J. Physiol. (1967), **190**, *pp.* 531–540
With 5 text-figures
Printed in Great Britain

ACTIVE TRANSPORT OF IONS BY THE GASTRIC MUCOSA OF THE RABBIT FOETUS

BY ANNE KENDALL (NÉE MASON) AND G. H. WRIGHT
From the Department of Physiology, Bedford College, London, N.W. **1**

(*Received* 6 *December* 1966)

SUMMARY

1. The short-circuit current and absolute fluxes of Na^+ and Cl^- across the gastric mucosa of the 28-day rabbit foetus have been measured *in vitro*.

2. Substitution of Na^+ in the solution bathing the mucosal surface by choline ion or K^+ resulted in a 70 % decrease in short-circuit current which was reversed when Na^+ was restored to the mucosal solution. The portion of the short-circuit current dependent on the presence of Na^+ in the mucosal solution was found to be equivalent to the net flux of Na^+ from mucosa to serosa.

3. The net flux of Cl^- from serosa to mucosa was compared with the short-circuit current persisting when Na^+ had been replaced in the mucosal solution. Averaged results from sixteen experiments indicated that the net flux of Cl^- was equivalent to 166 % of the Na^+ independent short-circuit current.

4. The results indicated that the component of short-circuit current associated with acid secretion was independent of the presence of Na^+ in the mucosal solution.

5. The small scale of the experiments and the secretion of mucus by the preparation did not permit successful simultaneous measurement of H^+ secretion and short-circuit current.

6. Replacement of Cl^- by SO_4^{2-} or glucuronate in the solutions on both sides did not result in a reversal or decrease in magnitude of the Na^+ independent short-circuit current, even after allowing time for the tissue to become depleted of Cl^-. It is suggested that a non-specific active anion transport was occurring.

INTRODUCTION

Previous *in vitro* work on whole foetal stomach of the rabbit (Wright, 1963) has shown that between 19th and 30th day of gestation Na^+ is transported from the solution bathing the mucosal side across the gastric

mucosa into the serosal solution. Furthermore, this transport was shown to occur against the gradient of electrochemical potential for this ion. On the 23rd day of gestation, concurrent with the appearance of oxyntic cells, H^+ and Cl^- were found to be transported into the mucosal solution against their gradients of electrochemical potential. These transport phenomena were found to be associated with a difference of electrical potential across the mucosa, the serosal side being positive with respect to the mucosal side, even when identical solutions were bathing each side of the stomach.

Ussing & Zerahn (1951) showed that a membrane across which active ion transport was occurring, resulting in the generation of a p.d., even with identical solutions on the two sides, could be short-circuited through a special external circuit and the current so obtained compared with the rate of active ion transport. Explicitly, the short-circuit current is equivalent to the rate of net ionic charge transfer across the membrane. Hogben (1955) applied this technique to the gastric mucosa of the frog and found that the short-circuit current (s.c.c.) was equivalent to the rate of net transport of charge carried by H^+ and Cl^-.

In view of the previous work it would appear that a deeper understanding of the transport phenomena occurring in the foetal stomach could be gained through application of the Ussing–Zerahn technique to this system.

METHODS

Stomachs of rabbit foetuses in the age range 28–30 days gestation age were obtained as described in a previous paper (Wright, 1963).

Some stomachs were used on the same day as the operation whilst others were stored at 2° C in Krebs bicarbonate Ringer solution for up to 5 days. This solution is carefully gassed with a 95 % O_2, 5 % CO_2 mixture to attain a pH of 7·4. The period of storage appeared to have no lasting effect on the electrophysiological properties of the preparation (open-circuit p.d., short-circuit current and resistance) apart from delaying the time of attainment of a maximum short-circuit current and p.d. after being brought to 36° C. After 24 hr storage this delay was about 20 min whilst after 5 days storage the delay was about 6 hr or even longer.

Stomachs were incised through the cephalic surface, opened out to a flat membrane and the *in vivo* contents washed away with Krebs bicarbonate Ringer. The washed membranes were then sandwiched between two half chambers constructed from Perspex and based on the design used by Ussing & Zerahn (1951). The useful area of membrane in these chambers was 0·6 cm^2 and the volume of each compartment was 5 ml. The solutions were stirred and oxygenated by the oxygen lift incorporated in this type of apparatus.

