFORMED DURING THREE CONSECUTIVE HOURS

The O_2 tension in the first and the third hour was always 700 mm Hg and that in the second hour was 300, 150 or 40 mm Hg. The mean values during the last 30 min of each hour ± S.E. are given.

pO_2 (mm Hg)	H^+ secretion (μequiv/h per cm^2)	Short-circuit current (μequiv/h per cm^2)	D.c. resistance (ohm·cm^2)	Electric potential difference (mV)	n
700	2.98 ± 0.22	2.18 ± 0.13	365 ± 21	24.2 ± 1.7	
300	2.42 ± 0.22	2.17 ± 0.56	410 ± 56	27.2 ± 1.5	17
700	2.61 ± 0.24	1.97 ± 0.11	404 ± 24	24.4 ± 1.4	
700	2.78 ± 0.41	1.95 ± 0.14	442 ± 45	23.9 ± 1.4	
150	1.19 ± 0.25	1.48 ± 0.17	570 ± 64	22.7 ± 2.2	12
700	2.04 ± 0.40	2.00 ± 0.16	378 ± 20	24.3 ± 2.0	
700	3.46 ± 0.35	2.01 ± 0.20	371 ± 38	22.7 ± 2.7	
40	0.27 ± 0.11	0.42 ± 0.11	724 ± 59	10.1 ± 1.8	8
700	1.90 ± 0.39	1.78 ± 0.15	481 ± 82	24.8 ± 1.8	

At an O_2 tension of 150 mm Hg the H^+ secretion and the short-circuit current were smaller than during the preceding control hour ($P < 0.01$ for both). There was an increase in the d.c. resistance ($P < 0.01$) but no significant change of the electric potential difference. The H^+ secretion was lower ($P < 0.01$) in the second than in the first control hour. The short-circuit current, the d.c. resistance and the electric potential difference did not change significantly.

At an O_2 tension of 40 mm Hg the H^+ secretion rate, the short-circuit current and the electric potential difference decreased and the d.c. resistance increased when compared with the preceding control hour ($P < 0.01$ for all). In the second control hour the H^+ secretion rate was lower ($P < 0.01$) and the d.c.resistance higher ($0.01 < P < 0.02$) than in the first. There was no significant change in the short-circuit current or the electric potential difference.

Provided that the short-circuit current is equal to the net transport of Cl^- *minus* the secretion of H^+ ions ($Cl^- > H^+$) a proportional reduction of both ionic transports would result in a concomitant decrease of the short-circuit current. The decrease of both H^+ secretion and short-circuit current found at O_2 tensions

of 150 and 40 mm Hg may thus indicate that there is a mutual relationship between the active component of Cl^- flux and the H^+ secretion in the frog gastric mucosa when the mucosa is made increasingly anoxic, as suggested by FORTE[19].

At an O_2 tension of 300 mm Hg, however, the short-circuit current remained unchanged although the H^+ secretion decreased. This could indicate the contribution of the active transport of some other ion to the short-circuit current at this O_2 tension and would thus reflect the observed active transport of Na^+ from the mucosal to the serosal side at this O_2 tension.

A transmucosal pH difference has been shown to decrease somewhat the rate of secretion of H^+ ions in experiments with the isolated frog mucosa, probably due to diffusional escape of H^+ along the chemical potential gradient[11]. No influence of a pH difference upon the short-circuit current was, however, found[11]. As the transmucosal differences in the experiments reported here and also by FORTE[19] were held constant in all experiments, they may be considered to be of no importance for the occurence of a reduction in the rate of H^+ secretion in hypoxic conditions.

In this study significant increases in d.c. resistance were found at O_2 tensions of 150 and 40 mm Hg, thus indicating some degree of hypoxia in the mucosa. At an O_2 tension of 300 mm Hg, however, the increase in the d.c. resistance was not significant. In order to obtain more complete information on the condition of the isolated gastric mucosa with different O_2 tensions in the surrounding solutions, the L-lactate production was measured and the impedance properties determined.

