Absorption and Secretion in the Foetal Stomach

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ABSTRACT.

An outline of the history of gastric electrolyte absorption and secretion along with the development of general electrolyte physiology has been presented and related to foetal electrolyte physiology.

A method has been described whereby net transfers of water and electrolytes across the gastric mucosa of the rabbit foetus may be studied in vitro. Net transfers of Na^+, H^+, Cl^-, K^+ and water were studied in relation to their differences of electrochemical potential across the mucosa. The results were then considered in relation to the developmental cytology of the mucosa.

The open circuit electrical potential difference and short-circuit current were measured using a method described in this thesis. Ionic concentrations were varied on the two sides of the mucosa and the resulting changes in open circuit p.d. were used to calculate the transport numbers of the principal ions in the system during their transfers across the mucosal cell membranes. The short-circuit current was studied in relation to the presence or absence of various ions on the two sides, and the effects of various drugs on it were described.

It is concluded from the results obtained that the oxyntic cells secrete H^+ and Cl^- against their gradients of electrochemical potential. The non differentiated cells were shown to effect an absorption of Na^+ against its gradient of electrochemical potential. All movements of water were passive and down osmotic gradients. The active anion transport was considered to be none specific. There was no evidence of an active transport of K^+. The physiological significance of these results has been discussed.

FOREWORD

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The experimental work described in this thesis is concerned with only one aspect of secretion and absorption in the foetal stomach; namely movements of water and simple inorganic ions across the gastric mucosa.

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Reprints:

Continuous recording of short-circuit current through frog skin. J.Physiol.146, 24-25P, 1959.

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Aerobic energy production and the stimulation of active sodium transfer across isolated frog skin by neurohypophysial extract. J.Physiol.152, 28-29P, 1961.

Absorption and secretion of electrolytes in the stomach of the rabbit foetus. J.Physiol. 155, 24-25P, 1961.

INTRODUCTION

Historical Background Prior to 1800.

Scientific interest in the inorganic properties of gastric secretion probably commenced in the first half of the 17th century. It was at this time thought that the phenomena of functional biology could be explained in terms of an adequate knowledge of acids and alkalis - the iatro-chemical school, or on the other hand, by a knowledge of the physical forces present - the iatro-physical school.

Sylvius, in 1650, stated that gastric digestion was describable by the chemical terminology of that time, and noted in particular the glandular nature of the gastric mucosa and the oozing of juice from it. Borelli, although belonging to the iatro-physical school, noted in 1680 that animals without teeth and with non 'fleshy' stomachs digest hard foods without crushing: 'These animals consume flesh and bones by means of a very potent ferment, much in the same way as corrosive liquids corrode and dissolve metals.'

Van Helmont recognised acidity in the gastric contents and distinguished this from the ferment itself. Spallanzani, however, failed to convince himself of the

acidity of gastric juice in spite of finding that sea-shells and corals were erroded by it. Once, after eating an excess of strawberries and wine, he found acid coming into his mouth, but attributed this to abnormal digestion.

By the middle of the 18th century the existence of acid in gastric juice was becoming widely accepted. John Hunter, in 1772 claimed that there was acid in the stomachs of a great variety of animals. He also noted that there was no acid in the stomachs of calves before birth. That was probably the first physiological investigation made on the secretory physiology of the foetal stomach. It is remarkable that practically no observations on the absorbtive and secretory activity of the foetal stomach have been made since.

Carminati in 1785 showed that the gastric juice was neutral during starvation and acid after feeding.

The developements from 1800 to 1900.

Considerable advances in knowledge of the inorganic constituents of gastric juice were made during this period, as a result of the techniques of chemical analysis which had been developed. Prout (1824) showed for the first time the presence of 'fixed' alkali in the gastric juice. Schmidt (1854) was able to confirm the findings of Prout in vivo using a human patient with a gastric fistula.

Heidenhain (1879), and later Pavlov (1910), using gastric fistulae on experimental animals, showed that the composition of gastric juice is dependant on the secretion rate: a finding that has received considerable attention ever since.

During the 19th century, it became apparent, with increasing use of the microscope, that the gastric mucosa was made up of a heterogeneous cell population. Thus Heidenhain (1879) recognised two principal cell types, the parietal cells and the argentiffin cells. In a short time the parietal cells became associated directly with the secretion of HCl. Thus Swiecki (1876) and Langley (1881) showed that in the frog, pepsin is secreted largely by glands in the oesophagus, whilst the glands of the gastric mucosa secrete acid almost entirely. The cells of the frog gastric mucosa were then shown to be of similar appearance to the parietal cells of the mammalian gastric mucosa.

Many attempts were made to identify the site of acid secretion using injected dyes. Claude Bernard (1859) injected a solution of iron lactate and potassium ferrocyanide into the jugular vein of a fasting rabbit, and later found a blue pigment on the surface of the gastric mucosa, particularly in the region of the lesser curvature.

pH indicators were injected by a large number of workers (Eddinger, 1890, 1892, Prinkler, 1895, Frankel, 1891,) and the results of these experiments indicated that the fundic region

of the stomach was the site of acid secretion. Since this region was known to contain the highest proportion of parietal cells, it was considered that these were the cells responsible for the secretion of HCl: they were termed 'oxyntic cells' by Langley in 1881. Zimmerman (1898) described small granules within these cells which had a low refractive power and did not decrease in number during secretion.

Progress in physical chemistry during this period produced laws and concepts which have since become of considerable value to physiologists wishing to describe the factors responsible for the difference in composition of gastric juice and plasma. The most useful advances were those in thermodynamics, particularly in relation to the concept of free energy, due to Helmholtz (1882) and Gibbs (1875), leading to the concept of thermodynamic potential and equilibrium.

At about the same time, Fick (1855) evolved his empirical law of diffusion, which has had considerable use by biologists ever since. However, indiscriminate use of this law can lead to serious errors of description.

A marriage of thermodynamics and electrochemistry produced the familiar equation relating the E.M.F. of a concentration cell to the activities of the electrolytes in the system (Nernst, 1889), and equations relating the magnitude of a diffusion potential to the transport numbers and activities of the electrolytes present (Planck 1890, Nernst 1888, Behn 1897).

Observations of electrical phenomena associated with secretory activity were made as long ago as 1834 when Donné observed an electrical p.d. across the gastric wall and associated it with the secretion of acid. Du Bois-Reymond (1848) first recognised that the living frog's skin is a seat of electro-motive force and could give rise to current flow. These observations were confirmed by Galeotti (1904) and extended to show that the p.d. depended on the presence of sodium (lithium) salts in the bathing solutions. Furthermore, the p.d. persisted with identical solutions bathing the two sides of the skin: thus the p.d. could not be produced by diffusion potentials or be explained in other simple physico-chemical terms.

The early interest in the electrical properties of frog skin has persisted until the present day, largely due to most workers in the field of active transport considering this tissue as a useful reference point; also the extreme robustness of the tissue makes it one of the most satisfying of preparations on which to work.

In spite of the considerable advances in physical chemistry which had taken place, physiologists realised that the phenomena of gastric secretion were not describable simply in terms of physical chemistry, but depended on other forces characteristic of living systems alone. Thus Heidenhain was led to postulate the participation of a 'Triebkraft' in the absorbtive and secretory processes of the gut.

The developments since 1900.

Interest in identification of the site of acid secretion continued with the work of Fitzgerald (1910), using the Prussian blue reaction, by injecting balanced amounts of ammonium-ferric citrate and potassium ferrocyanide. The blue colouration was in the gastric mucosa in the region of the lesser curvature. Vertical sections of the mucosa showed the colouration tobe localised in the upper third of the gastric pits only. It was also observed in the canaliculi of the parietal cells. As Conway (1958) points out it is surprising in view of the diffusion conditions present that such good localisation was obtained.

Using silver chloride deposition, Fitzgerald (1910), Monti (1913) and Leschke (1915) have shown that the highest chloride concentrations are found in the parietal cells and the intercellular canaliculi.

The canaliculi within the parietal cells have attracted interest since they were first described by Golgi in 1893 and confirmed on many occassions since (Moore and Bensley 1936, Hollander 1943, Plenk 1932). Conway(1958) points out that similar canaliculi are present within the yeast cells and are in fact a continuation of the cell wall. In the yeast cell at least, the cell wall has been shown to be a site of metabolic action (Rothstein, 1950). More recent studies on mammary gland secretory tissue, using the electron microscope, have confirmed the presence of intracellular canaliculi and invaginations of

the cell wall.

Since the gastric mucosa was composed of three cell types it was desirable to find means of determining the composition of the pure parietal secretion. Since the pure parietal secretion cannot be obtained in practice, it has been necessary to resort to indirect evidence. Pavlov (1910) observed that the acidity of the gastric juice increased as the rate of secretion increased. He interpreted this finding to mean that the parietal cells secreted acid at a high and constant concentration, and that this was neutralised and diluted by the non parietal secretions; this effect being most apparent at the onset and end of the secretory phase.

Hollander (1931, 1932, 1934) and Hollander and Cowgill (1931) confirmed Pavlov's findings and by plotting neutral chloride against total acidity showed that the HCl concentration was 170mM with zero neutral chloride: assuming then that there was no neutral chloride in the parietal secretion it was considered that the parietal cells secreted pure HCl at 170mM. However, the absence of neutral chloride in the parietal secretion is something that remains to be proved (Conway, 1958).

Gray (1943), plotted neutral chloride against secretion rate and extrapolated the line obtained to zero secretion

rate. It was found that the neutral chloride was not zero at zero rate of acid secretion but approached a value of about 7m.equiv/l. It was also shown that the potassium concentration remained constant at about 7m.equiv/l during large variations in acidity and secretory rate. Gray concluded that the parietal secretion has the following composition H^+159 m.equiv/l, Cl^-166 m.equiv/l, K^+7 m.equiv/l.

Fisher and Hunt (1950) using the data obtained by Ihre (1939) on young men in response to histamine and insulin, estimated the pure parietal and non parietal secretions to have the following compositions:

Parietal H^+ concentration	160m.equiv/l.
" neutral Cl^- "	10m.equiv/l.
Non parietal Cl^- "	125m.equiv/l.
" " HCO_3^- "	45m.equiv/l.

The above theories are usually classed together as the component theory of gastric acid secretion, and account for the variation of acidity with secretion rate. At low rates of acid secretion the neutralising effect of the (constant) parietal secretion is most apparent, whereas at the highest rates the gastric juice composition will tend to approach that of the pure parietal secretion.

Teorell (1939, 1940) has prepared an alternative 'diffusion theory' to account for the variation of acidity with secretory

rate. He suggests that the H^+ ions which have been secreted diffuse across the gastric mucosa, down their gradient of electrochemical potential, back into the blood. At the same time, Na^+ ions diffuse down their gradient of electrochemical potential, from the blood to the gastric lumen. The effect of this 'leakage' and $H^+ - Na^+$ exchange will be most pronounced at the lower secretion rates.

In order to test the diffusion theory, Teorell placed a measured amount of glycine buffer in the stomach, and at the end of the experimental period titrated the buffer to determine the amount of acid that had been produced. It was found that under these conditions the primary acidity varied between 170mN at the higher rates of secretion and 350mN at the lower rates. The main objection to this method is that the acid is being secreted against an abnormally low difference of electrochemical potential.

However, it would seem that there is little doubt that the gastric mucosa is permeable to hydrogen ions, and it would thus seem that both the component and diffusion theories help to explain these phenomena.

Although the osmolarity of the gastric juice remains close to that of plasma in spite of wide variations in osmolarity of the latter, (Gilman and Cowgill, 1932, Noble

and Robertson, 1938), slight variations of this parameter with secretion rate do appear. This result is explained by the component theory in the following way: the HCl is neutralised by the bicarbonate in the none parietal secretion with a resulting reduction in osmolarity:

$$HCl + NaHCO_3 = H_2O + NaCl. + CO_2$$

It is seen from the equation that the reduction in osmolarity will be greatest at intermediate secretion rates, a postulate which has been verified by experiment (Lifson et al, 1943).

Hirschowitz (1961) has proposed that the gastric juice is formed by a primary process whose products are consequently modified by a secondary process. The primary process is the secretion of NaCl at 160mM by the peptic cells. The secondary process involves the exchange of H^+ for Na^+ by the parietal cells. This theory is supported by the existence of a high (140mM) chloride concentration in the resting stomach and the inverse relationship between sodium and hydrogen ion concentration during acid secretion. Potassium ion concentration remained fairly constant at a level three or four times that of the plasma. It was also shown that the relation: $(Na^+ + K^+ + H^+) = Cl^-$ held. From this, and the linear relation between Cl^- and osmolarity of gastric juice passing through zero, it was also concluded that chloride was the principal anion in gastric juice and

that the amount and concentration of bicarbonate present was negligible, a view which has also been expressed by Rehm et al (1958) and Heinz and Obrink (1954).

Rehm et al (1959) have studied ionic movements across the resting stomach of the dog, using various solutions on the mucosal side. It was found with 0.05 M NaCl on that side Cl^- passed into the lumen against its gradient of electrochemical potential whilst Na^+ passed into the lumen down its gradient of electrochemical potential. With 0.10 M NaCl on the mucosal side there was no change in the amount of Cl^- in the lumen. With 0.15 M NaCl on the mucosal side there was a net decrease in amount of Na^+ and Cl^- on the mucosal side, Na^+ moving against its gradient of electrochemical potential and Cl^- moving with its gradient of electrochemical potential.

Water movement took place down an osmotic gradient, and in the absence of such a gradient, water movement was in the direction of net solute movement. The p.d. was monitored in these experiments and its magnitude was of the order of 60mV. with the mucosal side negative.

The authors point out that the results can be explained by assuming the existence of separate anion and cation 'pumps', both of which would be electrogenic and in parallel, with their positive poles aligned towards the serosal side. An alternative explanation given was that there may be a single electrogenic anion pump with its positive pole towards the

serosa and in parallel with it a channel through which NaCl could pass down its gradient of chemical potential by ion pair formation or by combination with a charged carrier $X^{\pm}$. These results are particularly pertinent to the present thesis.

That the p.d. originates in the mucosa has been demonstrated by Rehm (1946) by applying ethyl alcohol to the mucosal side and showing that it rapidly abolished the p.d., whereas the effect is much slower if the alcohol is applied to the serosa. Even more conclusive evidence for the mucosal origin of the p.d. is shown by stripping the mucosa off and showing that the full p.d. still exists across it (Hogben, 1955).

The concepts and techniques used so successfully by Ussing and his school for the study of active Na transport in frog skin have been of considerable use in studies of active transport across other epithelial membranes. In these studies the electrical properties of the membrane are related to the ion fluxes across it. It was shown that the ratio of the 'passive' unidirectional fluxes of a particular ionic species is equal to the ratio of the electrochemical potential of the ion on the two sides i.e.

$$\frac{M_{1\ 2}}{M_{2\ 1}} = \frac{A_1}{A_2} \exp\left(\frac{zEF}{RT}\right)$$

is the unidirectional flux from side 1 to side 2,

where $M_1 \rightarrow 2$

where $M_{2\rightarrow1}$ is the unidirectional flux* from side 2 to side 1,

A_1 " " activity of the ion on side 1

A_2 " " " " " " " " " 2

E " " electrical potential difference across the membrane,

Z " " valency of the ion in question

F " " Faraday (96, 500 coulomb)

R " " gas constant (erg./mol/^{o}AK)

and T " " absolute temperature.

Any deviation from this relationship is said to indicate active transport i.e. a source of free energy other than the kinetic energy of the ions and the potential energy due to the electric field is said to be available. The extra source of free energy is called the 'active transport potential' and may be written into the flux ratio equation to enable it to apply to an actively transported ion i.e.

$$\frac{M_{1\rightarrow2}}{M_{2\rightarrow1}} = \frac{A_1}{A_2} \cdot \frac{\exp(EZF/RT)}{\exp(E_aZF/RT)}$$

where E_a is the active transport potential. Measurement was made using two isotopic variants of the ion under consideration, simultaneously. It was found (Ussing, 1948) that the sodium ion was theonly one to be affected by an active transport potential. The experiments were carried out in vitro, using a piece of skin of known area, and bathing the two sides with oxygenated Ringer's solutions

* Rate of transfer of matter across unit area.

and modified Ringers.

A further important advance was made when in 1951 Ussing and Zerahn used the concept of short-circuit current to measure the rate of net active ion transport. The rationale of this is as follows: if the skin is bathed with identical solutions on the two sides, and at the same time active ion transport is taking place, the resulting movement of the charge will set up a p.d. which will then tend to be short-circuited by movement of the passive ions in the system. If the two sides of the skin are then connected by reversible electrodes and a circuit of very low resistance, a current will pass which will be exactly equivalent to the rate of active transport of charge. In practice the short-circuit current cannot be measured in this simple manner because the reversible electrodes that are available have too high a resistance to effect more than a partial shunt of the skin.

This difficulty was overcome, however, by applying an E.M.F. across the skin of appropriate sign and magnitude to reduce the spontaneous p.d. across the skin to zero. The current then passing in the external circuit is equal to the short-circuit. It was found that the rate of net sodium transport across the skin was exactly equal to the short-circuit current, thus proving that sodium is the only ion subjected to active transport in this system.

This electrochemical approach made it possible to describe (but not to explain) the active transport process in a convenient and consistent manner. Thus in the frog skin there was considered to exist a source of E.M.F. equal to the active transport potential and in series with it a resistance as in any other electrochemical cell. Across the source of E.M.F. and series resistance was a shunt resistance through which the circuit was completed by movement of the passive ions. The shunt resistance would cause the open circuit p.d. across the skin to be less than the active transport potential.

It was found however (Ussing, 1954) that the shunt resistance could be made very large by replacing chloride by sulphate in the Ringer solution, or by adding 10^{-5}M Cu^{++} to the Ringer on the mucosal side, the effect of this being to reduce the chloride permeability to practically zero. Under these conditions the open circuit p.d. is equal to the active transport potential, and no further active transport occurs with the skin on open circuit. The active transport potential was found to have a value of about 140mV, and the internal resistance (obtained by dividing the active transport potential by the short-circuit current) was about 1.5KΩcm^2.

Many of the early results obtained by the Ussing school

were confirmed by Linderholm (1952) who also derived expressions for the partial conductance of an actively transported ion.

Hogben (1955) working in Ussing's laboratory used the above techniques to study hydrogen ion, chloride and sodium ion transport across the gastric mucosa of the frog. He found that the short-circuit current was equivalent to the rate of net chloride transport from serosa to mucosa minus the rate of hydrogen ion transport in the same direction. The flux ratio for sodium ions was found to deviate slightly from the value to be expected if sodium transport was passive in this system. This latter result could be accounted for if thirty per cent of the sodium flux from mucosa to serosa was 'active'.

Heinz and Durbin (1959) showed that the frog gastric mucosa in vitro, bathed with solutions in which Cl had been replaced by sulphate, showed a reversed short-circuit current which was exactly equivalent to the rate of hydrogen ion secretion. Furthermore, the open circuit p.d. was of reversed sign under this condition.

An extensive study of the relationship between the electrical properties of the gastric mucosa and the rate of secretion of acid have been carried out by Rehm and his school, (Rehm et al 1943, 1945, 1948, 1957, 1955). More

recently (Rehm, 1959), it has been proposed that an active transport of chloride from serosa to mucosa gives rise to a p.d. which serves to drive a current of hydrogen ions from the serosal side to the mucosal side. On this theory, increasing the natural p.d. artificialy would be expected to increase the rate of hydrogen ion secretion, whilst decreasing the p.d. should decrease the rate of hydrogen ion secretion. This hypothesis was verified by experiment (Rehm et al, 1945), and confirmed by Crane, Davies and Longmuir(1948). It was also shown that the maximum current that could be drawn from the mucosa was electrochemically equivalent to the rate of hydrogen ion secretion.

An objection raised against this theory was that the resistance of the mucosa was too high to allow sufficient power to be available for the concentration of hydrogen ions against their gradient of electrochemical potential. However, Rehm analysed the complex impedance of the gastric wall and showed that it was analogous to capacitance in parallel with a resistance, these two components being considered to exist in the secreting cells. The serosal and muscular layers were considered to act as a higher resistance in series with the other two components. It was then shown that the parallel resistance was extremely small in the resting stomach (about 3 ohm.cm^2) and fell practically to zero

in the secreting stomach; thus showing that the E.M.F. could deliver an adequate hydrogen ion current. It was also shown that the parallel resistance became greater on death. The zero value of the parallel resistance was explained on the basis of their being active transport mechanisms for hydrogen and chloride ions (Rehm et al 1956, 1957). It is of interest to note that Hogben (1955) found a negative partial conductance for chloride ions in the secreting frog stomach, which would be compatible with an active transport process for these ions.

There would appear however, to be some degree of biochemical coupling between the chloride and hydrogen ion secretory processes, since Rehm et al (1963) have shown that the hydrogen ion secretion rate in sulphate Ringer, with the p.d. clamped to the same level as in chloride solutions, is about one third the rate with chloride Ringer bathing the mucosa: if the hydrogen ion secretion rate depended only on the E.M.F. across the mucosa (produced by chloride transport) it should be the same under the two conditions.