Measurement of net transport of NaCl. This was determined as the difference of the two absolute fluxes across the membrane. The absolute flux of Na from mucosal solution to serosal solution was measured first in each experiment by adding about 20 μc of ^{24}Na as isotonic $^{24}NaCl$ solution to the exactly known volume of mucosal solution. Duplicate samples of the musocal solution were taken and diluted 1:10. At the same time duplicate samples of serosal solution were taken and used for background and as a blank. After about 90–120 min a second pair of samples of serosal solution were taken for counting, and also a second pair of samples of mucosal solution which were diluted 1:10. The volume of serosal solution was

accurately dispensed. The concentration of Na in the mucosal solution was determined by flame photometry.

The samples of radioactive solution were dried and counted at infinite thinness on recessed planchettes for 1000 sec. Total counts obtained were greater than 4000 in the weakest samples. Count rates were at least 5 times background. The amount of Na transferred from mucosal solution to serosal solution was calculated from the equation:

$$\text{amount} = [\text{Na}]_m \cdot \frac{C_s}{C_m} \cdot V_s,$$

where $[\text{Na}]_m$ is the Na concentration in the mucosal solution, C_s is the count rate of the sample of serosal solution (corrected for background decay), C_m is the mean count rate of the first and second samples of mucosal solution after correction for dilution, background and decay, and V_s is the volume of serosal solution.

The flux of Na^+ from serosal solution to mucosal solution was then measured using $^{22}Na^+$ added to the serosal solution and then using analogous procedures to those used for determining the flux from mucosa to serosa. However, all counts for this part of the experiment were obtained 3 weeks later when the activity due to $^{24}Na^+$ had decayed to insignificant levels.

The net flux of Cl^- across the stomach wall was also measured as the difference of two absolute fluxes. Ideally this could have been carried out using $^{38}Cl^-$ and $^{36}Cl^-$ simultaneously or consecutively (as for Na). However, the use of $^{38}Cl^-$ with its short half life (38 min) was not practicable as no close source of this isotope was available. Instead the experiments were carried out on paired preparations from the same litter which had been stored over the same period, one preparation being used for measurement of $^{36}Cl^-$ transfer from mucosa to serosa and the other preparation being used for measurement of $^{36}Cl^-$ transfer from serosa to mucosa. The methods of sampling and counting were similar to those used for radioactive Na^+. $^{36}Cl^-$ was added as 0·10 M-KCl. These pairs of stored preparations always had similar open circuit p.d.s and short-circuit currents, agreeing to within 4%.

Solutions. The bicarbonate Ringer solution had the following composition (mM): Na^+ 135·7, Cl^- 132·2, K^+ 14·9, Ca^{2+} 3·5, HCO_3^- 25·3, glucose 24·0. Na^+-free solutions were 154 mM choline chloride or 154 mM-KCl, each with 24 mM glucose. There was no difference in the effects of these two solutions. Cl^- free Ringer was made up as above with Ca^{2+} present as the nitrate and all other Cl^- replaced by glucuronate or sulphate. Solutions which were Na^+ and Cl^- free were similar to the Cl^- free solution above, with K^+ replacing all of the Na^+.

Electrical measurements. The integrated s.c.c. was obtained as described previously (Wright, 1965). The Na^+ independent component of the s.c.c. was measured before and immediately after measurement with Na^+ present on the mucosal side, the two values usually being identical. When the preparations were dead, as judged by the absence of a p.d., no diffusion potential greater than 1·0 mV was observed when a Na^+ free solution was placed on the mucosal side.

RESULTS

The manner in which the s.c.c. was dependent on the presence of Na^+ in the mucosal solution is shown in Fig. 1. It was found that about 70% of the s.c.c. was reversibly dependent on the presence of Na^+ in the mucosal solution, the remaining 30% appearing to be independent of Na^+. The result shown was obtained with solutions containing Cl^-, but Cl^--free solutions gave the same result. The overshoot seen when Na^+ was returned to the mucosal solution was always observed and is at present unexplained.

The result of sixteen experiments in which the net flux of Na was

measured using isotopes, along with the Na^+ dependent s.c.c., are given in Table 1.