Determination of L-lactate production at different O_2 tensions

In the anoxic gastric mucosa (*i.e.* with no O_2 in the bathing solutions) DURBIN[20] found a rate of elution of lactate into the solutions of approximately 80 mμmoles/g per min and 90–100 % of the lactate appeared on the serosal side. In the present experiments the L-lactate concentrations in the mucosal and the serosal solutions were determined after the end of experiments performed during 1 or 4 h at a constant O_2 tension. In the 4-h experiments the volume of the solutions was 15 ml (otherwise 20 ml was always used). An elution rate of L-lactate of 1 μmole/h into the serosal side solution (*i.e.* roughly corresponding to that found by DURBIN[20]) would have resulted in a final concentration of 260 μM after 4 h. No L-lactate was detected, however, in any of the experiments performed during 1 or 4 h at O_2 tensions of 700, 300, 150 or 40 mm Hg.

This does not exclude, however, the possibility of a considerable L-lactate production in the preparation at a low O_2 tension. Due to the difference in lengths of the diffusion pathways of O_2 the inner parts or the gastric mucosa *in vitro* are probably less well oxygenated than the peripheral parts. L-lactate stimulates H^+ secretion in the frog gastric mucosa[11] and it is probable that L-lactate produced by the mucosa can be metabolized locally. In the thicker mammalian stomach wall it might be expected that the hypoxic regions would be relatively larger, and thus L-lactate production more easily detected.

Determination of the impedance properties at different O_2 tensions

The reason for undertaking these determinations was that the impedance at a series of different frequencies (*i.e.* an impedance run) gives more complete information on the electric properties of the mucosa than the mere d.c. resistance

determination. Furthermore, it cannot be excluded that the d.c. resistance may depend upon the current density used, as shown earlier for charged membranes by TEORELL (see ref. 18, p. 339) although such a phenomenon was not evident from the present results.

The O_2 tension was varied in the same manner as during the tracer experiments. Fig. 1 shows a typical experiment, where the O_2 tensions during three consecutive hours were 700, 40 and 700 mm Hg, respectively. As previously shown[21], the impedance run at an O_2 tension of 700 mm Hg forms part of a semicircle and the mucosa can be approximately represented by the equivalent circuit shown in Fig. 3A.

At lower O_2 tensions, however, the run formed parts of two semi-circles, indicating the appearance of two impedance loci, incompletely separated from each

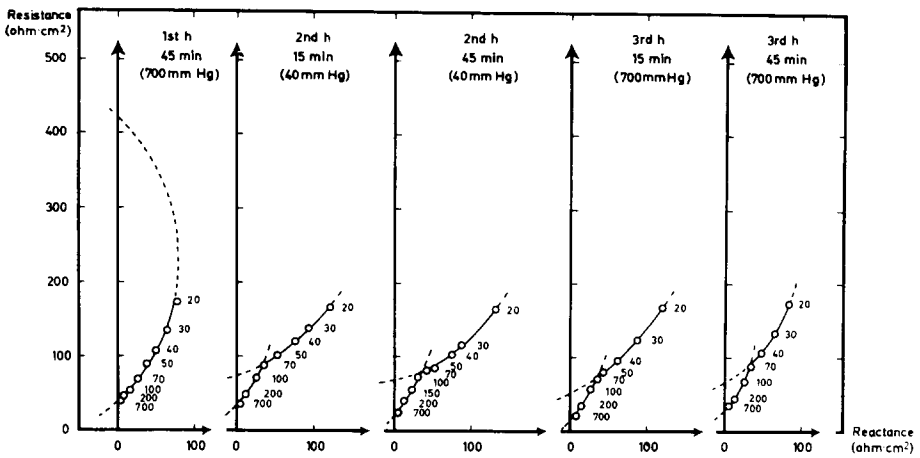

Fig. 1. Impedance runs obtained in an experiment performed during three consecutive hours with O_2 tensions of 700, 40 and 700 mm Hg, respectively. The time when each run was made is given in the figure.