Rehm et al (1963) have shown that histamine produces a lowering of the p.d. and resistance of the gastric mucosa which runs parallel with the increase in rate of acid secretion Crane, Davies and Longmuir (1948) have suggested that resonance of the iminazole ring of histamine enables it to act as a

hydrogen atom carrier in the electron transport cycle.

The nature of the p.d. across the gastric mucosa remains to be elucidated. However, there are three possibilities which can give rise to p.d.s across living membrane systems. The first of these occurs when the membrane acts as a concentration cell with respect to some of the ions in the system. This type of p.d. has been demonstrated to exist across many cell membranes and has been studied in great detail in nerve and muscle. In these cells a metabolic extrusion of sodium gives rise to an unequal distribution of potassium between the inside and outside of the cell (in order to preserve electroneutrality): the concentration of potassium on the inside gives rise to a diffusion potential which then becomes the equilibrium potential for potassium ions, and is described by the Nernst equation. The same argument applies to the other 'passive' ions which are able to pass through the membrane.

Koefoed-Johnsen and Ussing (1958) have used the above theory to describe the p.d. across frog skin. It was shown that the mucosal side of the skin would act as a sodium electrode (in sulphate Ringer) and the serosal side acted as a potassium electrode under the same conditions. These results led to the postulate that the mucosal side was permeable to sodium but not potassium, and the serosal side was permeable to potassium but not sodium (relatively speaking). The total p.d. across the skin was then considered to be the sum of a sodium and a potassium equilibrium potential. This type of theory is usually termed

non-electrogenic i.e. no current flow is involved.

Earlier, the p.d. across frog skin was considered to have an electrogenic origin (Ussing and Zerahn 1951): the active transport of sodium was considered equivalent to a current passing through a resistance. In the author's opinion both the electrogenic and non-electrogenic theories are applicable to frog skin. The post tetanic hyperpolarisation observed in non-myelinated nerve is also said to be electrogenic in nature and result from an increased rate of sodium extrusion(Ritchie and Straub 1957, Straub 1963).

A third possible source of p.d. is a redox system (Lund, 1947) which may simply involve electron translocation (pH independent) or hydrogen transfer as well as electron transfer (pH dependent). For many years it was thought that such systems could not exist across cell membranes because electrons could only 'flow' in metals. However, it is now well established that electronic conduction can occur in non metallic media of sufficiently periodic structure (Brillouin 1963). Recently, electronic conduction has been demonstrated in a large number of biological macromolecules, (Rosenberg 1963). The significance of such phenomenn has been considered by Szent-Gyorgii (1948).

Joseph, Reid, Kaplon and Stock (1948) have demonstrated the existence of a redox potential across the membranes of

the cells of synovial membrane of the dog. These authors suggest that a cytochrome system exists in these cell membranes.

Crane, Davies and Longmuir (1948) have suggested that a redox system of the type $Fe^{++} - Fe^{+++}$ may be involved in the production of hydrogen ions by the gastric mucosa, the effect of the system being to remove electrons from hydrogen atoms on the mucosal side of the oxyntic cells. In their scheme the p.d. would be at a maximum in the resting stomach and would fall during secretion: this fall was shown to occur.

Rehm (1963) concludes that the p.d. across the gastric mucosa results from the electrogenic 'pumping' of hydrogen and chloride ions from serosa to mucosa. The E.M.F. of the chloride pump tends to make the serosa positive whilst the E.M.F. of the hydrogen ion pump tends to make the mucosa positive. In the resting state the E.M.F. is at a maximum since only the chloride pump is operative. An activation of the hydrogen ion pump then tends to reduce the total p.d. It was shown in the same paper that the gastric mucosa does not behave as a chloride electrode thus making it difficult to explain the p.d. on a non-electrogenic basis.

Davies and Ogston (1950), using electrochemical methods, showed that the resting gastric mucosa is very nearly impermeable to hydrogen and chloride ions. This fact again makes it difficult to explain the p.d. on the basis of equilibrium potentials.

Modern theories of the mechanism of gastric acid secretion centre round a redox process involving a flavine enzyme (FH_2, F) and a cytochrome, Cyt.

$$FH_2 + 2\ Cyt^{+++} = F + 2Cyt^{++} + 2H^+.$$

Theories of this type have been proposed by Conway and Brady (1950), Crane and Davies (1948), and Rehm (1950). Davies and Ogston (1950) also suggested that this type of mechanism may be coupled to phosphate group transfer processes involving A.T.P.

Hogben (1951) has suggested that hydrogen ions may be actively secreted in an indirect manner by the active transport of bicarbonate from mucosa to serosa, with chloride exchanging for bicarbonate on a carrier system. The p.d. observed was then said to be due to a passive diffusion of bicarbonate from serosa to mucosa. However, Rehm (1954) has shown that the p.d. is insensitive to changes in the mucosal bicarbonate concentration.

Excellent reviews of the above theories have been given by Heinz and Ehrink (1954) and by Conway (1959).

Within recent years the subject of irreversible thermodynamics (de Groot 1951) has been applied to biological processes in general (Spanner 1954, Prigogine 1951) and to membranes systems of interest in biology (Kedem and Katchalsky 1958, 1961).

The theory is based on the principle of microscopic reversibility due to Onsager (1931) and states that all fluxes (or flows) in a system are, in general, dependent upon all the thermodynamic 'forces' in the system. Thus any flow Ji may be written

$$Ji = \sum_{j=1}^{j=n} LijXj$$

where the Xj is the thermodynamic force conjugated to the flow of species j and Lij is the Onsager cross coefficient relating the flow of i to Xj. Any combination of flows and forces may be chosen so long as it results in a positive rate of entropy production. In order to satisfy this condition straight coefficients, such as Lii and Ljj, must be positive, but the cross coefficients Lij may be positive or negative. Finally, in order to satisfy the second law of thermodynamics, the determinant of the matrix of the coefficients must be equal to or greater than zero i.e.

$$|L| \geqslant 0 .$$

In an isothermal system (the usual case in biology), the choice of flows and forces may be one that gives a

positive rate of free energy dissipation. Thus a typical case occurs if the flow is the rate passage of a molecular species across unit area of a membrane and the conjugate thermodynamic force is considered to be the difference in chemical potential of the substance across the membrane. If a second metabolic flow is taking place simultaneously in the membrane (e.g. electron translocation or phosphate group transfer) and a cross coefficient between the first and second flow exists, then the possibility of active transport arises (Spanner 1953, and Scheer 1959).

The theory of irreversible thermodynamics now makes it possible to determine whether a particular theory of biological mechanism is in fact feasible. In the past, gross oversimplifications have been made, particularly with regard to the use of Ficks' law of diffusion, which assumes that the flux in question is dependant only on its conjugate 'force' and that this force is unrelated to any other fluxes. Deviation from this law has been readily taken to indicate that some mechanism other than diffusion is operating, (Widdas 1951, Le Devre 19 48, Willbrandt 1938).

The alternative mechanism to diffusion which has been proposed by the above authors is a'carrier theory' in which the penetrating molecules are thought to become attached by loose bonds to a chemical constituent of the membrane and

ferried across. Such a scheme was first proposed by Ussing (1952). The carrier theory leads to relations between flux and concentration of the type:

$$y = K \left(\frac{C}{C + \delta} \right)$$

where y is the unidirectional flux, C the concentration, δ a half saturation constant and K a constant with the dimensions of a flux. It is seen that the variable part of the right hand side (in brackets) is dimensionless and unrelated to the chemical potential of the species in question.

There have been few studies of electrolyte transfers across foetal epithelia. Garby (1957) studied electrolyte movements across the isolated human amniotic membrane and was unable to demonstrate any active electrolyte transport or spontaneous p.d. across it. He concluded however, that sodium, potassium and chloride ions exchange across the membrane at a rapid rate. Wright (1959, unpublished) was unable to demonstrate a spontaneous p.d. across the isolated amniotic membrane (with identical Krebs bicarbonate Ringer on each side) of rabbit, sheep and human. In some of the experiments the chorion was left in contact with the amnion.

Crawford and McCance (1960) showed that the chorio-allantoic membrane of the pig exhibited a spontaneous p.d. in vitro and demonstrated that the short-circuit current was equivalent to the rate of net (active) sodium transport which was in the foetal to maternal direction. The rate of active transport was inhibited by high CO_2 tension and fall in pH on the foetal side, and consequently tends to be inhibited by the allantoic fluid itself, possibly providing a negative feed back mechanism. It was further shown that neurohypophysical extract has no effect on the rate of active sodium transport in this membrane. This latter result is somewhat unexpected since this extract is known to produce an enhanced rate of active sodium transport in frog skin (Koefoed-Johnsen and Ussing, 1958) and in amphibian bladder (Hays and Leaf, 1961). A certain amount of evidence for sodium and potassium electrode behaviour was also obtained i.e. the membrane appeared to be analogous to amphibian skin in this respect. Finally, it was shown that only the chorio-allantoic complex had an active transport function; if the chorion was stripped off this function was abolished. It was not possible however to study the chorion in isolation, owing to its fragility. A metabolic interdependence between the two membranes was suggested, analogous to that between corneal epithelium and stroma (Hermann and Pickman, 1949).

As far as this author is aware, no physiological study of electrolyte secretion and absorption has been carried

out on the foetal stomach, except that of Wright (1961, 1962). However, histological studies of the foetal gastric glands have been made over a number of years and this evidence has led to certain assumptions as to the function of the foetal stomach.

Kirk (1910) showed in the pig embryo that the oxyntic cells arise at a very early stage from an undifferentiated epithelium, whilst the peptic cells develop much later.

The foetal cat at birth has mucoid and oxyntic cells lining the gastric glands; the peptic cells appearing a week later. The human foetus has peptic and oxyntic cells fully developed at birth, whilst at 4½ months only mucoid and non mucoid cells are present (Lim, 1922). Hydrochloric acid and rennin (Dudin 1904) have been shown to exist in the 5 month stomach and pepsin (Keene and Hower 1929) is also present at this stage.

Mennies (1958) has studied the cytology of the gastric mucosa of the rabbit foetus from the 19th day until full term. He demonstrated the presence of only one cell type - undifferentiated cells - up to the 23rd day when a few oxyntic cells were shown to appear. On the 27th day the oxyntic cells suddenly became much more numerous and pitting of the epithelium became extensive. At birth there are still more oxyntic cells but peptic cells (certainly of the adult type) appeared to be absent although there were a few cells at the base of the pits which appeared to be precursors of the peptic cells.

On the basis of the above information it was thought it might be profitable to examine electrolyte transfers across the gastric wall of the rabbit foetus and attempt to correlate those findings with the cytological changes taking place. By this means it was hoped to determine the role of the foetal stomach in the water and electrolyte balance of the foetus; and also to determine the separate functions of the specific cell types present. In conclusion it is perhaps worth quoting Rehm (1959) on this latter point: 'I would like to point out ..... that I don't know in which cells these E.M.F.'s are located, and I don't know whether the same cells that produce the H^{+} ions also secrete the Cl^{-} ions. Some of you may think you know, but I am convinced that you don't know unless you have crucial data that have not been published as yet.'

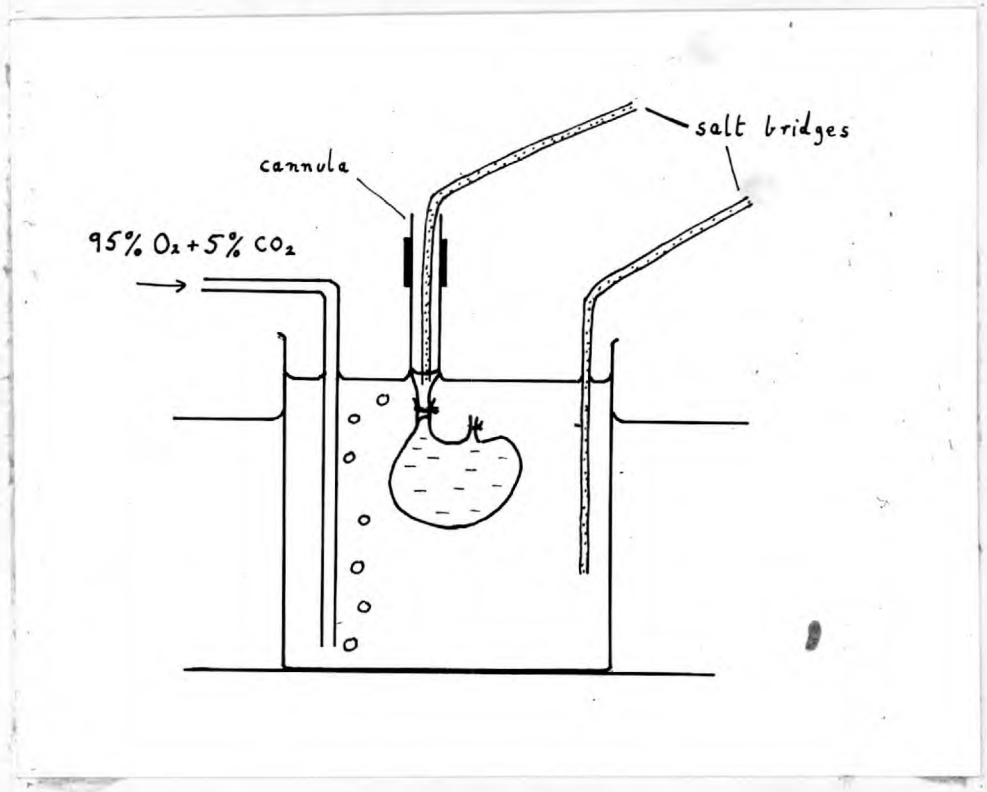


Fig.1. Method of mounting foetal stomach in vitro. The large container is placed in a water bath (not shown) at 38°C.

Nett Transfers of Water, Sodium, Chloride, and Potassium and Hydrogen Ions across the gastric mucosa of the Rabbit Foetus.

Experimental methods.

Adult female rabbits were mated overnight, fertilization occuring within a known 14 hr. period. The experiments were carried out on stomachs of foetuses of 20 - 31 days (full term) gestational age.

Operative procedure.

Pregnant rabbits were anaesthetised with pentobarbitone sodium (60mg/kg body weight) and procaine spinal anaesthetic. The foetuses were exposed by Caesarian section and quickly detached and killed by a blow on the head. The abdomen was opened and the viscera quickly cooled by washing with a modified bicarbonate Ringers solution at 20°C. The stomach was detached with 2 - 3 mm of duodenal and oesophageal stumps and was further cooled with bicarbonate Ringer solution.

Mounting procedure.

The duodenal stump was tied off and a fine glass cannula was tied into the oesophageal stump, the tip of the cannula being on the gastric side of the cardiac sphincter. The gastric contents were withdrawn through the cannula and the gastric lumen was washed out six or seven times with experimental solution and finally filled with this solution at 20°C. The stomach was then immersed in 100ml of modified bicarbonate Ringer's solution at 35°C for the duration of the experiment. (Fig.1.)

Solutions.

The bicarbonate Ringers solution used had the following composition (mM): Na^+ 145.8; K^+ 4.8; Ca^{++} 3.6; Cl^- 132.2; HCO_3^- 25.3; glucose 24.0. This solution had 95% O_2 + 5% CO_2 bubbled through it 1 hr. before being used, and during the experiment.

The experimental solutions were 154 mM - NaCl or 154 mM choline chloride. These solutions usually contained 24 mM glucose to make them iso-osmotic with the bathing solution. The solutions were shaken with air at 20°C before being introduced into the stomach. In some experiments, in which titratable acid was not measured, the Ringers solution was used as the experimental solution.

Analysis of experimental solutions.

Na and K were measured by flame photometry, Na to ± 2.0 m.equiv./l, K to ± 0.2m.equiv./l. Cl was determined by the Sanderson (1952) method of potentiometric titration to ± 0.5 m.equiv./l.

Titratable acid was determined by potentiometric titration with 0.01 N NaOH, a glass electrode and Cenmy microburette being used. The indifferent electrode was a Pt wire sealed into the tip of the burette, as in the Sanderson chloride method. The detection apparatus was a Vibron 33B electrometer of 10^{13} input impedance (Electronic Instruments Ltd.). The titration was carried out by adding 0.1ml. of the solution to be analysed to 2.0ml. of distilled water. This solution was

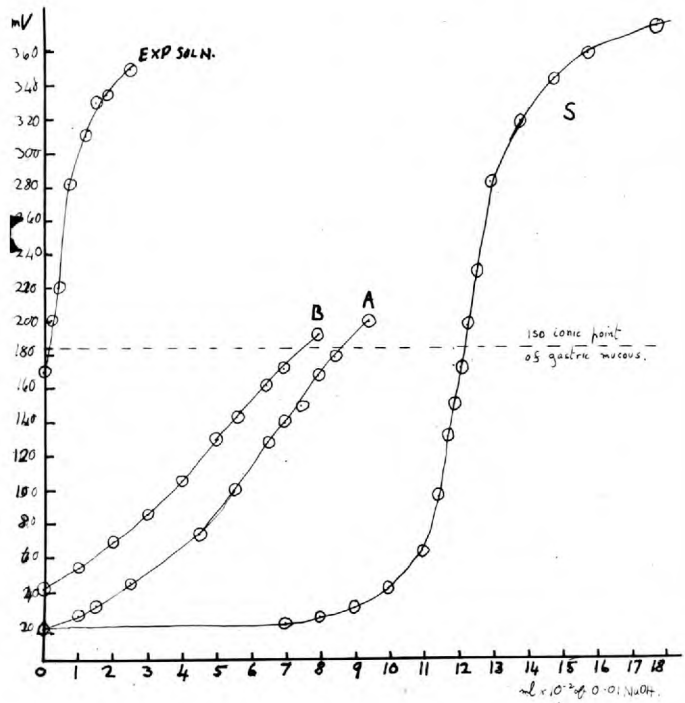


Fig.2. Potentiometric titration of gastric contents from two stomachs (A and B), experimental solution and standard solution (0.012N HCl in 154mM NaCl).

stirred with air and the NaOH slowly run in.

When an experimental solution was titrated after being in a stomach there was no clear point of inflexion on the potential - volume curve, (Fig.2). This was due to the presence of mucus which was buffering the acid in these samples. In these cases the iso-ionic point was determined by dialysis of gastric contents against 154mM NaCl or choline chloride, through cellophane at 1°C for 24hr; the value obtained was pH 7.4. This pH was then taken as the end point. By these methods titratable acid was determined to ± 0.2 m.equiv/l.

Volume changes.

Initial and final volumes were determined by one of two methods. In the first method the weight of the stomach, cannula and experimental solution was determined at the beginning and end of the experiment, after careful removal of surplus solution from the outside of the stomach and cannula with filter paper. After the second weighing had been carried out the stomach was incised, with a small pair of scissors, and the contents collected in a small specimen jar which was immediately stoppered. The stomach was then opened, the solution removed from the inside surface by blotting, and weighed with the cannula. From these weighings the initial and final volumes were determined.

In the second method, inulin (mol.wt.6,000) was added to the mucosal solution to give a known concentration (about 100mg./100ml). At the end of the experiment the inulin concentration in this solution was measured, and the final volume deter-

mined by blotting and weighing as in the first method. The initial volume was calculated from the final volume and the initial and final inulin concentrations.

Control experiments in which inulin was placed at high concentration on the serosal side only showed that none was detectable in the mucosal fluid after 6 hr.

In some experiments these two methods were used simultaneously and the results agreed closely, showing that tissue swelling, which would give anomalous results in the first method, was insignificant.

Inulin was determined by the method of Roe and Dell (1948) for fructose.

Electrical measurements.

The electrical potential difference across the stomach wall was measured with calomel electrodes which were connected to the solutions by 3M KCl in 2% agar contained in polythene tubing to form salt bridges. The electrodes were connected to a Vibron 33B electrometer. After checking for asymmetry the tip of one bridge was placed in the Ringer solution bathing the stomach, the other bridge was inserted down the cannula into the experimental solution in the lumen. This latter bridge was left in contact with the experimental solution only when a measurement was being made.