The same sixteen experiments gave the result expressed in Fig. 2. It should be noted that there is no significant intercept of the regression line on either of the axes and that the slope of the line is 45°, indicating that

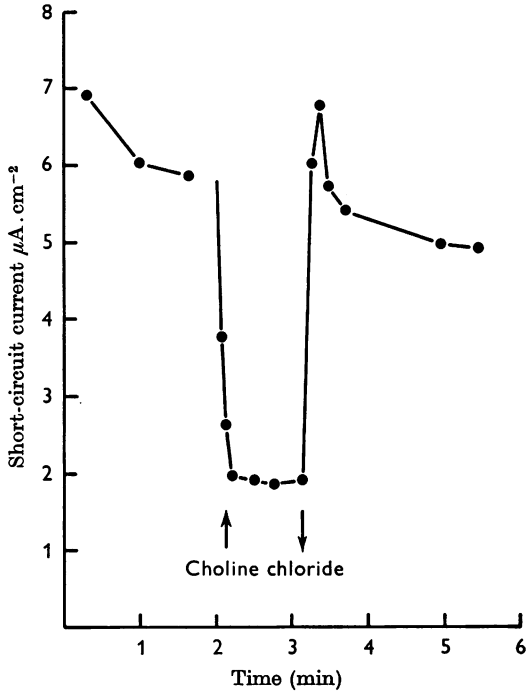

Fig. 1. Short-circuit current of a 28-day stomach showing the dependence of the s.c.c. on the presence of Na^+ in the mucosal solution. During the interval between the lines choline ion replaced Na^+ in the mucosal solution.

TABLE 1. Na^+ fluxes and short-circuit current. Influx is from serosa to mucosa, efflux is from mucosa to serosa. The units of flux are μ-equiv.cm.$^{-2}$hr^{-1}. The short-circuit current is expressed in the same units as the fluxes

Experiment	Influx	Efflux	Net flux	s.c.c.
10. ii. 65	0·46	4·48	4·02	4·47
12. ii. 65	1·17	5·15	3·98	3·98
16. ii. 65	1·93	3·68	1·75	1·36
17. ii. 65	0·44	2·58	2·13	1·49
18. ii. 65	0·54	1·54	1·00	0·91
19. ii. 65	0·47	3·01	2·54	2·48
23. ii. 65	0·57	2·17	1·60	1·50
25. ii. 65	0·40	3·44	3·04	2·28
26. ii. 65	1·73	5·56	3·88	3·87
2. iii. 65	1·08	4·47	3·39	3·07
3. iii. 65	0·92	5·63	4·70	3·10
16. iii. 65	0·46	1·20	0·74	0·99
19. iii. 65	0·10	1·78	1·68	2·00

the next flux of Na^+ from mucosa to serosa was equivalent to the Na current (I_{Na}). The equation of the regression line is $y = 0{\cdot}98x - 0{\cdot}01$, $P < 0{\cdot}001$.

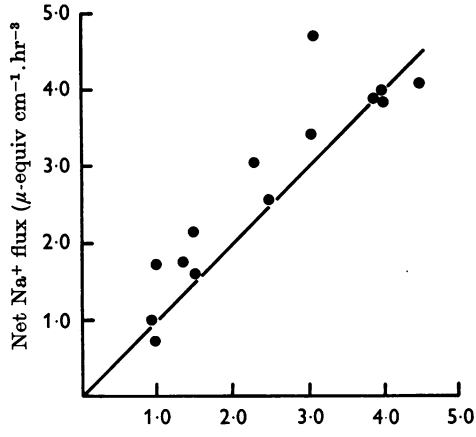

Fig. 2. The relation between net flux of Na^+ from mucosa to serosa and Na^+ dependent short-circuit current for sixteen foetal stomachs. The equation of the regression line is $y = 0{\cdot}98x - 0{\cdot}01$, $P < 0{\cdot}001$.