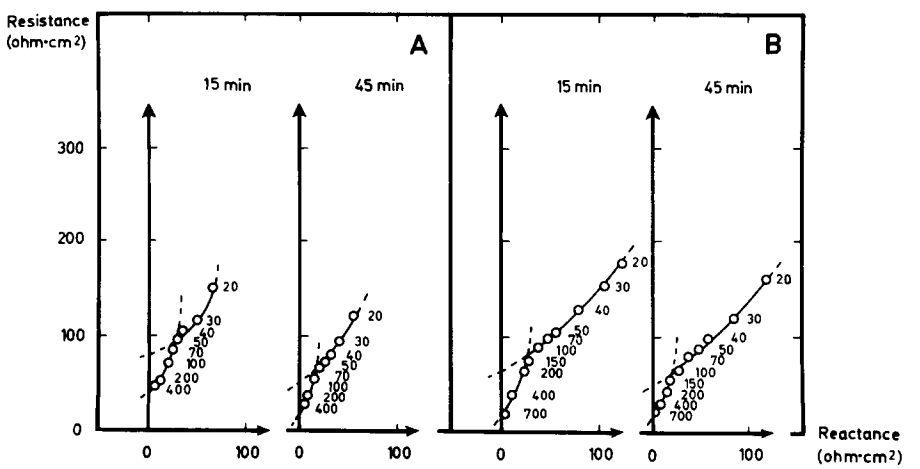

Fig. 2. Impedance runs obtained 15 and 45 min after change of the O_2 tension from 700 to 300 mm Hg in two separate experiments (A and B). In the preceding hours at an O_2 tension of 700 mm Hg the runs had the form of a single semicircle.

other. The separation point was at a frequency in the range 50–160 Hz. The radius of the semicircles obtained at low frequencies was greater than that obtained at higher frequencies. The impedance run, which was part of two semicircles at an O_2 tension of 40 mm Hg showed a tendency to reverse to the single semicircular form when an O_2 tension of 700 mm Hg was reestablished. The occurrence of simular complex impedance loci is known from other biological materials[22] and from collodion membranes[23]. During 2 out of 18 experimental hours at an O_2 tension of 700 mm Hg not preceded by a low O_2 tension the impedance locus showed deviations from the single semicircular form and became complex. This was also found during 5 out of 6 experiments at an O_2 tension of 300 mm Hg and in all experiments at O_2 tensions of 150 or 40 mm Hg. In Fig. 2 impedance diagrams from two experiments at an O_2 tension of 300 mm Hg are shown. The changes in the form of the impedance run typical for the lowest O_2 tensions already at an O_2 tension of 300 mm Hg seem to indicate that the frog gastric mucosa was in a somewhat hypoxic state when it actively transported Na^+.

The "polarization capacity" of a tissue is to a great extent due to the capacity of double layer membranes but "relaxation processes" due to other properties of the tissue are also of importance[22,24]. No definite conclusions can be drawn about the cellular location of resistive and capacitive elements in such a complex membrane as the gastric mucosa. An increase in the d.c. resistance of a tissue under anoxic conditions is often interpreted as a decrease of the extracellular space due to cellular swelling[25].

An equivalent electric circuit for the case of a high O_2 tension[21] is shown in Fig. 3A. Several equivalent electric circuits with capacitive, resistive and inductive components could represent approximately the frog gastric mucosa at low O_2 tension. A possible circuit, that has the advantage of being simple, is shown in Fig. 3B. For comparison the impedance diagrams of the two electric circuits in Figs. 3A and B are given in Figs. 4A and B. The values of the electric components used are given in the figures. As can be seen, two impedance loci, incompletely separated, will result if a small resistance is connected in series with part of the capacity. The change of the impedance run when an O_2 tension of 300 mm Hg or less was