Osmolality:

Osmolality of solutions was measured to $\pm$ 2.0 m.osmole/kg water by a cryoscopic method, a Stantel thermistor being used

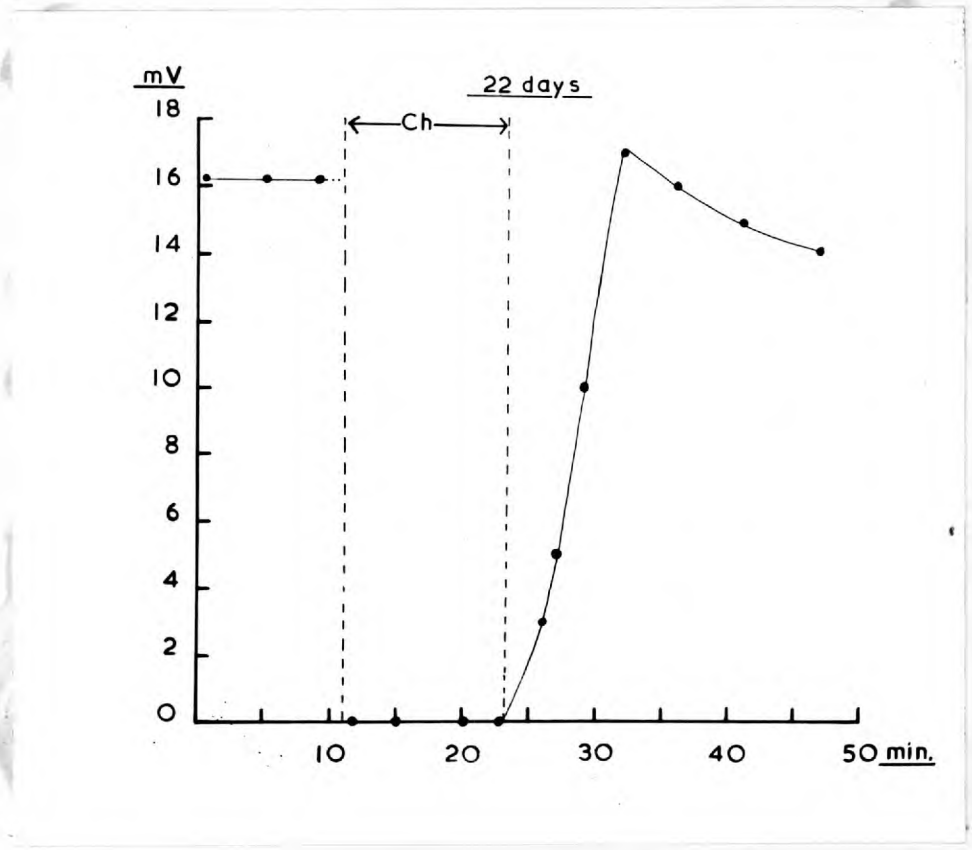


Fig.3. Dependence of the potential difference across the gastric mucosa upon the presence of Na in the mucosal solution at 22 days. Between the interrupted lines the mucosal solution was 154mM choline chloride: outside the lines it was 154mM NaCl. The mucosal side was negative with respect to the serosal side.

as the temperature sensitive element. These determinations were carried out on the bathing Ringer solution at the beginning and end of the experimental period, on the experimental solution before it was placed in the stomach and on its removal from the stomach. (see Fig.2).

Correction for osmotic transfer due to loss of water from the bathing solution by evaporation.

In all experiments the osmolality of the mucosal solution and the bathing solution increased during the course of the experiment, the latter increase was due to evaporation occuring as a result of gassing. The final osmolality of the mucosal solution always approached but never exceeded the osmolality of the bathing solution. If the osmolality of the mucosal solution increased by x%, then the net water loss due to osmotic gradient was equal to x% of the initial volume of mucosal solution.

Difference of electrochemical potential.

The difference $/u_s - /u_m$ of electrochemical potential on the serosal and mucosal sides of the stomach for a particular ion was calculated from the equation

$$/u_s - /u_m = RT \ln \frac{C_s}{C_m} + (E_s - E_m)ZF \quad \ldots\ldots(1)$$

where C_s and C_m are the concentrations (in m.equiv/l.) of the ion on the serosal and mucosal sides respectively.

$E_s - E_m$ is the measured difference of electrical potential of the serosal and mucosal sides

Z is the valency of the ion.

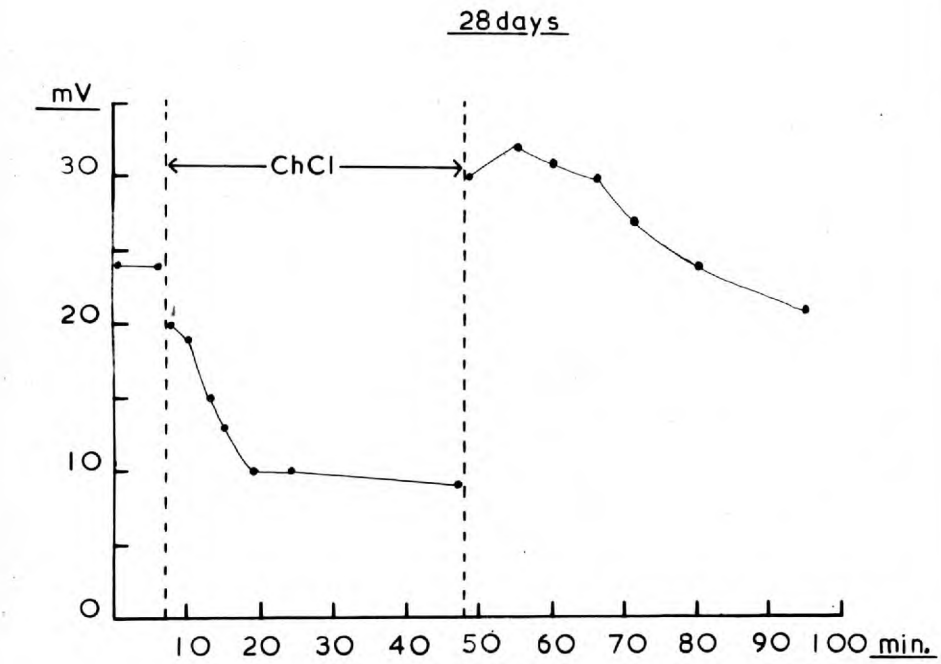


Fig.4. Results obtained from a stomach at 28 days on carrying out the same procedures as those described under Fig.3. The sign of the potential difference is the same as in the 22 day stomach.

F is the Faraday.

R is the gas constant (8.3×10^7 erg mole $^{-1}$ $^{\circ}$ A $^{-1}$)

and T is the absolute temperature.

The equation assumes equality of activity coefficients on the two sides.

RESULTS.

Differences in electrical potential.

A p.d. across the foetal gastric mucosa, with identical solutions on the two sides was found at all ages studied, the mucosal side being negative with respect to the serosal side in all cases. This p.d. was dependent on the presence of Na in the mucosal solution. Figure 3 shows the effect on the p.d. across a stomach of 22 days of replacing 154 mM Na Cl on the mucosal side by 154 mM choline chloride. It is seen that the p.d. rapidly falls to zero, but on replacing the Na the p.d. returns to a value close to the original one, after exhibiting a small overshoot.

Figure 4 shows the result of carrying out this procedure on a stomach of 28 days. In this case the effect was reversible as before, but the p.d. fell to about 30% of its initial value instead of to zero when choline replaced Na. This result was typical of those obtained from stomachs of 23 days gestational age up to full term.

There was no significant difference in p.d. with bicarbonate Ringer solution or 154 mM NaCl, with or without 24mM glucose on the mucosal side.

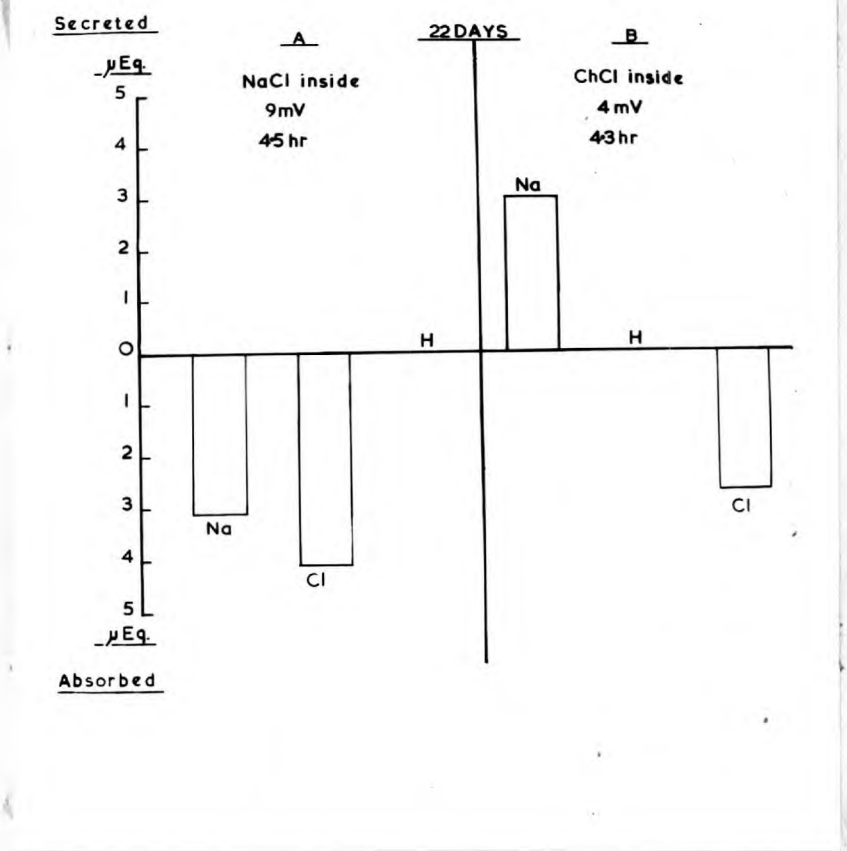


Fig.5. The pattern of net transfers of electrolytes across the gastric walls of a pair of stomachs from the same litter at 22 days. The serosal solution was bicarbonate Ringer's. A: mucosal solution 154mM NaCl; mean p.d. 9mV (range 8.0 - 9.0); time 4.5 hr. B: mucosal solution 154mM choline chloride; mean p.d. 4mV (1.5 - 11); time 4.3 hr.

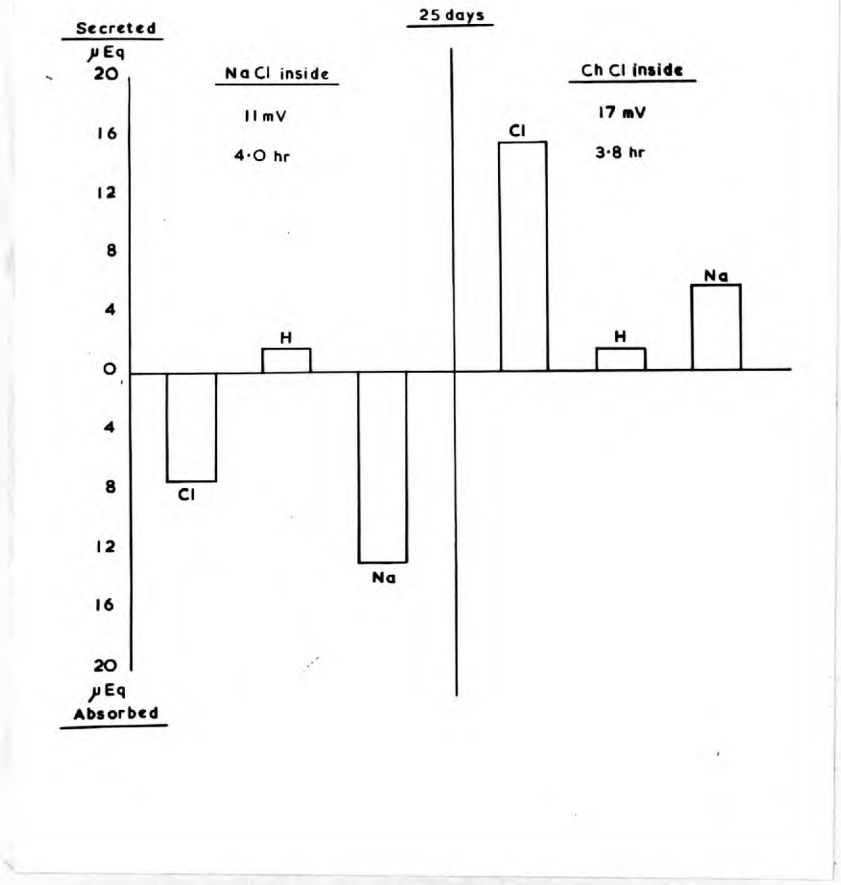


Fig.6. The pattern of electrolyte transfers in a pair of stomachs at 25 days. Solutions were the same as those described under Fig.5. A: NaCl inside; mean p.d. 11mV (10.0 - 16.0); time 4.0 hr. B: choline chloride inside; mean p.d. 17mV (15 - 22) time 3.8hr.

At 22 days the range of p.d.s observed was 8 - 18mV; after 22 days the range was 9 - 35mV. After equilibration the p.d. would remain for 4 - 6 hr.

Net transfer of electrolytes.

Figure 5 shows the results obtained from two stomachs at 22 days. In this experiment the stomach A, with 154mM NaCl showed a nt absorption of Na^+ against a gradient of electrochemical potential (7.5mVP initially and 9.0mVP finally) during the experimental period.

Stomach B, with 154mM choline chloride on the mucosal side, showed a net gain of Na, which passed down its gradient of electrochemical potential. Cl passed out of both stomachs down its gradient of electrochemical potential. The initial and final differences of electrochemical potential (for Cl) were 4.5mVP for stomach A and 4.4 and 4.5mVP respectively for stomach B.

No titratable acid was detected in the contents of either stomach at the end of the experiment. These results were typical of those obtained from stomachs of less than 23 days.

Figure 6 shows the results of experiments carried out on two stomachs of 25 days. The net transfers of Na were qualitatively similar to those occuring in the 22 day stomach: Na was absorbed from the mucosal side of stomach A against initial and final differences of electrochemical potential of 9.5 and 10.4mVP, respectively; and passed into the lumen of stomach B down a gradient of electrochemical potential. In the case of stomach

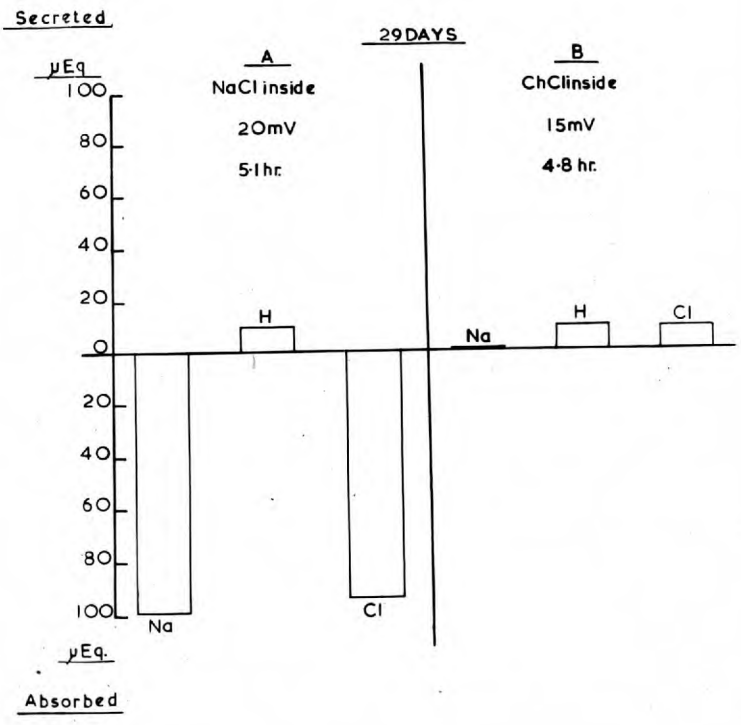


Fig.7. Results obtained from a pair of stomachs at 29 days. The solutions were as described under Fig. 5. A: NaCl inside; mean p.d. 20mV (17 - 23); time 5 hr. B: choline chloride inside; mean p.d. 15mV (13 - 21); time 4.8 hr. Note the change of scale on the ordinate.

A, Cl^- was absorbed down a difference of electrochemical potential of 6.5mVP initially and 5.5mVP at the end of the experiment. In the stomach B, Cl^- was secreted into the lumen against a difference of electrochemical potential (12.5mVP initially and 7.7mVP finally).

Figure 7 shows the results of a similar experiment carried out on a pair of stomachs at 29 days. The results were qualitatively the same as those shown in fig.6. although quantitatively the net transfers are about 5 times greater.

The results shown in Figures 6 and 7 are typical of those obtained from all stomachs of greater than 22 days gestation age (over 30 pairs).

The pH of the gastric contents was not measured. However, at the beginning of all determinations of titratable acid carried out on stomachs from 23 days onwards, the pH of the gastric contents, after being added to 2.0 ml of distilled water, was at least 0.5 pH unit below the iso-ionic point of the buffer material. Thus, for net H^+ transfer into the post 22 day stomachs to have been passive, the observed p.d. would have to have been greater than 32.0mV. Under the conditions of these experiments a p.d. of this magnitude was not usually observed. It must be concluded then that H^+ was actively secreted into the lumen of the stomachs of more than 22 days gestation age.

In all of the above experiments a net transfer of K into the lumen occured; the maximum concentration of K being less

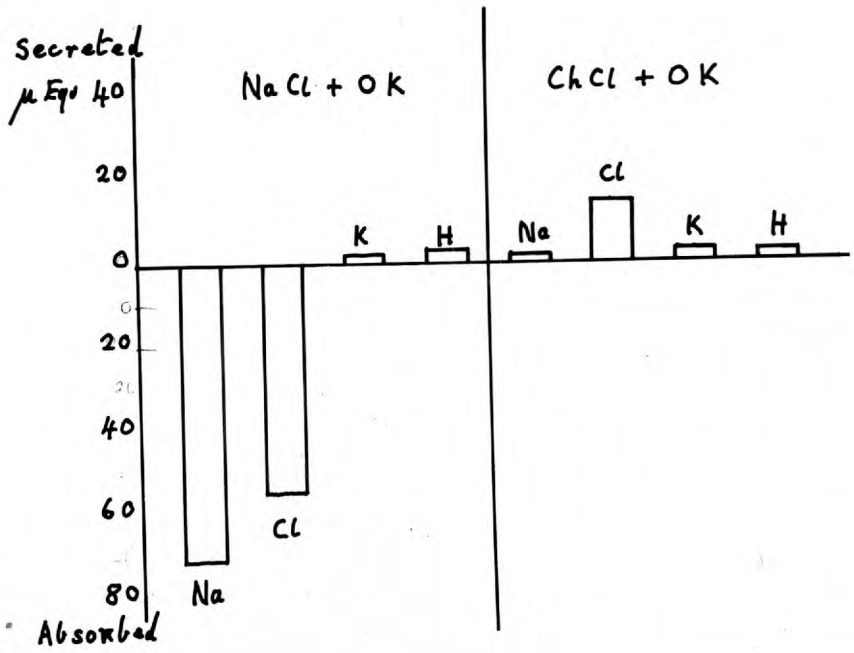


Fig.8. Net gain of K^+ by a pair of stomachs at 26 days with low mucosal K^+ concentration. A: NaCl inside; $/u_s = /u_m = 53.8$mV.F initially, 12.6mV.F finally. B: choline chloride inside; $/u_s = /u_m$ initially +ve and indeterminate $(K)=0_{mucosal}$, 28.2 mV.F finally.

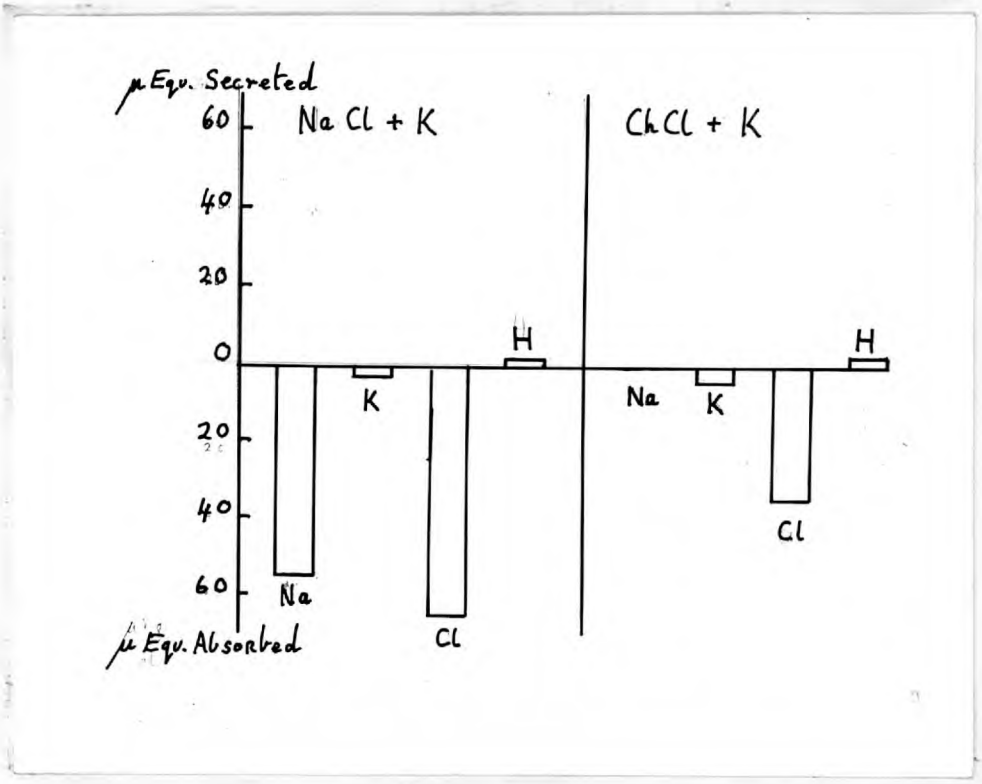


Fig.9. Net loss of K^+ from a pair of stomachs at 27 days with high mucosal K^+ concentration. A: NaCl inside; $/^{u}_{s} - /^{u}_{m} = -1.3mV.F$ initially, $-19mV.F$ finally. B: choline chloride inside; $/^{u}_{s} - /^{u}_{m} = 4.0mV.F$ initially, $+1.0mV.F$ finally.

than that in the bathing solution at the end of the experimental period. Remembering the sign of the electrical p.d., this means that the net K transfers under these conditions were down the gradient of electrochemical potential: at the beginning of an experiment this gradient would be enormous whilst the final difference of electrochemical potential was usually of the order of 10 - 30mVP.