TABLE 2. Cl^- fluxes and Na^+ independent short-circuit current. Fluxes and units are defined as in Table 1. The net flux is defined as positive when in the direction serosa to mucosa

Experiment	Influx	Efflux	Net flux	s.c.c.
15. iii. 66	4·37	6·56	2·19	1·37
4. iv. 66	3·81	5·86	2·05	1·27
4. iv. 66	7·31	4·00	−3·31	0·64
5. iv. 66	5·62	7·58	1·95	1·15
6. iv. 66	2·08	4·91	2·83	0·69
25. iv. 66	5·39	8·20	2·81	0·78
26. iv. 66	5·94	6·95	1·01	2·14
27. iv. 66	6·44	7·72	1·28	2·58
28. iv. 66	5·95	7·81	1·86	1·89
2. v. 66	9·14	6·12	−3·02	1·46
3. v. 66	5·30	9·06	3·76	1·64
3. v. 66	4·53	7·00	2·47	1·32
9. v. 66	4·41	6·14	1·73	0·79
10. v. 66	3·97	8·28	5·31	1·25
10. v. 66	4·56	6·06	1·50	1·19
16. v. 66	4·27	6·57	2·30	1·72
16. v. 66	3·37	5·31	1·94	1·24
17. v. 66	4·78	6·33	1·55	1·01

Determinations of the s.c.c., in the absence of Na^+ on the mucosal side, and the radioactive Cl^- absolute fluxes produced the results shown in Table 2.

The use of two separate preparations for each experiment gave rise to a greater variation of results. The over-all result obtained from this second

series of sixteen experiments, shown in Fig. 3, indicated that the mean net flux of Cl^- from serosa to mucosa was 166% of the Na^+ independent s.c.c. The statistical significance of this result was investigated using the t test for related means, which gave $t_{n-1} = 2{\cdot}24$, $0{\cdot}01 < P < 0{\cdot}025$: the difference of the means is considered to be significant.

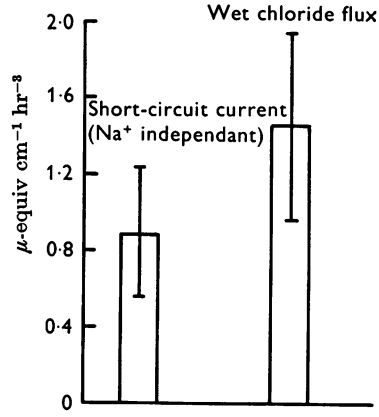

Fig. 3. The left-hand column shows the mean Na^+ independent short-circuit current and S.D. for sixteen experiments. The right-hand column shows the mean net flux of Cl^- from serosa to mucosa and σ for the same sixteen experiments. The t test for related means gives $t_{n-1} = 2{\cdot}24$, $0{\cdot}01 < P < 0{\cdot}025$ which shows that the difference of the means is significant.

Since the net flux of Cl^- from serosa to mucosa was found to exceed the Na^+ independent component of the s.c.c., it was necessary to determine the extent to which active transport of H^+ into the lumen contributed to this component of the s.c.c. Attempts to measure directly the rate of appearance of H^+ on the mucosal side, simultaneously with s.c.c., using a glass electrode and 0·01 N-KOH, were unsuccessful owing to the high degree of frothing occurring when the volume of this solution was reduced to a level at which measurable pH changes would be detected. Oxygenation of the serosal solution alone proved to be inadequate.

An attempt was made to determine indirectly the contribution of H^+ secretion to the s.c.c. by replacing Cl^- by an anion species that would not be expected to be subject to active transport. Under this condition, with Na^+ free solution on the mucosal side, the gastric p.d. and s.c.c. should be reversed in direction if H^+ transport was the only active process occurring: results of this nature have been obtained from frog gastric mucosa when Cl^- was replaced by SO_4^{2-} (Heinz & Durbin, 1959; Rehm, Davis, Chandler, Gohmann & Bashirelahi, 1963). Neither of the anions used in the present series of experiments (SO_4^{2-} or glucuronate) produced a reversal of p.d.

and s.c.c. when used to replace Cl^-: in fact there was always a large increase in p.d. and s.c.c. as shown in Fig. 4 (compare Durbin, 1964). It is noteworthy that even after 4 hr, there was no fall in the s.c.c. observed in these experiments.