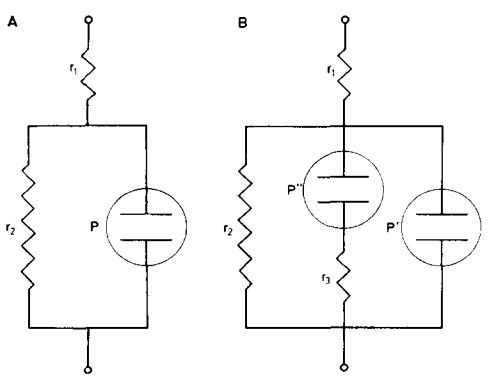

Fig. 3. Electric equivalent circuits as suggested for the frog gastric mucosa at a high tension (A) and at lower tensions (B).

used might be interpreted as the occurrence of a resistive element in series with a capacitive element and thus undetectable by d.c. resistance determination. One possible location of such a resistive element might be within the cell or in its membranes. The equivalent circuit in Fig. 3B might explain why a change in the impedance diagram but no significant increase in d.c. resistance at an O_2 tension of 300 mm Hg took place.

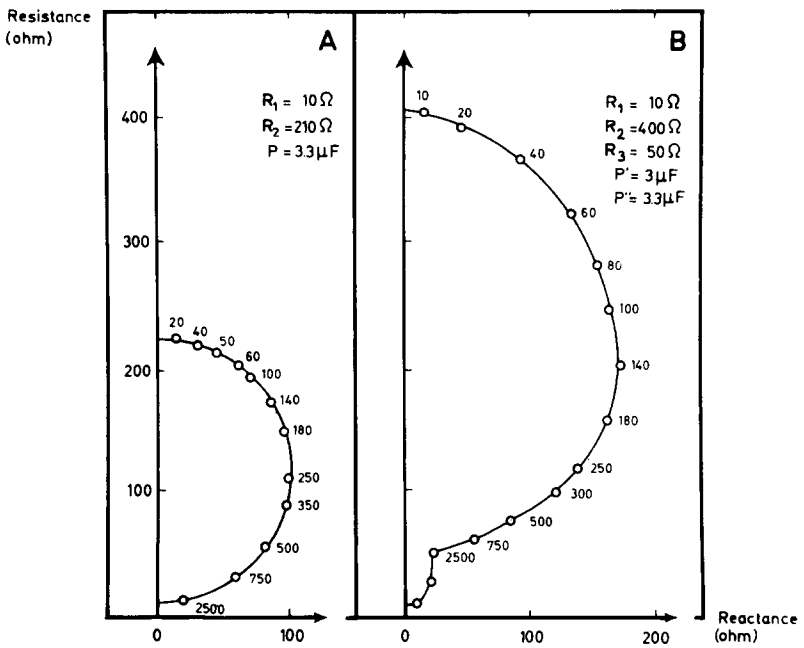

Fig. 4. Impedance diagrams for the electric equivalent circuits in Figs. 3A (A) and 3B (B). The values for the electric components are given in the figures. R_1 is the mean of the high frequency resistances obtained in 6 experiments at O_2 tensions of 700 (A) and 40 (B) mm Hg. R_2 is the mean of the d.c. resistances in the same experiments *minus* R_1. R_3, P, P' and P'' are not calculated from experiments.

Boundary phenomena at the interfaces between membranes and surrounding solutions will contribute to the total membrane capacity in the low frequency region necessary for such determinations in the frog gastric mucosa[26]. For this reason, the capacity was not determined and the values of the electric components chosen in Figs. 4A and B need not represent the physiological ones. The principal appearance of the impedance runs will, however, be the same if other values for the same components are chosen.

DISCUSSION

Na^+ was found to be actively transported from the mucosal to the serosal side of 300 mm Hg in the bathing solutions. The short-circuit current remained unchanged although the H^+ secretion decreased when the O_2 tension was reduced from 700 to 300 mm Hg which might be interpreted as a contribution of the active transport of Na^+ to the short-circuit current.