This information does not tell us whether K can be actively 'secreted' by the gastric mucosa. In order to clear up this point, experiments were carried out in the same way as described previously but with K added to the experimental solutions to bring the concentration to about 15m.equiv./l; the effect of this being to produce a gradient of electrochemical potential for K in the direction of mucosa to serosa, the difference of electrochemical potential being of the order of 5.0mVP.

Figure 9 shows the results obtained from an experiment with a high K concentration in the experimental solution. It is seen that in both experiments (one with Na and one without Na on the mucosal side) there is a net transfer of K from mucosa to serosa, down the gradient of electrochemical potential. Figure 8 shows the results obtained from a pair of stomachs of the same age but without K in the experimental solution. It is seen that the net transfer of K is from serosa to mucosa, down the gradient of electrochemical potential. It would appear then that K movements are 'passive' under the conditions of these experiments.

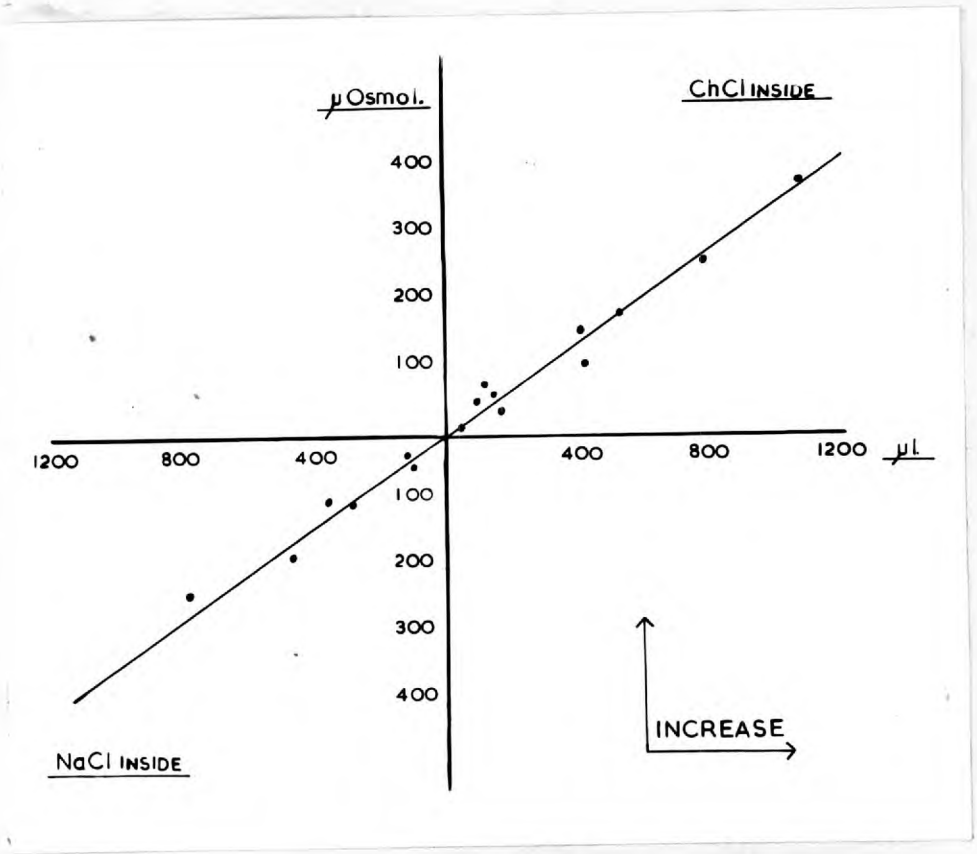


Fig.10. Net solute transfer during the experimental period is plotted against net water transfer for sixteen post 23 day stomachs. The top right hand quadrant shows increase in amount of solute and water in the lumen with 154mM choline chloride on the mucosal side. The bottom left hand quadrant shows a decrease in amount of solute and water when the mucosal solutions were 154mM NaCl or bicarbonate Ringer's solution. The slope of the line drawn by eye is 332 m.osmole /kg. (Water transfers have been corrected for transfers down osmotic gradients).

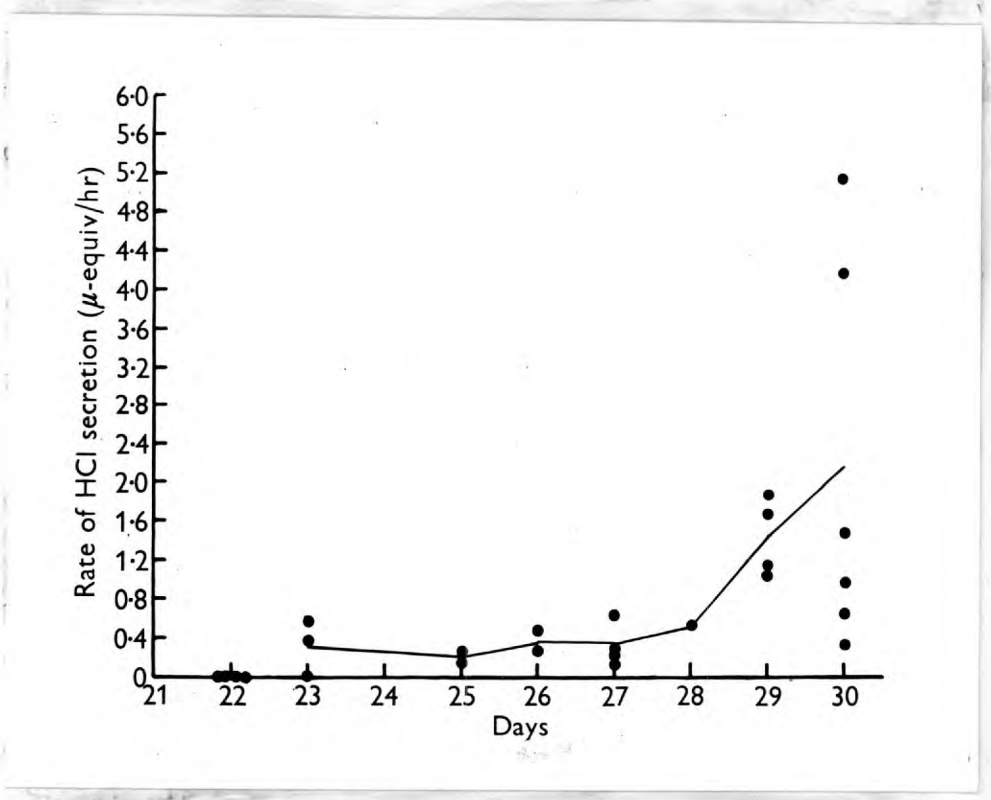


Fig.11. Rate of HCl secretion in twenty-six stomachs from 22 to 30 days. The solid line is drawn through the mean secretion rate at each age.

Net water transfers.

Figure 10. shows the relation between net solute transfer and net water transfer in 16 post 22 day stomachs after correction for osmotic water transfer due to loss of water from the bathing solution by evaporation. In those experiments where the mucosal solution was choline chloride there occurred a net increase in amount of solute and water on the mucosal side. When the mucosal solution contained NaCl there was a net decrease in amount of solute and water on the mucosal side.

It is seen that there is a linear relation between solute transfer and solvent transfer over the range of observations, and that the line passes through the origin and has a slope of 332 m.osmole/kg water.

It thus appears that water is free to pass down an activity gradient and that solute is transferred as iso-osmotic solution. Since the line passes through the origin there can be no active transport of water under these conditions.

Rate of acid secretion as a function of gestation age.

Figure 11 shows the rates of acid secretion of 26 stomachs. There was no acid secretion before the 23rd day, after which the mean rate of acid secretion was of the order of 0.4 μu.equiv/hr of H^+ up to the 28th day. The mean rate of H^+ secretion was then raised to about 2.0 μu.equiv/hr. at full term. Considerable variation in the secretory rate occurred at the greater gestation ages, the range being 5.0 to 0.2 μu.equiv/hr. at 30 days.

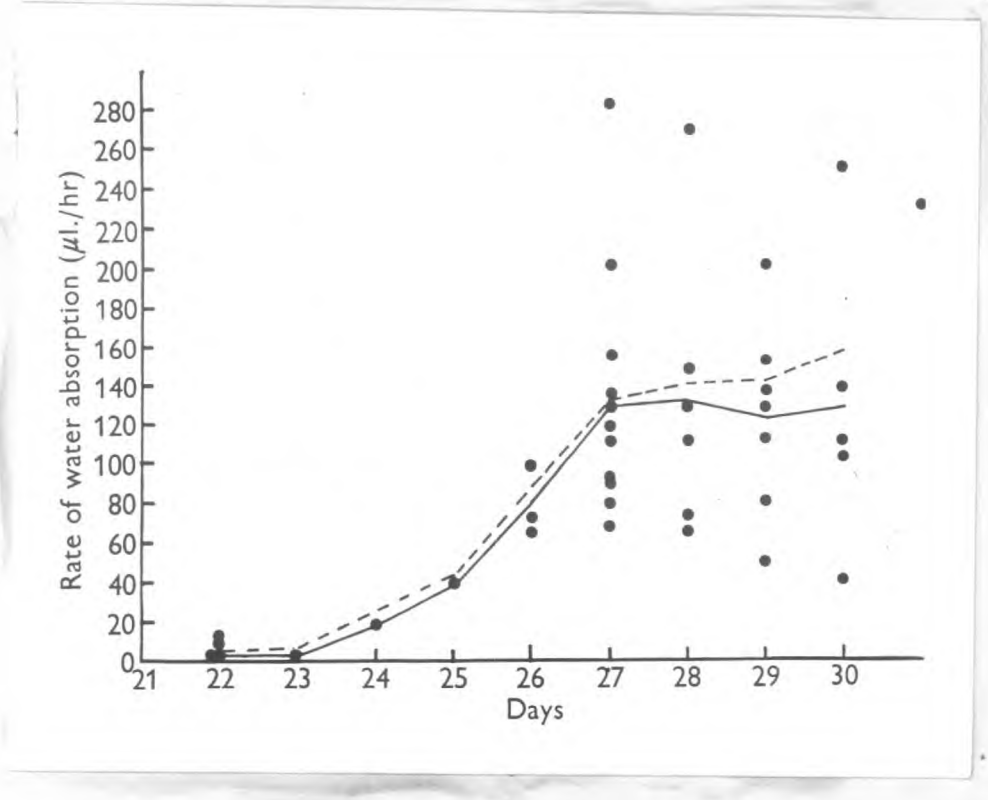


Fig.12. Rate of water absorption in forty stomachs from 22 to 30 days. The solution in the lumen contained 154mM - Na^+ in all cases. The solid line is drawn through the mean rate of absorption at each age. The dashed line is drawn through points representing the mean rate of water absorption at each age had there been no secretion of HCl.

Rate of water absorption as a function of gestational age.

The rates of water absorption of 40 stomachs are shown in Figure 12. It is seen that the mean net rate of water absorption was rapidly raised from a value of the order of 10 - 20 µl./hr. before the 25th day up to a more or less constant value of 130 µl./hr. from the 27th day until full term.

Each point in Figure 12 shows the algebraic sum of the secretory and absorbtive processes in each stomach. By using the values shown in Fig.11 and considering iso-osmotic HCl as the primary secreted acid the effects of the absorptive and secretory processes can be seperated, as is shown in Fig.12. The mean absorption rate in isolation is seen to increase after the 27th day up to a value of 150 µl./hr. at 30 days.

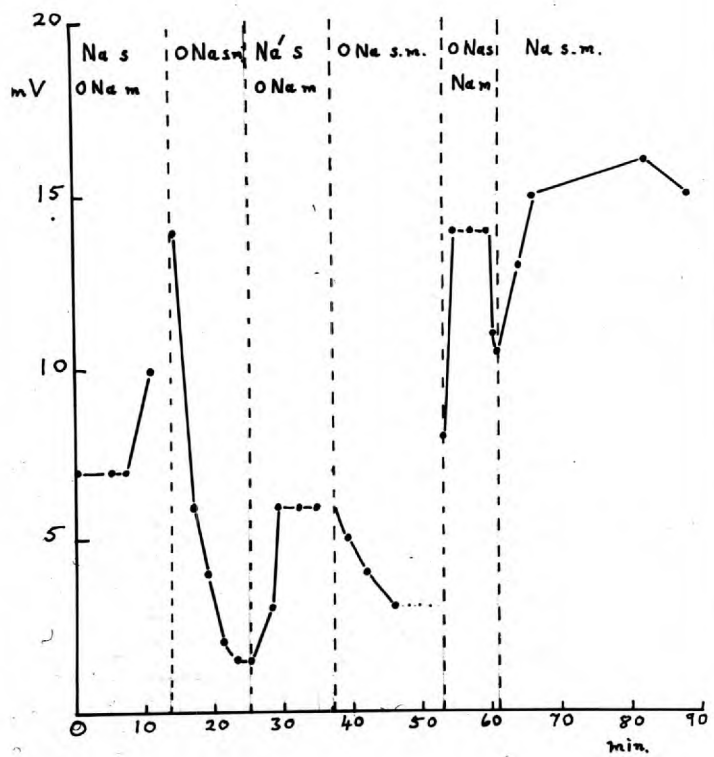


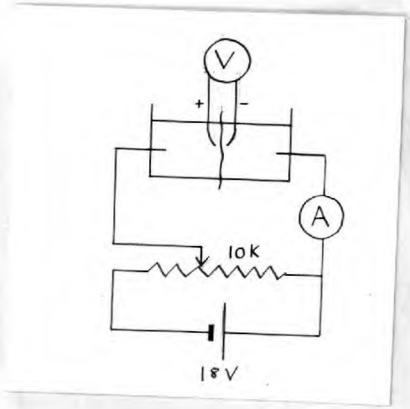
Fig.13. Effect of replacing Na^+ by choline$^+$ in serosal and mucosal solutions at 28 days. Serosal solutions were low in HCO_3^- (10 m.equiv./l). Na_s and ONa_s denote presence and absence of Na^+ (154m.equiv./l) respectively on the serosal side. Na_m and ONa_m denotes presence and absence of Na on the mucosal side.

Analysis of the electrical potential difference.

Fig.13 shows the results of placing Na free solutions on the mucosal and serosal sides of a stomach at 28 days. The serosal solutions were low in bicarbonate (10mM/l.) in order that NaCl could be replaced by choline chloride.

It is seen that the maximum p.d. occurred with Na(150 mM/l.) on both sides and that the minimum p.d. occurred with Na free solutions on both sides. The increase in p.d. when Na is added to either side is not a diffusion potential of Na. Thus when Na is present on the serosal side only, the diffusion potential of this ion would lower the observed p.d. - not increase it as is actually observed. Similarly, the increase in p.d. when Na is present on the mucosal side is not simply a diffusion potential of Na, since it is not abolished (but in fact increased) when Na is present at the same concentration on the serosal side.

It was decided to investigate more extensively the permeability properties of the serosal and mucosal sides of the gastric epithelium by the determination of the transport numbers of the principal ions in the system. This was done in a manner analogous to that used by Hodgkin and Katz (1949) to determine the relative permeabilities of the nerve membrane to Na^+ and K^+ and by Nafsed-Johnsen and Ussing (1950) to determine the relative permeabilities of the mucosal and serosal sides of the frog skin to Na^+ and K^+. The method



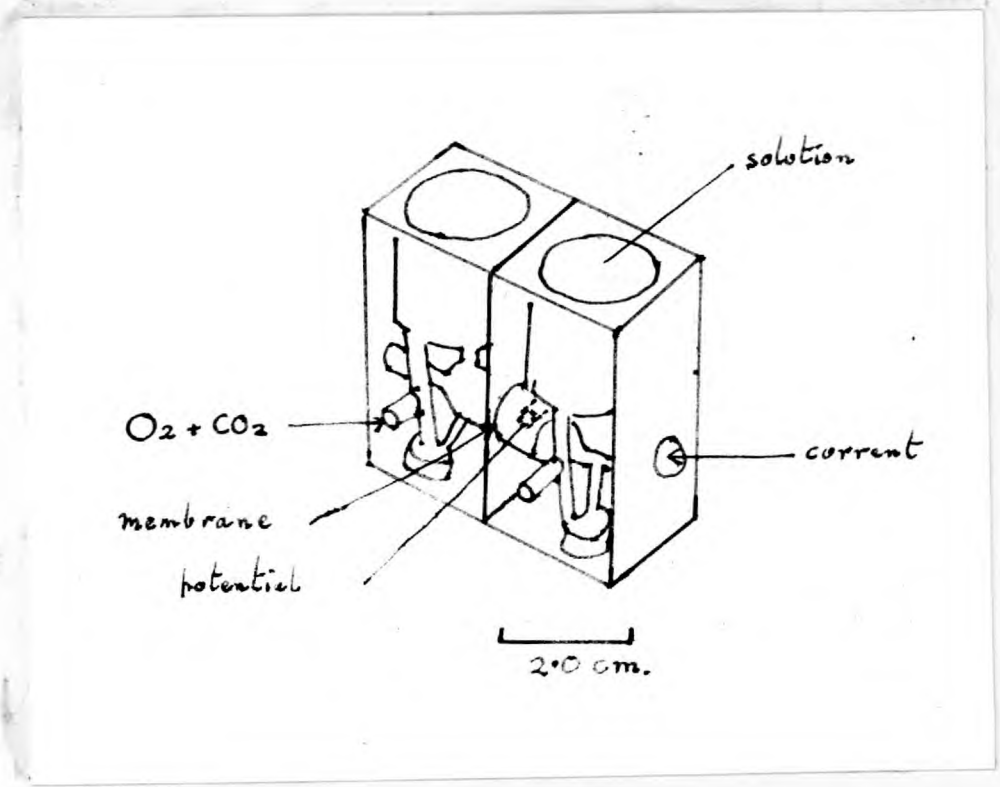


Fig.14. Showing the perspex chambers between which the stomach membrane was clamped. See text for details.

The small figure shows the circuit used for passing current and recording the p.d.

is based on the use of the general equation for the electrical potential difference across a membrane which is written (Staverman 1952):

$$E = -\int_{x=0}^{x=d} \sum_{k=1}^{n} \frac{t_k}{Z_k F} \cdot d/u_k \qquad \text{.....(2)}$$

where t_k is the transport number of the k^{th} ionic species, Z_k is the valency of that species and $/u_k$ its chemical potential. Integration of the equation over the thickness x of the membrane results, in special cases, in expressions of the type

$$E = -\frac{RT}{F} \sum_{k=1}^{n} \frac{t_k}{Z_k} \cdot \ln \frac{A_{kx}}{A_{ko}} \qquad \text{.....(3)}$$

where A_{ko} is the activity of the species k on side o and A_{kx} is the activity of k on side x of the membrane. By plotting E against $\ln A_{kx}$ or $\ln A_{ko}$. t_k can be obtained from the slope of the curve.

Experimental methods.

Each experiment was carried out using a piece of stomach wall (mucosa and muscle) sandwiched between two perspex chambers of the type used by Ussing and Zerahn (1951), Fig.14. The piece of stomach formed a membrane of 0.293cm^2 separating the chambers which were of 10ml capacity each. Each chamber was provided with an oxygen lift which oxygenated

and stirred the solutions. Holes were drilled into the chambers in order that salt bridges of the type previously described could be inserted so that their tips were close to the membrane. The other ends of these bridges were connected to calomel electrodes in order that the electrical potential difference could be measured. Two other holes were drilled so that a second pair of salt bridges could be inserted with their tips lying along the normal to the plane of the membrane at its centre. The other ends of these latter bridges were connected to silver - silver chloride electrodes which were in turn connected to a circuit for passing a current through the system. By means of these two pairs of electrodes it was possible to measure the short circuit current and D.C. resistance of the preparation as well as the open circuit p.d.

The two chambers were placed in a bath of liquid paraffin containing an aquarium heater and thermostat and a rapid stirrer. The temperature of this bath was adjusted so that the solutions in the chambers remained at a constant temperature of 35°C ($\pm$0.3°C).

The composition of the solutions on the serosal side was the same as that used previously except that in certain cases methyl sulphate was substituted for chloride and Ca^{++} was then added as $Ca(NO_3)_2$. When choline was substituted for sodium on the mucosal side choline chloride was added in place of sodium chloride (154mM), glucose at 24mM also being present. In

chloride-free solutions, sodium was replaced by adding potassium methyl sulphate in place of sodium methyl sulphate, potassium being regarded as an 'inert' ion on the mucosal side. Control experiments showed that in post 22 day stomachs the p.d. was unaffected by replacing choline chloride on the mucosal side by KCl.

When the solutions in the chambers were changed care was taken to see that no disturbances in temperature equilibrium occurred.