TABLE 3. Effect on Na^+ independent s.c.c. of replacing Cl^- by SO_4^{2-} or glucuronate

Experiment	Anion	p.d. mV		s.c.c. μA	
		Before	After	Before	After
17. v. 66	SO_4^{2-}	5·5	17·5	30	140
18. v. 66	SO_4^{2-}	4·2	34·0	22	90
23. v. 66	Glucuronate	12·5	21	56	100
24. v. 66	Glucuronate	10·0	26	54	100
25. v. 66	Glucuronate	12·0	30	52	100

Mucosal solutions were Na^+ free. The values of p.d. and s.c.c. are those existing immediately before replacement of Cl^- and 2 hr after replacement.

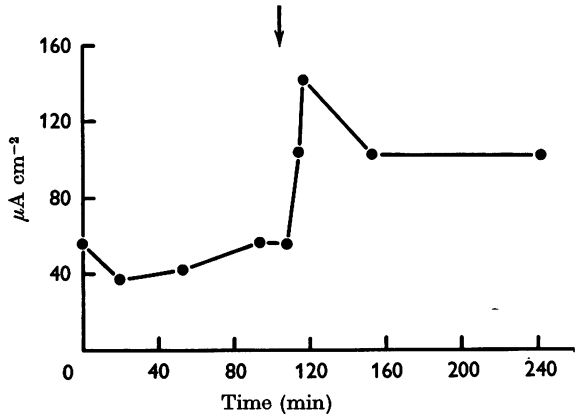

Fig. 4. The effect on short-circuit current of a 28-day stomach of replacing Cl^- by glucuronate in mucosal and serosal solutions. The mucosal solution was Na^+ free at all times. Area of membrane = 1·15 cm^2.

Previous work has shown that replacement of Cl^- by methyl sulphate or ethyl sulphate also fails to reverse the p.d. and s.c.c. (Wright, 1964).

DISCUSSION

The measurements of net flux of Na^+ and s.c.c. have shown that I_{Na} can be used as an exact measure of the net flux of Na^+ from mucosa to serosa. Although this result might have been anticipated from Fig. 1, there would still have remained the possibility that the value of the Na^+ independent component of the s.c.c. was in fact altered when Na^+ was present in the mucosal solution: the results presented in this paper have shown that this possibility is not realized.

It was shown in an earlier paper (Wright, 1963) that the active transport of Na^+ from mucosa to serosa was a function performed by the non-differential cells. In the same paper it was shown that the onset of HCl secretion into the mucosal solution coincided with the appearance of oxyntic cells in the mucosa (on the 23rd day of gestation). Before the appearance of the oxyntic cells there was no p.d. across the mucosa when Na^+ was absent from the mucosal solution: under this condition no s.c.c. would be obtained. It is concluded that the Na^+ independent component of the s.c.c. arises from oxyntic cell activity associated with acid secretion.

The results presented in this paper have shown that the Na^+ independent component of the s.c.c. and the net flux of Cl^- are in opposite directions and that the latter is 166 % of the former. If H^+ and Cl^- were secreted at the same rate there would be no s.c.c. associated with acid secretion. The following explanations of this result may be considered:

(*a*) Active transport of Cl^- is electrogenic and active transport of H^+ is non-electrogenic and no other electrogenic process is occurring. H^+ and Cl^- may or may not be secreted in equivalent amounts.

(*b*) Active transport of both H^+ and Cl^- is electrogenic, but Cl^- is secreted in excess of H^+. No electrogenic transport of any other ion species is considered to occur.

(*c*) Equivalent electrogenic secretion of H^+ and Cl^- is occurring and some other cation is being electrogenically transported from mucosa to serosa.

(*d*) Electrogenic transport of H^+ and Cl^- in equivalent amounts is occurring and some other anion is being transported electrogenically from serosa to mucosa.

(*e*) H^+ and Cl^- secretion is non-electrogenic and some other cation is subject to electrogenic active transport from mucosa to serosa.

(*f*) H^+ and Cl^- secretion is non-electrogenic and some other anion is subject to electrogenic active transport from serosa to mucosa.