In agreement with HOGBEN[2], no active transport of Na^+ was found, at an O_2 tension of 700 mm Hg. Neither was such transport observed at the lowest O_2 tensions of 150 and 40 mm Hg.

Although no production of L-lactate was detected, impedance determinations provided evidence that the frog gastric mucosa was in a somewhat hypoxic state when actively transporting Na^+. These experimental findings might be of importance in evaluations of experiments on the isolated mammalian gastric mucosa and stomach, which have been found to produce large quantities of L-lactate indicating tissue hypoxia. Accordingly, in the mammalian gastric mucosa under conditions *in vivo* no appreciable active transport of Na^+ has been shown[27,28].

It is accepted fairly generally that a $(Na^+ + K^+)$-dependent membrane ATPase with a pH optimum of about 7.4 participates in the outward transport of Na^+ and the inward transport of K^+ common to most cell membranes[29]. $(Na^+ + K^+)$-dependent ATPase activity has been demonstrated in the rat[5] and the cat[30] gastric mucosa. In the frog gastric mucosa such ATPase activity has not been found[31,32]. MOZSIK AND OYE[33], however, prepared $(Na^+ + K^+)$-dependent ATPase in the human gastric mucosa by treatment with sodium iodide. In untreated preparations the activity of the enzyme was probably masked by other ATP-hydrolyzing systems. This might also be the case for the frog gastric mucosa, in which an increase of the mucosal content of Na^+ was observed when ouabain was administered on both sides[34].

KASBEKAR AND DURBIN[31] demonstrated an ATPase with a pH optimum of 8.3 and other activity characteristics in the frog gastric mucosa. This enzyme was stimulated by HCO^-_3 and halide ions and was ouabain-insensitive, but was inhibited by SCN^-, which is a much studied inhibitor of HCl secretion. It is not improbable that this ATPase is associated with the Cl^- transport mechanism[32].

No detailed knowledge about the pH in the cells lining the gastric lumen is available. Since one bicarbonate ion is delivered to the serosal side of the mucosa for each H^+ ion appearing on the mucosal side, it can probably be assumed that there is an intracellular liberation of base during H^+ secretion (*cf.* TEORELL[35]). The pH in the acid-secreting cells, long known to be acidophilic cells, may thus be high.

Assuming that the mucosal side of the acid-secreting cells is a specific HCl-secretory membrane, as has been suggested earlier[36] and for this reason has no Na^+ transport mechanism, the following hypothesis for active Na^+ transport from the mucosal to the serosal side is proposed. Under conditions of full oxygenation the Na^+ pump is less effective due to an intracellular pH which is high and well above the pH optimum of the $(Na^+ + K^+)$-sensitive ATPase. When the O_2 tension is moderately reduced there would be a small reduction of intracellular pH thus favouring the ATPase and stimulating the Na^+ pump mechanism (but inhibiting the active transport of Cl^-). On account of an asymmetric distribution of the Na^+ pump (*i.e.* presumed not to be present on the mucosal side of the cells) this would result in active transport of Na^+ ions from the mucosal to the serosal side. When the O_2 tension is further reduced, both Na^+ and Cl^- transport are inhibited together with all other aerobic cell activities.

This hypothesis would be in good agreement with the results obtained in this study and the finding[6] that ouabain depressed the electric potential difference more effectively when added on the serosal than on the mucosal side of the isolated cat gastric mucosa.