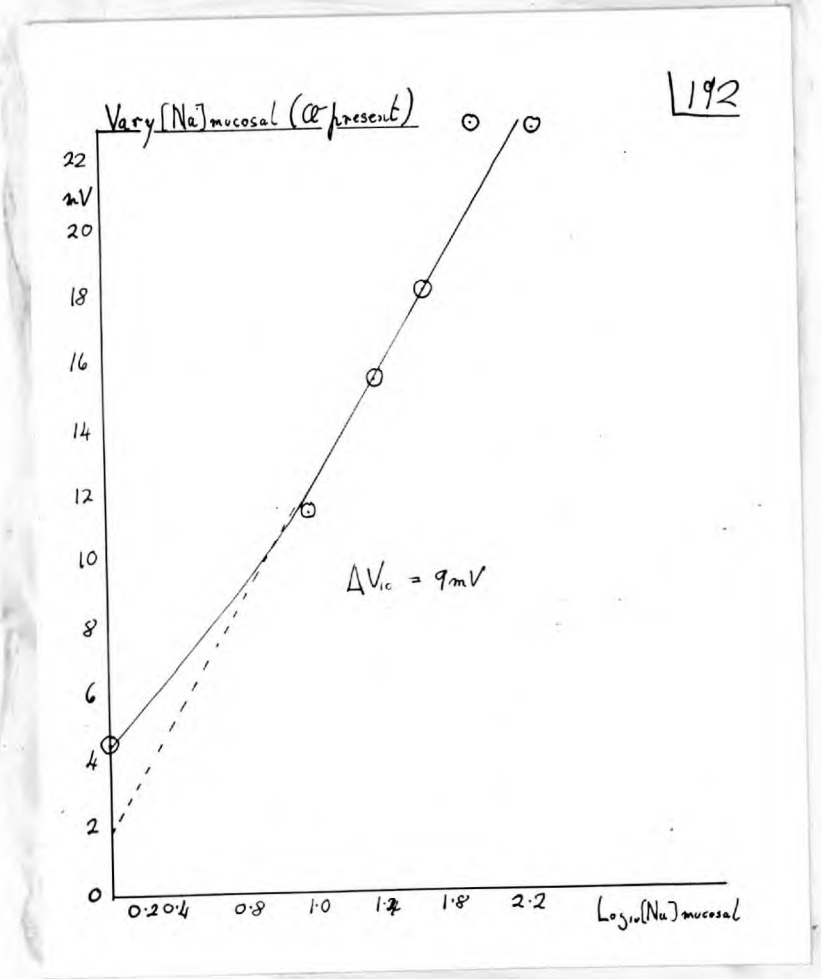


Fig.15. The relationship between gastric p.d. and mucosal Na^+ concentration. Cl^- was present on both sides.

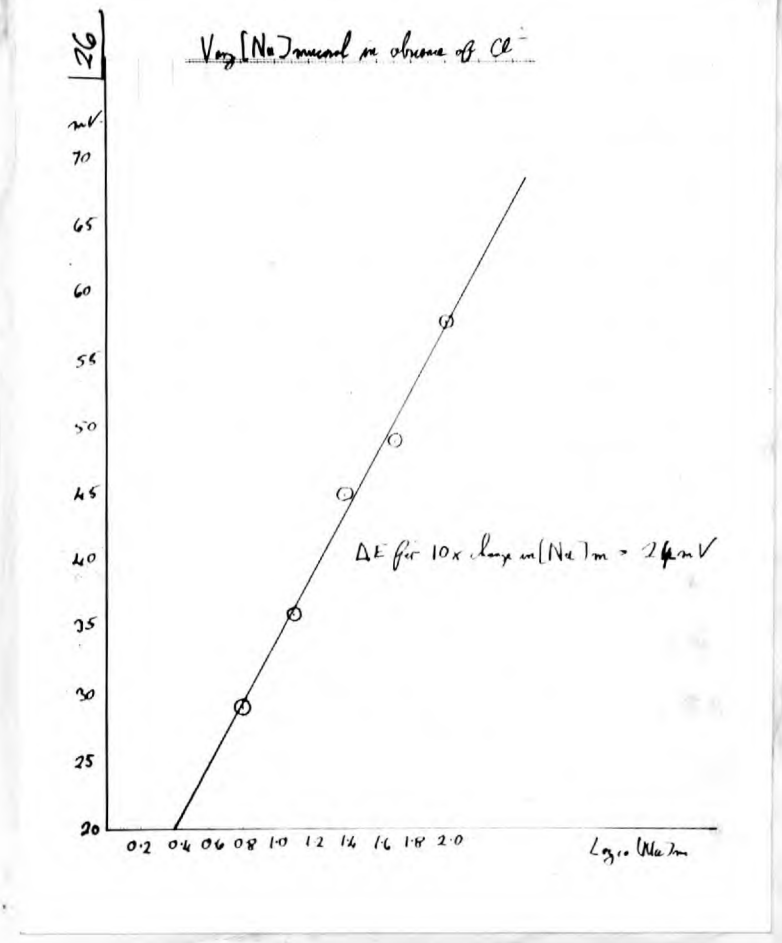


Fig.16. The gastric p.d. as a function of the Na^+ concentration on the mucosal side. Serosal and mucosal solutions were Cl^- free.

The gastric p.d. as a function of the Na concentration on the mucosal side.

Fig. 15 shows the effect on the p.d. of changing the mucosal Na concentration in the presence of chloride (154mM). It is seen that at the higher Na concentrations a linear relation exists between the p.d. and Na concentration and the slope of the line shows an increase in p.d. of 9.0mV for a 10 fold increase in Na concentration. If the transport number of Na^+ was unity under these conditions the p.d. would change by 60mV for a 10 fold change in Na concentration (see the equation 3); however it appears that Na is able to diffuse across the cell membrane on the mucosal side, but that other ion movements can also contribute to the current. The transport number for Na^+ is then equal to 9.0/60 i.e. the fraction of current carried by Na^+ across the cell membranes on the mucosal side is 0.15; which leaves 85% of the current carried by Cl^-.

The reduction in slope at the lower Na^+ concentrations was to be expected since other ions in the system become relatively more significant (see discussion). These results are typical for all such experiments.

Fig.16 shows the results of a similar experiment carried out with Cl^- free media on both sides. The straight line again indicates that Na is free to diffuse across the cell membranes on the mucosal side of the gastric epithelium but the greater slope under these condition indicates a

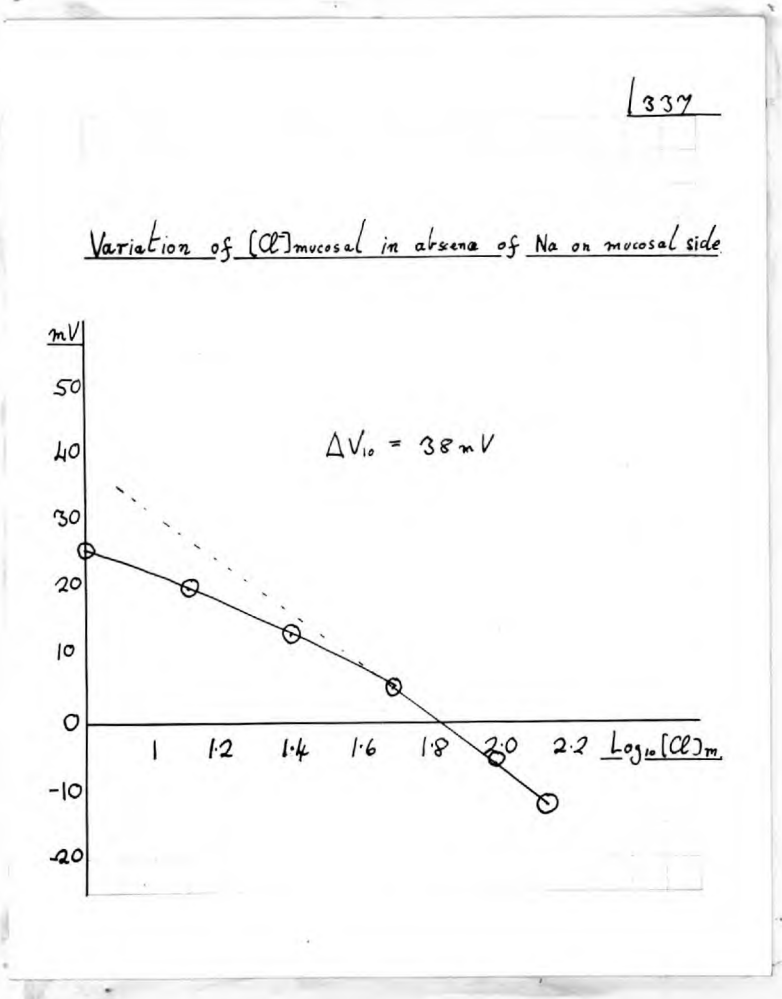


Fig.17. The gastric p.d. as a function of Cl^- concentration on the mucosal side in the absence of Na^+ on the mucosal side and Cl^- on the serosal side.

transport number for Na of 24/60 i.e. 40% of the current passing is carried by Na, the remaining 60% being carried by the methyl sulphate ion. Combining this result with the previous one the relative diffusibilities of Cl^- and methyl sulphate ions in the cell membranes on the mucosal side can be calculated as 85/60 i.e. the Cl^- ion is 1.41 times more mobile than the methyl sulphate ion in this system.

The gastric p.d. as a function of chloride concentration on the mucosal side.

Fig.17 shows the effect on the p.d. of replacing chloride by methyl sulphate on the mucosal side. The mucosal solutions were Na^+ free and the serosal solution was Cl^- free. It is seen that a linear relationship is obtained at the higher Cl^- concentrations, the slope of the line in this region showing a decrease in p.d. of 30mV for a 10 fold change in Cl^- concentration, which corresponds to a transport number of 0.63 for chloride relative to methyl sulphate i.e. the Cl^- ion is 1.58 times more mobile than the methyl sulphate ion. This value is in reasonable agreement with the result recorded in the previous experiment.

In this experiment, with Cl^- free serosal solution, it is to be noted that the p.d. becomes reversed at the higher mucosal Cl^- concentrations.

Fig. 18 shows the results of a similar experiment

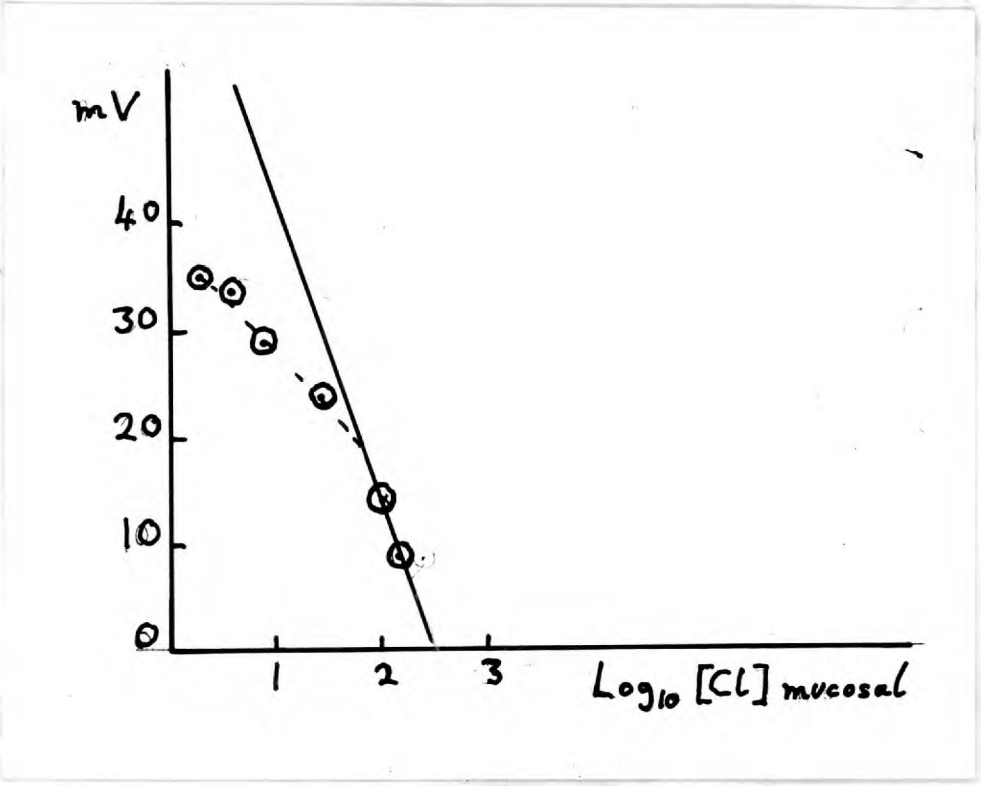


Fig.18. The gastric p.d. as a function of Cl^- concentration on the mucosal side. Cl^- was present on the serosal side and Na^+ was absent on the mucosal side.

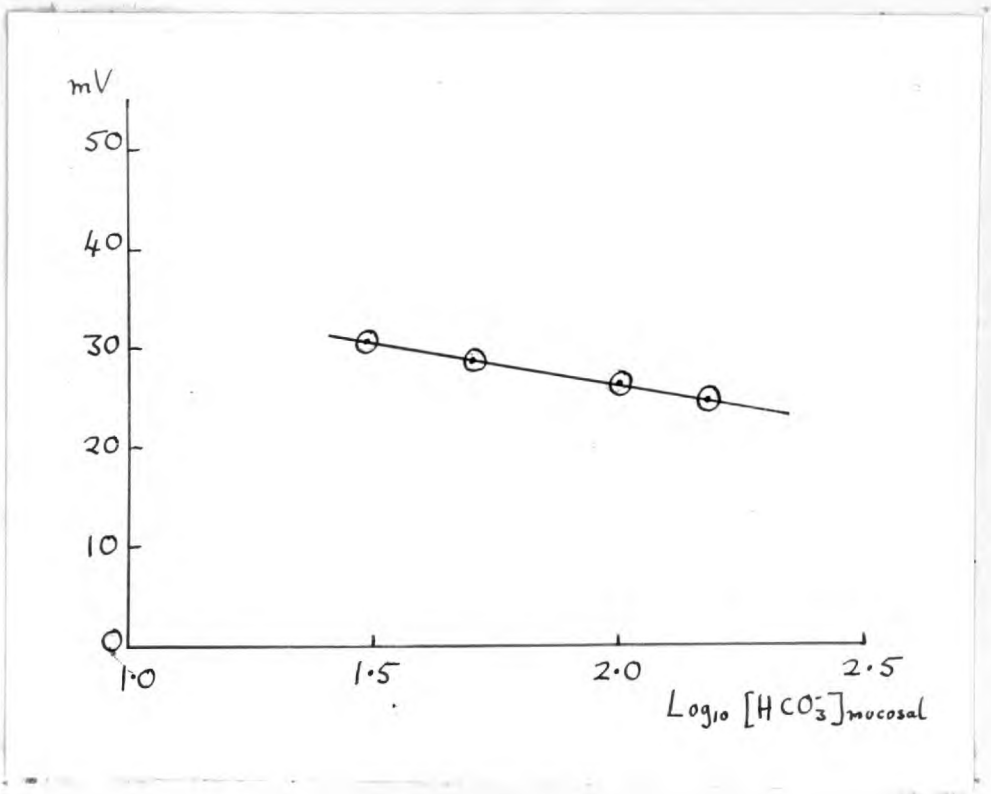


Fig.19. The gastric p.d. as a function of HCO_3^- concentration on the mucosal side. See text for details.

carried out with a normal chloride concentration on the serosal side. The slope at the higher Cl^- concentrations is virtually the same as before (37.5mV for a 10 fold change in Cl^- concentration) but no reversal of p.d. occurred this time.

The reduction in slope at the lower concentrations in both types of experiment is again predicted by the general equation, (see discussion).

The gastric p.d. as a function of the bicarbonate concentration on the mucosal side.

Fig. 19 shows the result of a single experiment in which the mucosal bicarbonate concentration was varied in a 28 day stomach. The serosal solution was bicarbonate Ringer containing chloride. On the mucosal side potassium methyl sulphate was substituted for $KHCO_3$.

It is seen that a linear relationship existed between the p.d. and the log of the mucosal bicarbonate concentration and that the slope of the line corresponded to a transport number of 8/60=0.133 for bicarbonate relative to methyl sulphate. From previous results the transport number for bicarbonate relative to chloride can be calculated at 0.133/1.41 = 0.095. The negative slope of the line helps to confirm that the changes in gastric p.d. resulted from changes in the bicarbonate diffusion potential at the cell membranes on the mucosal side.

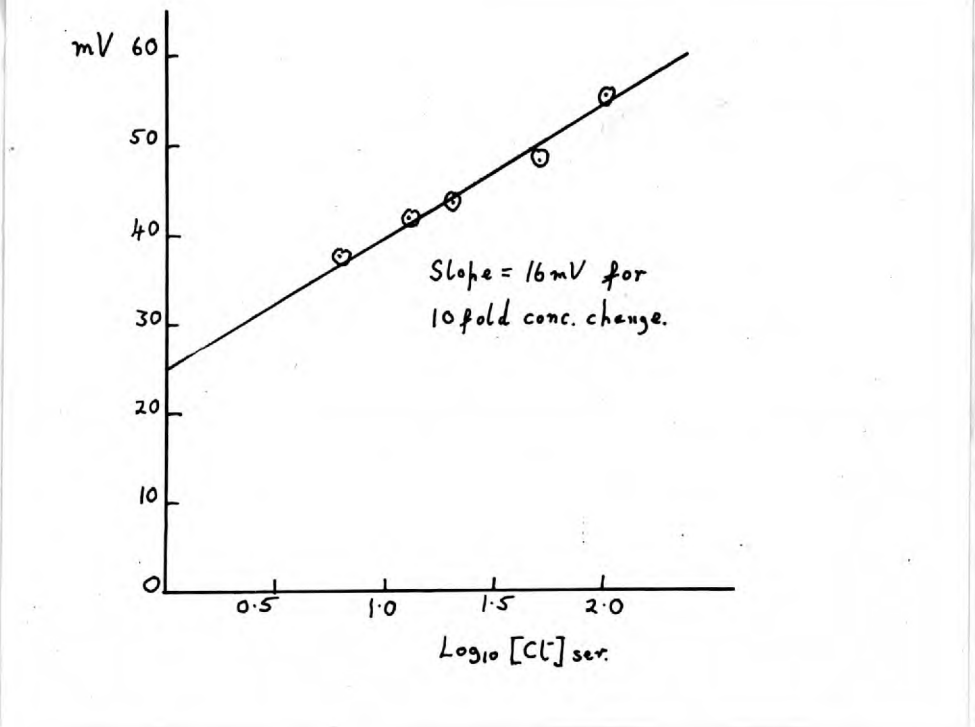


Fig.20. The gastric p.d. as a function of Cl^- concentration on the serosal side. The mucosal solution was Na^+ free.

The effect of changes in pH of the mucosal solutions on the gastric p.d.

In several experiments the pH of the mucosal solution was varied in steps between pH 1.0 and pH 7.4 by addition of HCl or H_2SO_4. Above pH 2.5 the p.d. was unaffected by changes in pH within this range. Below pH 2.5 the p.d. became irreversibly reduced or even abolished. All such experiments were carried out on stomachs of about 28 days and the mucosal solutions contained Na in some experiments. These results indicate that the mobility of H^+ ions in the cell membranes on the mucosal side is very low relative to those of the other ions in the system.

The gastric p.d. as a function of the chloride concentration on the serosal side.

Stomachs of 28 to 30 days were used as previously. The mucosal solutions were Cl^- free in all the experiments (5) and Na^+ free in three of them. The serosal solutions were bicarbonate Ringer with methyl sulphate substituted for Cl^- to varying extents.

In the three experiments free of Na^+ on the mucosal side a linear relation between the p.d. and the log of the serosal chloride concentration was seen and the line had a positive slope consistent with a Cl^- diffusion potential. The mean slope was 10mV for a 10fold change in serosal Cl^- concentration with a range of 7 - 16mV. Fig.20 shows the results of one of these experiments.

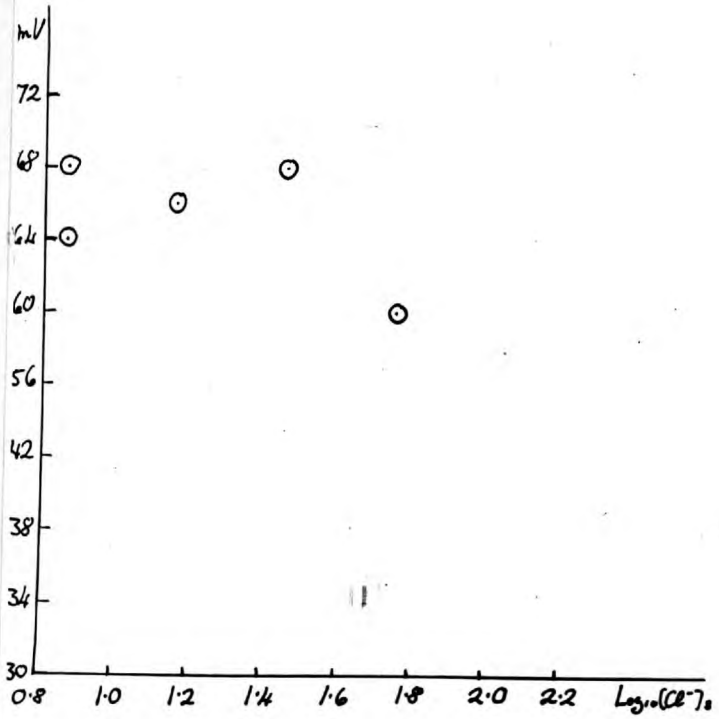


Fig.21. The gastric p.d. as a function of Cl^- concentration on the serosal side. The mucosal solution contained 154mM Na^+.