Previous work (Wright, 1964) has shown that K^+ only passes across the foetal gastric mucosa in either direction, down its gradient of electrochemical potential; and also in experiments with Na^+ free solution on the mucosal side there was no alteration in s.c.c. when $choline^+$ was substituted for K^+. The Na^+ independent s.c.c. reported in this paper would not therefore have received any contribution from choline ion or K^+ transport, thus ruling out alternatives (*c*) and (*e*). It has also been shown (Wright, 1963, 1964 and unpublished) that Cl^- secretion always occurs in excess of H^+ secretion, hence ruling out alternatives (*c*) and (*d*). Alternative (*a*) would only apply if the Na^+ independent s.c.c. was exactly equivalent to the rate of net transport of Cl^-; this was shown not to be the case. Since net flux of Cl^- was found to bear a definite relation to Na^+ independent

s.c.c. it would appear that active transport of Cl^- is electrogenic, thus ruling out alternatives (*e*) and (*f*). It could appear then that alternative (*b*) is the correct one to apply to this preparation.

If alternative (*b*) was correct, then replacement of Cl^- by non-actively transported anions should have reversed the Na^+ independent s.c.c. The failure to obtain this reversal showed that electrogenic active transport of H^+ could not be demonstrated by these means and that the s.c.c. which persists is presumably due to a non-specific active transport of anions: this point requires direct verification using various species of labelled anions. This s.c.c. might have been due to extrusion of an intracellular pool of Cl^- from the oxyntic cells. However, calculation of the maximum amount of Cl^- which could have been contained within these cells at the beginning of an experiment could not provide the amount of charge carried by the s.c.c. over the experimental period. Considering the result shown in Fig. 4, an s.c.c. of 100 μA over a period of 4 hr is equivalent to the charge carried by 14·8 μ-equiv. of Cl^-. A typical value for the wet weight of the preparation is 0·14 g and 20 % of this is generously assumed to be intracellular water of oxyntic cells in which Cl^- is assumed to be present at a concentration of 150 m-equiv/l., then the amount of Cl^- which could have been contained in these cells initially was 4·2 μ-equiv.

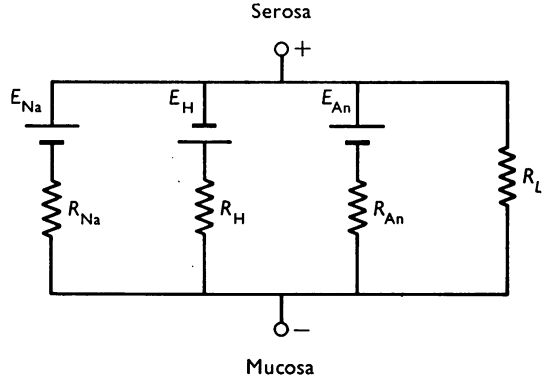

Fig. 5. An equivalent circuit describing the properties of the gastric mucosa of the late rabbit foetus. E_{Na}, E_H and E_{An} represent the E.M.F.s of the Na^+, H^+ and anion transport systems respectively whilst R_{Na}, R_H and R_{An} represent the internal resistances of these systems. R_L is the 'passive' leakage resistance of the mucosa to all ions.

The results presented in this paper, considered with earlier results, lead to the conclusion that the major part of the s.c.c. of the foetal rabbit stomach of 28–30 days gestation age is accounted for by a specific active transport of Na^+ from mucosa to serosa. The remainder of the s.c.c. is associated with HCl secretion from serosa to mucosa, the Cl^- perhaps

being transported by a non-specific anion transport mechanism. An equivalent circuit which describes the results obtained is shown in Fig. 5. E_{Na}, E_{An} and E_H are the E.M.F.s of the Na^+, anion and H^+ transport systems. R_{Na}, R_{An} and R_H are the internal resistances of these systems and R_L is the leakage pathway through the mucosa. This circuit is similar to one proposed by Bornstein, Dennis & Rehm (1959) to describe the transport phenomena occurring in the resting stomach of the dog, in which active transport of Na^+ was observed.

The calculation of E_{Na} and E_{Cl} from the flux ratio (Ussing, 1949) was not made as it has been shown by Kedem & Essig (1965) that the value obtained is only valid when the resistance of parallel leakage pathways is very high, as indicated by a high flux ratio ($> 100:1$). The Na^+ flux ratios obtained in these experiments of about 10:1 are low and give a value of E_{Na} which is perhaps only 25% of the true value (see Fig. 1 of Kedem & Essig, 1965). The flux ratios for Cl^- were even lower and subject to greater variation.

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