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REFERENCES

1 C. A. M. HOGBEN, *Proc. Natl. Acad. Sci. U.S.*, 37 (1951) 393.
2 C. A. M. HOGBEN, *Am. J. Physiol.*, 180 (1955) 641.
3 E. HEINZ AND R. P. DURBIN, *Biochim. Biophys. Acta*, 31 (1959) 246.
4 G. H. WRIGHT, *J. Physiol.*, 163 (1962) 281.
5 J. T. CUMMINGS AND B. E. VAUGHAN. *Biochim. Biophys. Acta*, 94 (1965) 280.
6 S. KITAHARA, *Am. J. Physiol.*, 213 (1967) 819.
7 S. KITAHARA, K. R. FOX AND C. A. M. HOGBEN, *Am. J. Digest. Diseases*, 14 (1969) 221.
8 C. A. M. HOGBEN, *J. Gen. Physiol.*, 51 (1968) 240s.
9 H. W. DAVENPORT AND V. JENSEN CHAVRE, *Gastroenterology*, 15 (1950) 467.
10 G. FLEMSTRÖM AND K. J. ÖBRINK, *Biochim. Biophys. Acta*, 203 (1970) 184.
11 G. FLEMSTRÖM, *Acta Physiol. Scand.*, in the press.
12 R. P. DURBIN AND E. HEINZ, *J. Gen. Physiol.*, 41 (1958) 1035.
13 B. DANIELSON, J. DELAHAYES AND U. SJÖSTRAND, *Acta Soc. Med. Upsalien.*, 71 (1966) 173.
14 R. SCHOLZ, H. SCHMITZ, T. BÜCHER AND J. O. LAMPEN, *Biochem. Z.*, 331 (1959) 71.
15 D. ALONSO, K. NIGON, I. DORR AND J. B. HARRIS, *Am. J. Physiol.*, 212 (1967) 992.
16 T. TEORELL, *Arch. Sci. Physiol.*, 3 (1949) 205.
17 H. H. USSING, *Acta Physiol. Scand.*, 19 (1949) 43.
18 T. TEORELL, *Progr. Biophys. Biophys. Chem.*, 3 (1953) 305.
19 J. G. FORTE, *Am. J. Physiol.*, 216 (1969) 167.
20 R. P. DURBIN, *J. Gen. Physiol.*, 51 (1968) 233s.
21 T. TEORELL AND R.WERSÄLL, *Acta Physiol. Scand.*, 10 (1945) 243.
22 K. S. COLE, *Membranes, Ions and Impulses*, University of California Press, Berkeley and Los Angeles, 1968.
23 E. HEINZ, *Biochim. Biophys. Acta*, 15 (1954) 489.
24 H. P. SCHWAN, in W. L. NASTUK, *Physical Techniques in Biological Research*, Vol. 4, Academic Press, New York and London, 1963, p. 323.
25 H. H. USSING, *Harvey Lectures*, 59 (1965) 1.
26 H. P. SCHWAN AND K. S. COLE, in O. GLASSER, *Medical Physics*, Vol. 3, Year Book Publ., Chicago, 1960, p. 52.
27 C. F. CODE, R. C. LA FORCE AND J. F. SCHLEGEL, *J. Physiol.*, 205 (1969) 34 P.
28 A. M. BORNSTEIN, H. DENNIS AND W. S. REHM, *Am. J. Physiol.* 197 (1959) 332.
29 J. C. SKOU, *Physiol. Rev.*, 45 (1965) 596.
30 S. L. BONTING, K. A. SIMON AND N. M. HAWKINS, *Arch. Biochem. Biophys.*, 95 (1961) 416.
31 D. KASBEKAR AND R. P. DURBIN, *Biochim. Biophys. Acta*, 105 (1965) 472.
32 G. SACHS, W. E. MITCH AND B. I. HIRSCHOWTIZ, *Proc. Soc. Exptl. Biol. Med.*, 119 (1965) 1023.
33 G. MOZSIK AND I. ØYE, *Biochim. Biophys. Acta*, 183 (1969) 640.
34 H. W. DAVENPORT, *Proc. Soc. Exptl. Biol. Med.*, 110 (1962) 613.
35 T. TEORELL, *J. Physiol.*, 114 (1951) 267.
36 L. VILLEGAS, *Biochim. Biophys. Acta*, 88 (1964) 227.