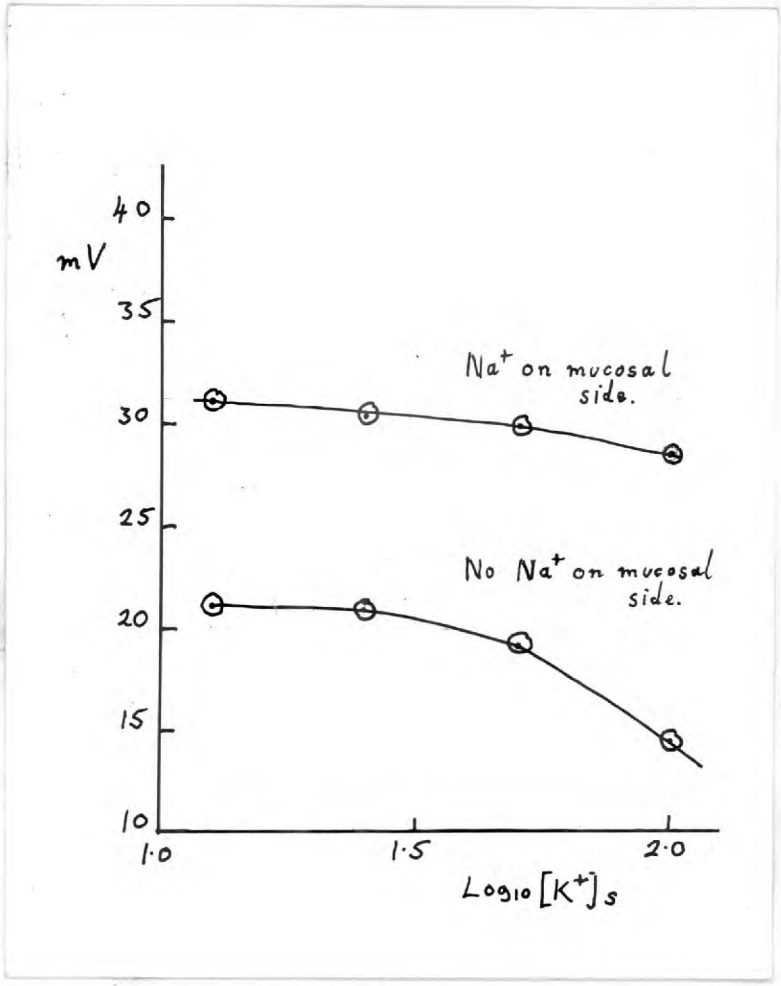


Fig.22. The gastric p.d. as a function of the K^+ concentration on the serosal side. The upper curve was obtained with Na^+ present on the mucosal side. The lower curve was obtained with Na^+ absent on the mucosal side.

In the two experiments in which the mucosal solutions contained Na^+ there appeared to be no relationship at all between the p.d. and the serosal chloride concentration. Fig.21 shows the results of one of these experiments.

The gastric p.d. as a function of the bicarbonate concentration on the serosal side.(1 experiment).

Changing the serosal bicarbonate concentration from 10 to 100 m.equiv/l in the absence of Cl^- on both sides and the absence of Na^+ on the mucosal side produced no change in the p.d.

The gastric p.d. as a function of the Na^+ concentration on the serosal side.(1 experiment).

Substitution of choline$^+$ for Na^+ on the serosal side (with a normal Cl^- on the serosal side and potassium methyl sulphate on the mucosal side) immediately raised the p.d. from 40mV to 46mV the p.d. remaining at this level for half an hour. A similar result is shown in Fig.13.

The gastric p.d. as a function of the K^+ concentration on the serosal side.(4 experiments).

Fig.22 shows the results of one of these experiments in which sodium methyl sulphate on the serosal side was replaced by potassium methyl sulphate in varying amounts. Both serosal and mucosal solutions were Cl^- free. The result shown is typical for all the experiments. It is seen that the curve has a negative slope which is greater and constant at the higher K^+ concentrations

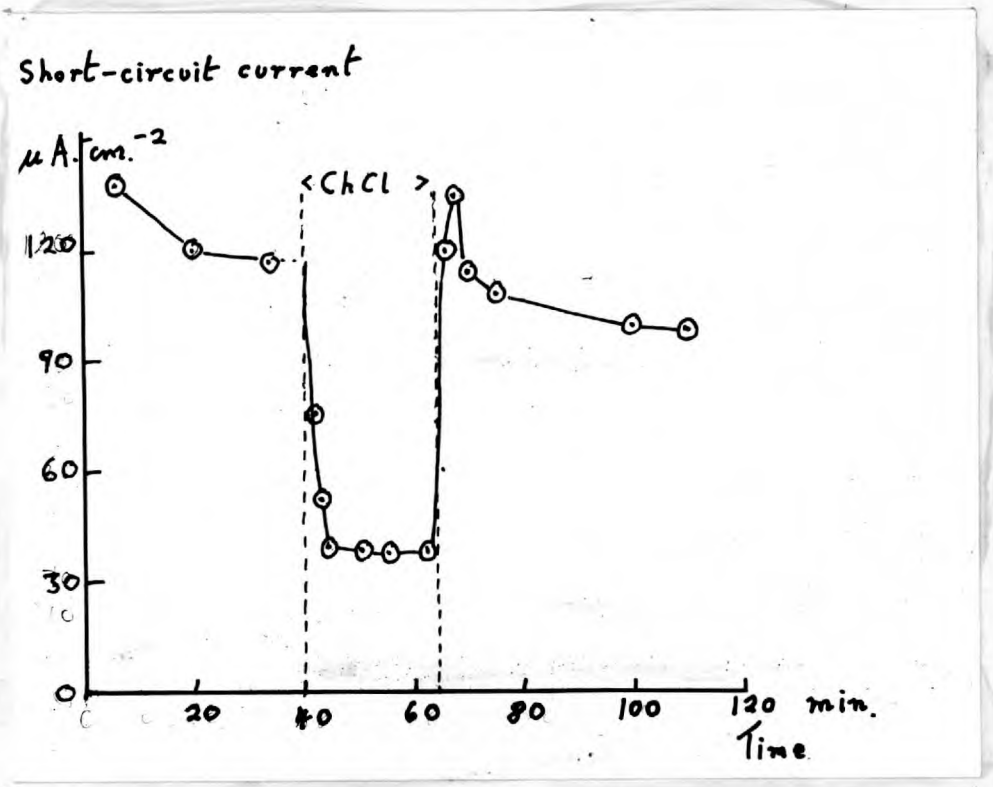


Fig.23. The relationship between short-circuit current and the presence of Na^+ on the mucosal side. Area of stomach = 0.293 cm^2. Between the dashed lines the mucosal solution was 154mM choline chloride. Outside the lines the mucosal solution was 154mM NaCl. The serosal solution was bicarbonate Ringer.

The maximum constant gradient of the curve obtained from two experiments which were free of Na^+ on the mucosal side was 14mV and 15mV for a 10 fold change in serosal K^+ concentration. In two experiments in which Na^+ was present on the mucosal side the maximum observed constant gradients were 9.0mV and 11.0mV for a 10 fold change in serosal K^+ concentration. In Fig.22 both curves were obtained from the same stomach: the other two curves were obtained from separate stomachs.

The short-circuit current of the foetal gastric mucosa.

The short-circuit current of the gastric mucosa was measured using the apparatus shown in Fig.14, a piece of stomach wall being set up as described previously. Current was passed through the preparation using the circuit shown so as to reduce the positivity of the serosal side until the p.d. was zero: the current passing under this condition is defined as the 'short-circuit current' and is equivalent to net rate of charge transport through the membrane (see page 14).

The experiments described in this section were carried out on stomachs of 27 to 30 days gestation age. The various solutions used were made up in the same way as described in the previous section and were used at 35°C.

The dependence of the short-circuit current on the presence of Na^+ on the mucosal side.

Fig.23 shows the result of replacing 154mM NaCl on the mucosal side by 154mM choline chloride; the serosal solution

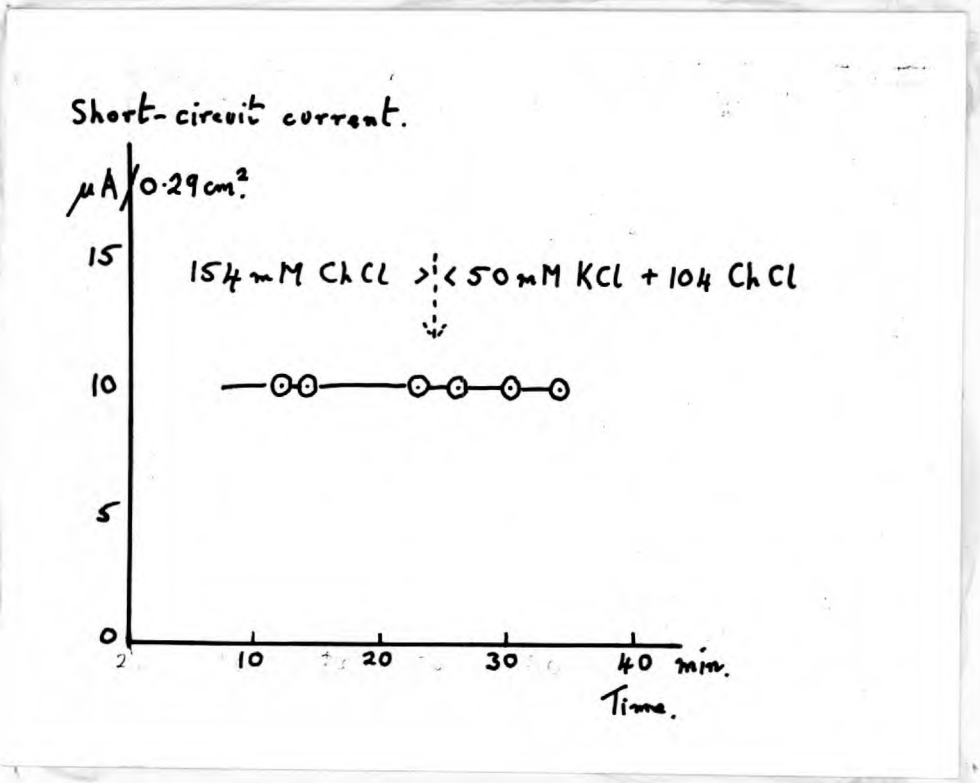


Fig.24. Showing no effect on gastric p.d. on partial replacement of choline chloride by KCl in the mucosal solution.

being bicarbonate Ringer. It was seen that as a result of this replacement the short-circuit current (s.c.c.) falls from 133 μ A cm^{-2} to 41 μ A cm^{-2}. The effect is reversed on replacement of Na^+ on the mucosal side, with a small 'overshoot' occurring during the reversal.

By making use of the Faraday F, (=96,500 coulombs) which is the amount of charge carried by one gm. ion equiv, the rate of passage of charge (current) through the membrane can be related to the ion fluxes occurring: thus 1 μ A is equivalent to an ion flux of 37.2 m.μ.equiv.hr^{-1}.

The above result can be explained on the basis of an active transport of Na^+ from the mucosal to the serosal side accounting for a s.c.c. of 133 - 41 μ A cm^{-2}. The remaining s.c.c. of 41 μA cm^{-2} can be explained on the basis of there being an active transport of Cl^- from the serosal side to the mucosal side. This interpretation would be in qualitative agreement with the direct chemical measurements of net ionic fluxes since the current passes in the direction of mucosa to serosa. An alternative explanation would be that the residual s.c.c. of 41 μA cm^{-2} would be a measure of a net flux of $choline^+$ from mucosa to serosa. This explanation is unlikely however, since replacement of 33% of the $choline^+$ on the mucosal side by K^+ has no effect on the s.c.c. (Fig.24) and since direct chemical measurements showed that no active transport of K^+ occurs in this system it appears that neither

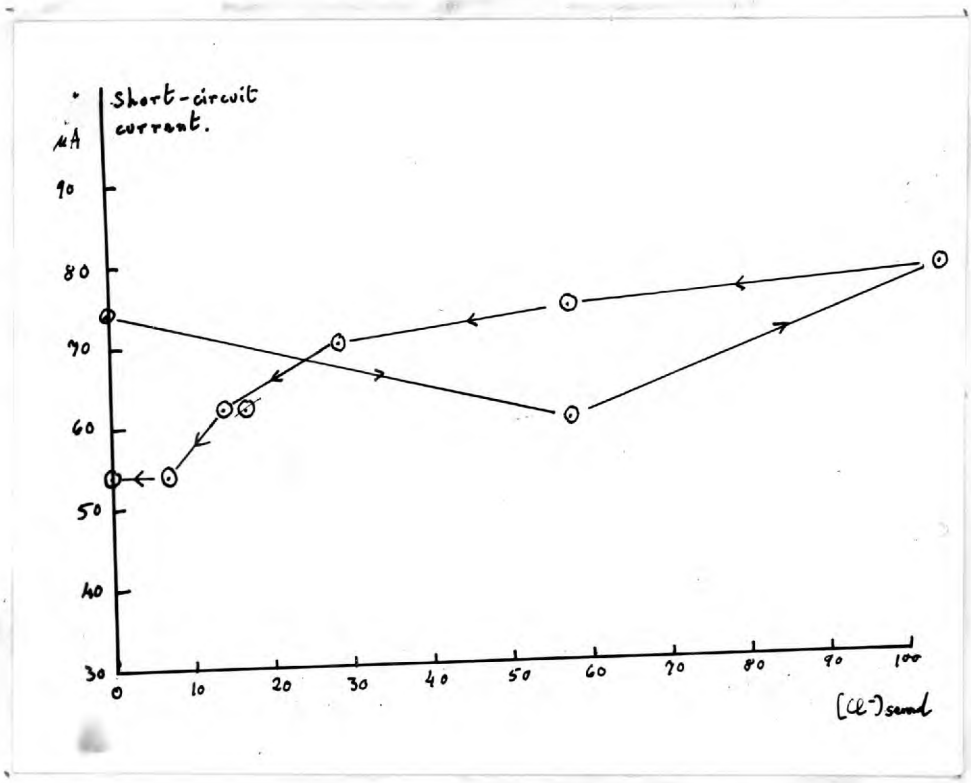


Fig.25. Short-circuit current (ordinate) and concentration of Cl⁻ (abscissa). Arrows indicate increases or decreases in Cl^- concentration. The mucosal solution was Cl^- free Ringer. Gestation age was 29 days. Area = 0.293cm^2.

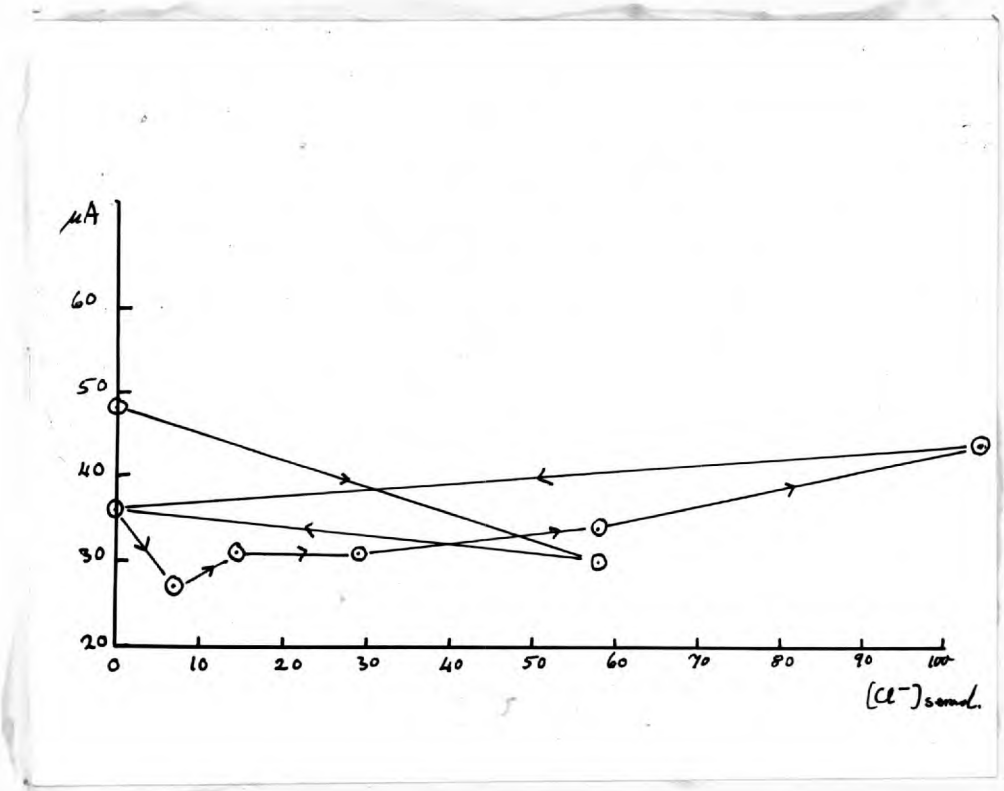


Fig.26. Same procedure as for Fig.25. Contation age days. Area = 0.293 cm^2.

K^+ or choline$^+$ ion movement can contribute to the s.c.c. to any significant extent: the residual s.c.c. of 41 uA cm^{-2} is therefore considered to represent anion (Cl^-) flux from serosa to mucosa.

To determine whether the residual s.c.c. resulting from a net anion flux from serosa to mucosa was specific for Cl^- (and perhaps the other halides) the s.c.c. was measured with Cl^- free media on both sides, methyl sulphate being substituted for Cl^-.

Fig.25 shows the result of an experiment on a stomach of 29 days with Cl^- free Ringer on the mucosal side. It was seen that the s.c.c.was not related in any direct manner to the Cl^- concentration on the serosal side although there appeared to be some hysteresis. However it was seen that the s.c.c. could be as high with methyl sulphate on the serosal side as it was with Cl^- on the serosal side. Fig.26 shows the result of an identical experiment carried out on a stomach of 25 days. It appears from these results that no part of the s.c.c. was due to a specific active transport of Cl^- from serosa to mucosa but the residual s.c.c. was due to a non specific active anion transport from serosa to mucosa.

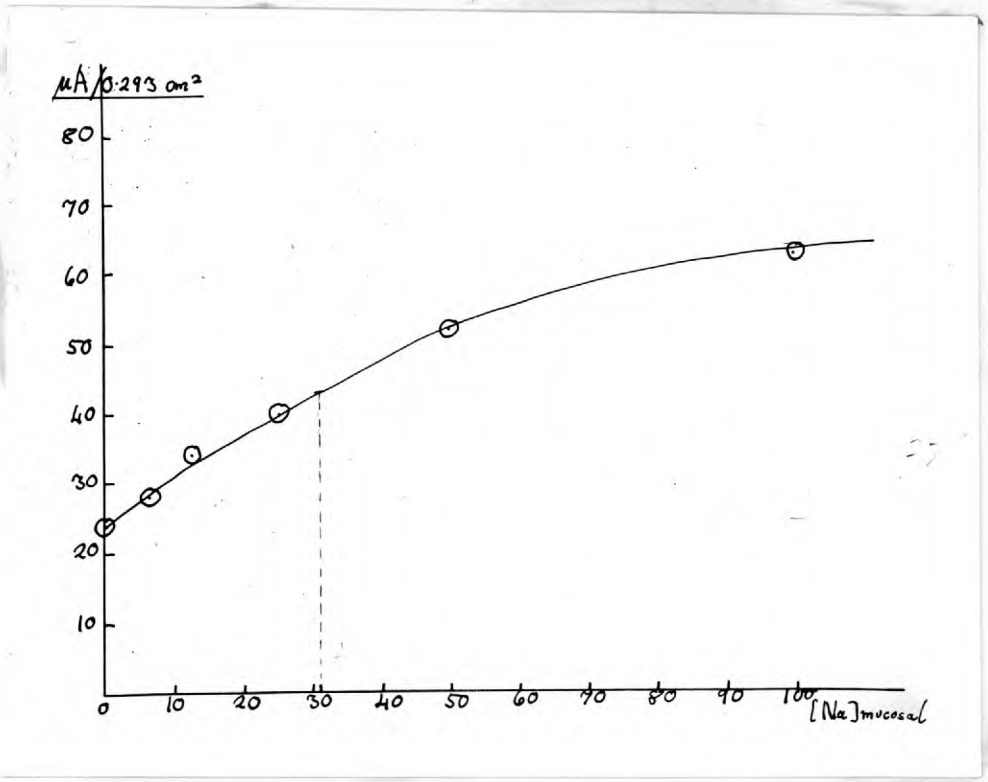


Fig.27. Short-circuit current as a function of Na^+ concentration on the mucosal side.

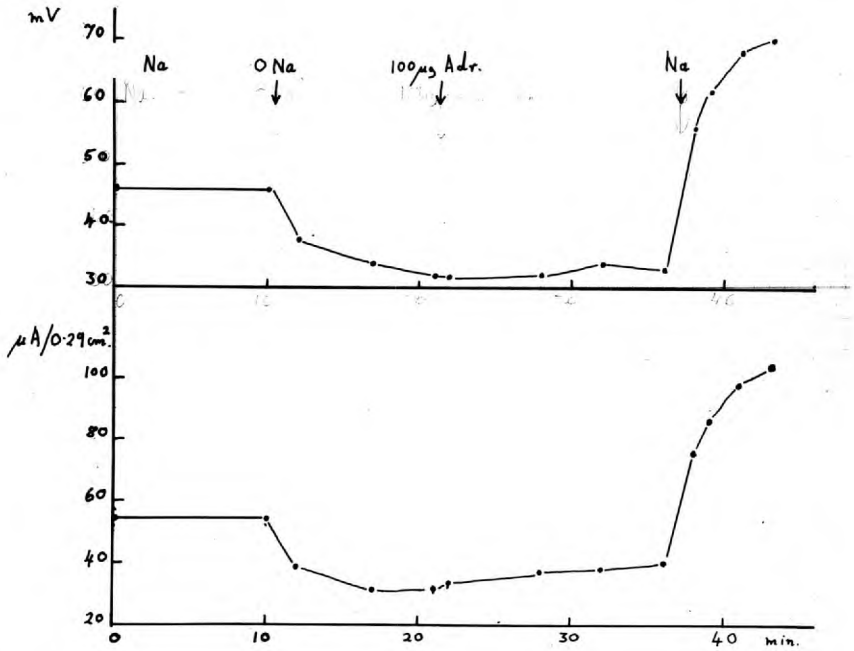


Fig.28. Effect of adrenalin on short-circuit current of a 28 day stomach. See text for details.

The short-circuit current as a function of the Na^+ concentration on the mucosal side.

Fig.27 shows the result of an experiment carried out on a stomach of 30 days under Cl^- free conditions. Sodium methyl sulphate was substituted for potassium methyl sulphate on the mucosal side. It is seen then that the s.c.c. increased from 82 $\mu A\ cm^{-2}$ with no Na^+ on the serosal side to 212 $\mu A\ cm^{-2}$ with 100 m.equiv/l Na^+ on the mucosal side, this being a asymptotic value. It is also seen that half the maximum increase in s.c.c. occurred at a Na^+ concentration of 31m.equiv/l on the mucosal side: this value was obtained in all the experiments of this type (12 experiments).

The effect of drugs on the s.c.c.

Adrenalin.

The effect of adrenalin on seven stomachs of 27-30 days gestative age was investigated under Cl^- free conditions on both sides. In two of the experiments it was possible to determine separately the effect of adrenalin on the two components of the s.c.c. (the one due to anion transport, the other due to Na^+ transport). Fig.28 shows the results of one of these experiments on a stomach of 28 days. The s.c.c. was measured first in the presence of Na^+ on the mucosal side then in its absence and from the two values the anion and cation transport components were reported. Adrenalin hydrochloride (100 μg) was added to both sides in the absence of Na^+ on the mucosal

Expt.No.	Age (days)	Before Adrenalin p.d. (mV)	Before Adrenalin s.c.c. /uA.0.293cm^{-2}	After Adrenalin p.d. (mV)	After Adrenalin s.c.c. /uA.0.293cm^{-2}	mV % (cation)	mV % (anion)	s.c.c. % (cation)	s.c.c. % (anion)	Soln.
41	30	22	22	24	27	-	+9	-	+23	-Na
37	28	43 32	42 32	64 33	94 40	+282	+3	+540	+25	-Na
42	30	36	36	36	38	-	0	-	+5.5	-Na
40	30	29 57	34 64	31 58	43 78	+2	+7	+22	+26	-Na
25	27	26	27	26	28	-	0	-	+4	-Na
335	30	32	45	48	108	+50		+240		
36	28	25	18	30	30	-	+20	-	+67	-Na

Table 1.

Effect of adrenalin (100/ug) on anion and cation components of p.d. and s.c.c. The extreme right hand column indicates when Na was absent on the mucosal side. All solutions were Cl^- free.

side: after the s.c.c. reached a new steady value Na^+ was added to the mucosal side and a further increase in s.c.c. observed. It is seen that the anionic component increased in the presence of adrenalin from 32 to 40 μA - an increase of 25%, whilst the open circuit p.d. increased from 32mV to 34mV - an increase of 6.2%. The Na component increased from 54-32=22 μA to 104-40=64 μA - an increase of 190%. The open circuit p.d. associated with Na transport increased from 46-32=14mV to 70-34=36mV - an increase of 157%.

The results of the seven experiments are summarised in Table 1. It is seen that adrenalin can stimulate both components of the s.c.c. It is interesting to note that whereas the effect of adrenalin was greatest on the Na^+ current in experiment 37, the effect was greatest on the anion current in experiment 40. The % increase in s.c.c. was always greater than the % increase in p.d.

Adrenalin appeared to be effective whether added to either the serosal or mucosal side.

Neurohypophysial extract (Pituitrin).

0.3 units of Pituitrin (Parke-Davis) was added to the serosal solution (4.0ml in volume) in 3 experiments. The open circuit p.d. and s.c.c. were measured in two of the experiments whilst in the other only the open circuit p.d. was measured. The results are summarised in Table 2. It is seen that in the experiment with Na^+ and Cl^- on the mucosal

Expt.No.	Age (days)	Before Pituitrin p.d. (mV)	Before Pituitrin s.c.c. (μA.0.293cm^{-2})	After Pituitrin p.d. (mV)	After Pituitrin s.c.c. (μA.0.293cm^{-2})	mV (%)	s.c.c. (%)	
265	29	15	33	16	34	+6.7	+3	
335	30	48	92	43	72	-10.4	-21.8	$-NaCl_m$ $-Cl_s$
293	30	22	-	23	-	+5	-	

Table 2.

Effect of Pituitrin (0.3 units), added to serosal side, on p.d. and s.c.c. Extreme right hand column indicates when Na and Cl were absent on mucosal or serosal sides.

(and serosal) side 1 administration of Pituitrin is followed by an increase in open circuit p.d. and s.c.c., the percentage increase in open circuit p.d. being the greater of the two: there was therefore an increase in resistance of the preparation also.

In the experiment with Na^+ and Cl^- absent on the mucosal side the s.c.c. and open circuit p.d. both decreased after addition of Pituitrin, the percentage reduction in s.c.c. being the greater of the two: there was therefore an increase in resistance of the preparation.

In the third experiment with Na^+ and Cl^- on the mucosal side the open circuit p.d. increased on addition of Pituitrin to the serosal side.

Histamine.

Histamine acid phosphate (0.5mg) was added to the serosal solution in 3 experiments. The results are summarised in Table 3 where it is seen that in the two experiments with Na^+ present on the mucosal side there was a decrease in s.c.c. and an increase in resistance following administration of histamine whilst the open circuit p.d. increased in one case and was reduced in the other.

In the experiment in which Na^+ was absent on the mucosal side there was an increase in s.c.c. and a fall in resistance and open circuit p.d. following administration of histamine.

Expt.no.	Age (days)	Before Histamine p.d. (mV)	Before Histamine s.c.c. (μA.0.293cm^{-2})	After Histamine p.d. (mV)	After Histamine s.c.c. (μA.0.293cm^{-2})	mV %	s.c.c. %	
335	30	43	72	38	58	-11.6	-19.5	$-Cl_s$
265	29	15	32	16	28	+6.7	-12.5	
5	29	24	29	22	30	-8.3	+3.5	$-Cl_s$ $-NaCl_m$

Table 3.

Effect of Histamine acid phosphate (0.5mg), added to serosal solution, on p.d. and s.c.c. Extreme right hand column indicates absence of Na or Cl on serosal or mucosal sides.

Discussion

The previous section has shown that the stomach of the rabbit foetus may be studied in vitro over a period of several hours with a greater degree of experimental control than would be possible in vivo. The only reason for experiments not having been carried out on stomachs of less than 20 days gestation age was that the techniques of dissection, mounting and chemical analysis available at that time were too cumbersome to be used on these stomachs, bearing in mind that the volume of the lumen of a 20 day stomach is less than 0.1 c.c.

As far as the author is aware, the only other mammalian stomach on which it has been found possible to make experiments in vitro is the mouse stomach (Crane and Davies 1948). The author has found that stomachs of small adult rats, foetal guinea pigs and rabbits more than one day post partum are unable to maintain a spontaneous p.d. for more than a few minutes under in vitro conditions. Furthermore, the author has found that if stirring of the solution in which an adult mouse stomach is suspended is stopped, the spontaneous p.d. declines immediately. If this procedure is carried out on a foetal rabbit stomach the onset of the decline in p.d. is delayed for 2-3 minutes. It appears then that it is impossible to provide adequate oxygenation for most mammalian stomachs in vitro, although the mouse stomach is at a critical point where adequate oxygenation is just achieved: the

foetal rabbit stomach can survive under slightly less critical conditions. It is suggested that adequate oxygenation depends on the muscle and connective tissue layers on the serosal side not forming a diffusion barrier to oxygen above a critical level: these layers are certainly thin, relatively, in the mouse and foetal rabbit stomachs.

In the case of the foetal stomach it is also possible that greater use is made of anaerobic energy sources than occurs in the adult: as is well known with other foetal tissues (Shelley 1961).

The chemical measurements of net transfers of water and electrolytes have shown that the rabbit foetus's stomach has an absorptive function during the last third of gestation. Since water movement was shown to follow solute movement i.e. it was down an osmotic gradient, and since at all ages studied there was a net absorption of Na^+ followed by passive movement of Cl^- in order to preserve electroneutrality, it is apparent the active transport of Na^+ is the prime mover in the absorptive process.

On dissection all stomachs contained Na^+ (see appendix 1); it therefore seems reasonable to suggest that the absorptive process is occurring in utero.

By measuring the area under the solid curve in Fig.12 the volume of fluid which the stomach can absorb from the

22nd day until full term under in vitro conditions, with 154mM NaCl inside, can be calculated and comes to 13.5ml. The maximum volume of amniotic fluid is about 8.0ml at 26 days, falling to about 1.0ml at full term. Since all the stomachs were filled to varying degrees on dissection, it appears that there is, in utero, a continual absorption and formation of amniotic fluid, as has often been postulated, but with the stomach playing a highly significant role in this process.

As well as a decline in the volume of amniotic fluid towards term it can be seen by a study of the concentration of the principal electrolytes in the fluid (Appendix ii), that the amount of these present also diminishes. Again it seems that the stomach may play a major role in the absorption of these. It seems reasonable to postulate that the stomach plays a major part in the absorption of these electrolytes which may then be assimilated into the foetal body fluids. The amniotic fluid may act as a reservoir from which this assimilation may occur at a rapid rate towards term with the minimum disturbance to the water and salt balance of the mother.

It was shown that the foetal rabbit stomach is able to secrete HCl during the latter stages of gestation. The fact that the onset of acid secretion occurs on the 23rd day which

is the same time that the oxyntic cells appear (Menzies 1958) is of considerable significance in that it provides direct evidence in favour of the classical view that HCl is secreted by these cells. It should also be emphasised that the evidence presented indicates that both hydrogen and chloride ions are secreted by the same cells: this is a matter on which, with regard to the adult stomach, some doubt had existed in the past. The results showing the relation between net solute transfer and water transfer indicate that the oxyntic cells secrete HCl as an isotonic solution, the water movement being passive.

It is doubtful whether the HCl secretion is of any significance in utero, since the pH of the gastric contents in the foetus was seldom below 5.0 due to buffering by the mucus which was present. The mucus may be highly significant in that it allows the oxyntic cells to develop without their producing a low pH which may be harmful under the in utero conditions.

A continuous secretion of HCl in utero will of course in the first instance produce a metabolic alkalosis in the foetus which must be compensated for. There would appear to three principal means by which compensation could occur. The first of these would be by reabsorption of the secreted HCl lower down in the gut. However, since most of this HCL is

buffered by mucous it would be necessary for the mucous to be broken down in this region so liberating hydrogen ions free for absorption. Alternatively, a high pH (7.4) in this region produced by a 'secretion' of $KHCO_3$ or $NaHCO_3$ would liberate hydrogen ions and enable the reaction $NaHCO_3+HCl=NaCl+(H_2O+CO_2)$ to take place with perhaps absorption of NaCl following. A secretion of $NaHCO_3$ into the intestine would counteract the metabolic alkalosis caused by the gastric secretion and the consequent absorption of NaCl would alleviate any net loss of electrolyte during the process. Whether the intestine of the rabbit foetus is capable of absorbing NaCl remains to be demonstrated. However, Nixon and Wright (1961) (appendix iii) have shown that Na^+ and Cl^- ions can be rapidly absorbed from the intestine of the sheep foetus from 100 days gestation age until full term (145 days).

A second means of compensating the metabolic alkalosis resulting from HCl secretion may be by a renal excretion of Na^+ and conservation of Cl^- with a resulting alkaline urine. However, it is well known that the foetal urine of many species is acidic in utero (Nixon and Alexander 1961). It therefore seems that the foetal kidney is not being concerned with compensation for gastric acid secretion: the compensation must have occurred elsewhere.

The third site at which compensation could occur is the

placenta, where an exchange of Cl^- from the mother for HCO_3^- from the foetus would be effective. However, there is evidence that bicarbonate ions are not readily transferred across the placental barrier(Blechner et al 1960). Furthermore, Huggett, Britton, Nixon and Wright (1959) have shown that the sheep foetus can remain in a state of acidaemia for several hours (as a result of lactic acid concentration produced by infusion or by asphyxia) while the composition of the maternal blood remains normal. It thus seems likely that the placenta can only contribute to the foetal acid-base balance by transfer of CO_2 and also of Na^+ and K^+ which have been shown to exchange rapidly between maternal and foetal compartments (Plentl 1958).

The finding that active absorption of Na^+ occurs before the 23rd day when the mucosa consists only of non differentiated cells (Menzies 1958) indicates that these cells are concerned with active transport of Na^+. When the oxyntic cells appear active HCl secretion is superimposed on the absorption. However, the absorptive process, with 150mM NaCl on the mucosal side, is considerably larger than the secretory process.

The only previous finding of active transport of Na^+ in any gastric mucosa is that of Bornstein, Dennis and Kohn (1959) who found an active absorption of Na^+ from the resting dogs stomach. However, in this case it is not known which cells are responsible for this function; and since there are no

non differentiated cells in the adult gastric mucosa (Menzies, personal communication). From the results presented in this thesis it would appear that the oxyntic cells are unlikely to be absorbing Na^+ therefore leaving the possibility that the peptic and or mucous neck cells are associated with active Na^+ absorption, which seems quite likely since the capacity for active Na^+ transport exists before differentiation it might well continue afterwards. To resolve this problem in the adult it would be necessary to study Na^+ transfers across segments of the stomach containing greater or lesser proportions of peptic cells relative to mucous neck cells. An exchange of Na^+ for H^+ by the oxyntic cells as suggested by Hirschowitz (1961) seems unlikely however, since it has been seen in this thesis that hydrogen secretion continues in the absence of Na^+ on the mucosal side.

The pattern of electrolyte transfers in the post 23 day foetal stomach can be explained along the same lines as those used by Bornstein et al to explain their results, in the case of their first theory (anion and cation pumps in the mucosa), but not in the case of their second theory (anion pump only; see page 11). This is because the p.d. before the 23rd day is entirely and reversibly dependent on the Na^+ concentration on the mucosal side. Further, Na^+ is actively absorbed: thus at this stage there is a cation 'pump'. After the 23rd day the p.d. is greatly increased in magnitude with Na^+ on the

mucosal side; active anion secretion occurring concurrently. It would appear then that after the 23rd day there are both anion and cation 'pumps' and that the undifferentiated cells are associated with the latter and the oxyntic cells with the former. The present results do not preclude the possibility of net transfers of NaCl down its gradient of chemical potential by some means.

The results presented do not yeild any new information about the mechanism of the well known secretion-rate dependent variations in electrolyte composition of gastric juice. However, it should be noted that neither the component theory of Pavlov (1910) nor the diffusion theory of Teorell (1933) takes into account the presence of a Na^+ absorbing mechanism in the gastric mucosa. Investigation of the rate dependence of foetal gastric fluid composition should throw more light on this problem especially since the peptic cells are not present until after birth in the rabbit (Menzies 1958).

It has been known for many years that the concentration of K^+ is three or four times higher in gastric juice than in plasma. This fact has led many writers to refer to a 'secretion' of K^+ into the gastric lumen. As far as this author is aware however, the difference in concentration of K^+ plasma and gastric juice, or rather the ratio of the concentrations, has not been considered in relation to the difference of electrochemical potential of this ion in plasma

and gastric juice. The results presented in this thesis have shown that the K^+ concentration tends to rise in the gastric juice above the plasma concentration under normal conditions: but no net movement of K^+ can take place against a gradient of electrochemical potential. It would therefore be incorrect to refer to K^+ 'secretion' in the foetal stomach if the word 'secretion' is meant to imply the participation of an active transport process.

Typical values quoted for the concentration of K^+ in adult gastric juice and plasma in many mammals are 15.0 and 5.0 m.equiv./kg water respectively, with a p.d. within the usual range 40 -60mV (serosa + ve). Using these values in the Nernst equation it is seen that the gradient of electrochemical potential is downwards going from plasma to gastric juice: there is therefore no evidence suggesting the existence of K^+ 'secretion' in the adult. It would be of value to reverse the direction of the gradient of electrochemical potential in the adult to see if there was still a net transfer of K^+ into the gastric juice.

The directions of net active transport of Na^+ and Cl^- observed in the foetal stomach, along with the direction of H^+ active transport enable the transport functions of the foetal gastric mucosa to be described in terms of the circuit shown in Fig. 29a before the 23rd day; and in terms of the two alternative circuits shown in Fig. 29b and 29c in the case of

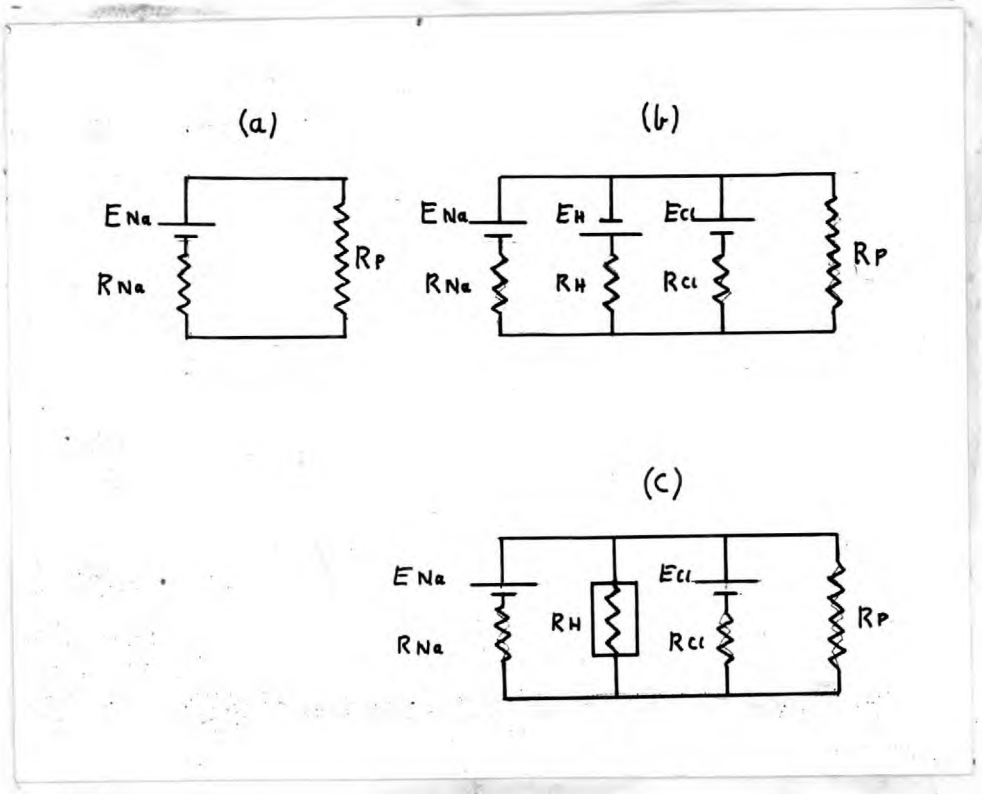


Fig.29. Equivalent circuits for foetal gastric mucosa. a) before 23rd day. b) and c) alternative circuits for post 23rd day stomach. In b) there is considered to be a H^+ pump present whilst in c) the energy for H^+ transport is derived from E_{Na} and E_{Cl}, with R_H capable of providing a very low resistance pathway for hydrogen ions. See text.

the post 23 day stomach. E_{Na}, E_H and E_{Cl} represent the E.M.F.s of the Na^+, H^+ and Cl^- active transport systems respectively. R_{Na}, R_H and R_{Cl} represent the internal resistance of the respective systems and R_p represents the resistance of the path for 'passive' ions.

If the power necessary for H^+ secretion is derived from the E_{Cl}, producing current flow through the H^+ path, the circuit in Fig. 29c is applicable. Whether or not this is so depends on the value of the resistance R_H which cannot be measured directly. If R_H is not very small then Fig. 29b will apply, with E_H a H^+ 'pump' coupled to metabolic free energy sources.

Equations attempting to describe electrochemical diffusion through membranes have been used by biologists to determine the 'permeability' of the membrane to the penetrating ionic species and also the transport numbers of the ions present in the system. Until recently the method has been to write down an equation for 'flux' or current flow due to movement of a particular ion i of the form

$$\phi i = - u^i . c^i . \frac{d\,u}{dx} \qquad \text{(Teorell 1951)} \quad \ldots\ldots(4)$$

where ϕi is the flux of species i through unit area of a particular membrane along a normal to it,

u^i is the mobility of i in the membrane (velocity / force),

c^i the concentration of i in the membrane,

and $\frac{d/u^i}{dx}$ the gradient of chemical potential of i in the membrane.

The chemical potential μi is then split into two parts, one associated with the electric field $\frac{dV}{dx}$ in the membrane and the other with the thermal energy of the ions in the membrane i.e. RT ln Ai where Ai is the activity of i. Multiplying both sides of the equation by the valency Zi of i gives an expression for the current carried by i through unit area of the membrane.

For the equation to be useful it must be integrated over the thickness x of the membrane. This requirement produces mathematical difficulties which can only be overcome by assuming a constant electric field in the membrane $(\frac{d^2V}{dx^2} = 0)$ (Goldman 1943) or a constant concentration gradient or better still a constant activity gradient, in the membrane i.e. $\frac{d^2(\ln Ai)}{dx^2} = 0$. (Teorell 1951, Linderholm 1952). There are no means of determining which, if either, of these assumptions is valid and therefore the integrated equations are only of limited value. However, when there is no current flow i.e. the membrane is on open circuit; integration of the flux equation reduces to the simple Nernst equation in both cases when only one mobile ion species is present.

When more than one ion species is present, all of which have numerically equal valency, +Z or -Z, the constant field equation integrates across the membrane thickness to

$$E_{1\rightarrow 2} = \frac{RT}{ZF} \ln \left[\frac{\sum_i Pi\, Ai_2}{\sum_i Pi\, Ai_1} \right] \qquad \text{.....(5)}$$

where P_i is the permeability coefficient of the membrane to the i^{th} ionic species and Ai_2 is the activity of the i^{th} cation on side 2 or the i^{th} anion on side 1. Equation 5 applies only when there is no net current flow through the membrane. Hodgkin and Horowicz (1959) point out that this equation is more general than its derivation implies and is not itself subject to the constant field restriction.

It is to be noted that the permeability coefficients are related to the mobilities u (diffusion constants) of the ions in the membrane as follows

$$P_i = \frac{u_i}{x_i}$$

and have the dimensions of velocity.

It can be shown that the transport numbers of two ionic species a and b in a membrane system are related to the permeability coefficients P_a and P_b as follows:

$$\frac{P_a}{P_b}\frac{a^+_1}{b^-_2} = \frac{t_a}{t_b} \qquad \text{.....(6)}$$

if a is a cation and b an anion (Hodgkin and Horowicz 1959).

The experiments in which the p.d. was studied as a function of the Na^+ concentration on the mucosal side showed that the cell membranes of the gastric epithelium on this side were significantly permeable to Na in a 'passive' sense. The fall in slope at the lower Na concentrations can be accounted for by other cations in the system contributing

to the p.d. Thus from the constant field equation

$$E_{i \to m} = \frac{RTt\ln}{F} \left[\frac{Na^+_m + \alpha C^+_m}{Na^+_i + \alpha C^+_i} \right] \quad \text{.....(7)}$$

Hodgkin & Horowicz (1959)

when t is the transport number of Na in this system,

$_{i}E_{m}$ is the electric potential of the cell interiors with respect to the mucosal solution,

C^+ is the concentration of a cation adding a contribution to the p.d.

and α is equal to $\frac{P_{C^+}}{P_{Na^+}}$

It is probable that in these experiments choline$^+$ is able to penetrate the cell membranes of this system and therefore this ion may be equivalent to C^+ in equation 7.

It is interesting to note that there was no decline in slope at the lower Na concentrations when this procedure was carried out with methyl sulphate substituted for Cl^-.

When the Cl^- concentration was changed on the mucosal side it is seen that the system behaves , at the higher Cl concentration, in the simple manner predicted by equation 3. At the lower Cl^- the decline in slope may be described by an equation similar to 7, with methyl sulphate contributing to the p.d. The dotted line in Fig.17 shows the result obtained if $_{i}E_{m}$ is plotted against $\ln\left(\left[Cl^-\right]_m + \alpha\left[MeSO^-_4\right]_m\right)$, where the subscripts m refer to mucosal concentrations and α

$$= \frac{P_{MeSO_4^-}}{P_{Cl^-}} = 0.63$$

At the higher Cl^- concentrations on the mucosal side the transgastric p.d. becomes reversed in the absence of Cl^- on the serosal side.

When the Na^+ or Cl^- concentrations were changed on the serosal side there was very little change in p.d. observed, and therefore neither of these ions appeared to have a significant role in determining the p.d. when present on this side.

The reversible decrease in p.d. when the K concentration was raised on the serosal side indicated a simple passive permeability to K which enabled the variation of p.d. to be described by Equation 3 at the higher K^+ concentrations by Equation 7 at the lower concentrations.

At the gestation age at which the stomachs were used for these experiments designedto analyse the p.d., the mucosa is made up largely of non differentiated cells and a very small proportion of oxyntic cells (Monsies 1958). Since the p.d. is absent when Na^+ free solutions are present on the mucosal side of pre 23 day stomachs (no oxyntic cells present) it is tentatively assumed that most changes in p.d. occurring in the later stomachs when Na^+ is absent on the mucosal side are associated with the oxyntic cells. When Na^+ is present on the mucosal side it is postulated that the observed changes

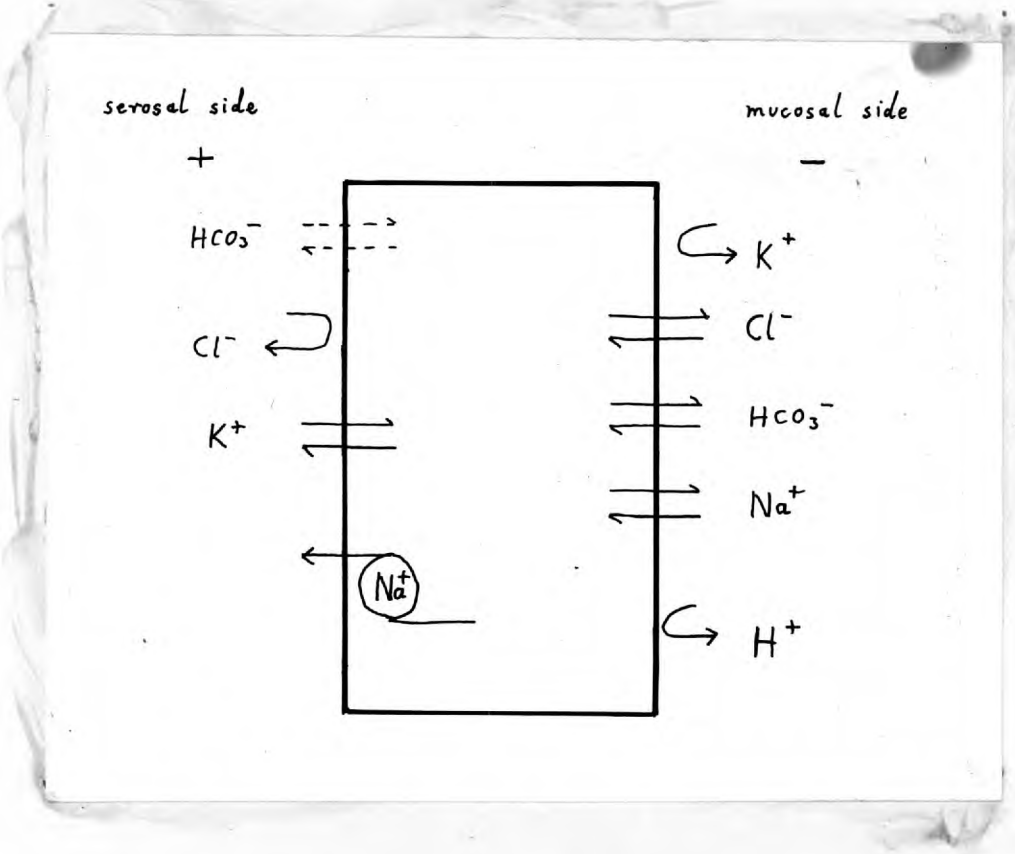


Fig.30. Scheme for ion transfers across non differentiated cells. A Na^+ pump is present on the serosal side. Dashed arrows indicate low transport numbers, solid arrows indicate high transport numbers, bent arrows indicate non penetration.

in p.d. are associated with the non differentiated cells since these form the majority in the cell population being considered (see appendix iv).

The results obtained from the analysis of the p.d. in terms of relative ionic mobilities in membranes can be fitted into a scheme analogous to that used by Koefoed-Johnsen and Ussing (1958) to describe the p.d. across living frog skin (see page 19).

Fig 30 shows this scheme applied to the non differentiated cells in the light of the evidence presented in this thesis. It is assumed that from a functional standpoint these cells form a sheet one cell thick. The mucosal side of these cells appears to be permeable to Na^+, Cl^-, HCO_3^- and (relatively) impermeable to K^+ and H^+. The serosal side of these cells appears to be permeable to K^+ and impermeable to Cl^-. It is also suggested that there is a Na^+ transport mechanism associated with the serosal membrane which transports Na from the cell interior to the solution on the serosal side.

Fig. 31 shows the scheme applied to the foetal cryptic cells. The cell membranes on the mucosal side are considered to be permeable to Cl^- (and perhaps Na^+ and HCO_3^-) but impermeable to K^+ and H^+. The serosal membranes are considered to be permeable to Na^+, K^+, Cl^- and impermeable to HCO_3^-. A metabolic Cl^- transport mechanism is thought to be associated with the cell membranes on the serosal side, and a metabolic

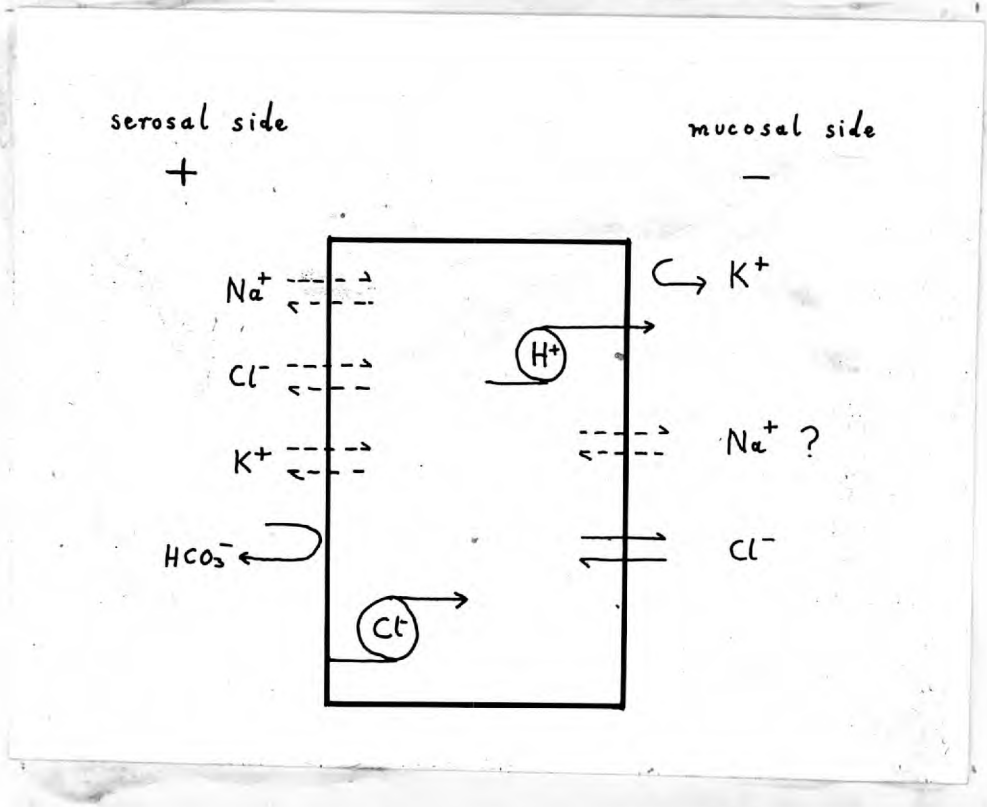


Fig.31. Scheme for ion transfers across foetal oxyntic cells. A H^+ pump is present on the mucosal side and a Cl^- pump is present on the serosal side. Dashed arrows indicate low transport numbers whilst solid arrows indicate high transport numbers. Bent arrows indicate non penetration.

H^+ transport mechanism is thought to exist across the whole cell or in association with the mucosal cell membrane.

It would seem reasonable to postulate that if an ion was not distributed across a membrane in a manner described by equations 3 or 5 and yet a net flux of the ion in question takes place across the membrane, then there would exist some sort of transport mechanism for that ion, involving forces other than the gradient of electrochemical potential of the ion; the transport mechanism(s) being located in association with the membrane being studied.

The p.d. across the two cell types of the foetal gastric mucosa, unlike that across the frog skin, is probably not simply equal to the sum of two potential drops, each of which is described by equation 5. The high sensitivity of the mucosal side to changes in concentration (activity) of certain ions, as judged by changes in p.d. may mean that equation 5 describes the p.d. across the cell membranes on the mucosal side. However, the low sensitivity of the serosal side to changes in concentration of any of the principal ions in the system indicates that equation 5 does not describe the p.d. across this membrane.

It is likely that the Na^+ and Cl^- 'pumps' situated on the serosal sides of the non differentiated and oxyntic cells respectively, are electrogenic and responsible for a potential drop across this membrane (cell contents -ve with respect to the

serosal solution).

The measurements of short circuit current, which were carried out on stomachs of 27 to 30 days gestation age are consistent with the chemical measurements of net transfers occurring at this age. Thus on the basis of the chemical measurements a short circuit current equal to the sum of the active Na^+, Cl^- and H^+ currents was to be expected. The dependence of the s.c.c. on the Na^+ concentration on the mucosal side was clearly seen. It is interesting to compare the relation between mucosal Na^+ concentration and s.c.c. in the foetal stomach with this relationship in frog skin. In this latter organ the transport mechanism becomes half saturated at 40mM Na^+. In the foetal stomach the Na^+ transport mechanism is half saturated at 31mM Na^+ and just about completely saturated at 100mM Na^+.

If the transport is by a carrier type mechanism Michaelis-Merton kinetics can be applied (Kirschner 1955) and the half saturation figure appears in the equation relating active Na flux to mucosal Na^+ concentration $\left[Na^+\right]_m$:

$$\text{Active flux of } Na^+ = a.K\left(\frac{\left[Na^+\right]_m}{\left[Na^+\right]_m + f}\right) \quad \ldots\ldots\ldots\ldots(8)$$

where f is the half saturation constant expressed in M and K is a rate constant (mols/unit area/unit time) and a is the surface area of the transport system exposed to Na^+ on the mucosal side.

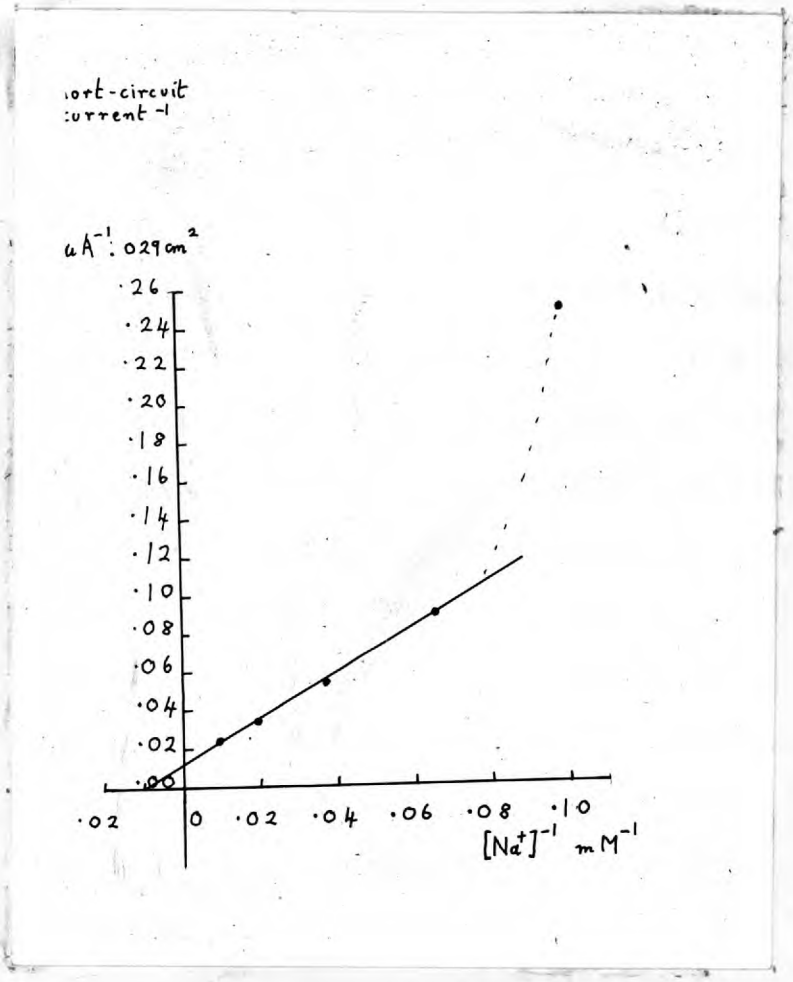


Fig.32. Lineweaver-Burke plot of result shown in Fig.27.

Using a Lineweaver-Burke plot the applicability of the above equation can be tested and the values of K and ϕ determined from the intercept on the ordinate (giving K^{-1}) and the intercept on the abscissa (giving ϕ). Figure 32 shows the result of this procedure when applied to the results shown in Fig.27. It is seen that the above relationship applies at Na^+ concentrations above about 15mM (on the mucosal side). The departure from the above kinetics at the lower concentrations is probably due to the passive Na^+ flux from the serosal to mucosal side becoming significant in relation to the active flux.

It was interesting to find that the s.c.c. was as large with methyl sulphate solutions on the two sides as it was with Cl^- solutions. The analysis of the gastric p.d. indicated that methyl sulphate passed across the mucosa less readily than Cl^-. If there was no active transport of methyl sulphate a reversal of p.d. across the mucosa (with Na^+ free solutions on the mucosal side) would be expected due to activity of the H^+ pump. It seems therefore, that active anion transport in the foetal gastric mucosa is non specific and that as a result of this methyl sulphate can be transported in place of Cl^-.

It would be interesting to determine how many other anionic species can be actively transported in this system. Unpublished work by the author indicates that ethyl sulphate can replace Cl^- in the transport process.

The trans-mucosal p.d. of frog stomach is reversed when the organ is bathed in Cl^- free (SO_4^{--}) media (Heinz and Durbin 1959, Rehm et al 1963) indicating strongly the electrogenic nature of the H^+ pump. The reversal of the p.d. under these conditions would indicate that there was now little active transport of anions, although a small degree of active SO_4^{--} transport across frog gastric mucosa has been demonstrated (Hogben 1961).

The observed stimulation of the Na^+ free component of the s.c.c. by adrenalin is similar to the effect of this hormone on frog skin, where it has been shown to evoke active Cl^- transport by the flask shaped glands (Johnsen, Ussing and Zerahn 1952). The stimulatory effect on the Na^+ component of the s.c.c. of the foetal gastric mucosa appears to be unique since as far as the author is aware adrenalin has not been reported to stimulate active Na^+ transport in any other tissue.

The effect of histamine on the Na^+ free component of the s.c.c. is similar to that described by Rehm for the frog gastric mucosa. In both cases there is a fall in resistance and p.d. and an increase in the p.d./resistance ratio. Similar results have been reported for the dog stomach (Rehm 1953).

The effect of histamine on the non differentiated cells was to decrease the p.d./resistance ratio. There are no other reports with which to compare this observation.

The effect of pituitrin on the non differentiated cells was similar to its now well known effect on frog skin (Ussing

1959) and toad bladder (Leaf and Hayes 1962). The hormone increases the open circuit p.d. and the p.d./ resistance ratio. Detailed analysis indicates that the internal resistance of the active transport mechanism becomes lowered (Duhrman and Ussing 1951). Leaf and Hayes (1962) have shown that vasopressin alters the permeability of the membrane on the mucosal side of the cells of the toad bladder wall. The effect of this is to make Na^+ on the mucosal side more readily available to the transport mechanism.

The inhibitory effect of pituitrin on the secretion by the oxyntic cells, as measured by the reduction in s.c.c. in the absence of Na^+ on the mucosal side was associated with a decrease in the p.d./resistance ratio. An effect of this nature has not been reported previously.

The results presented in this thesis indicate that the non differentiated cells have certain properties in common with the Na^+ transporting cells of frog skin and toad bladder, whilst the oxyntic cells have many properties in common with those attributed to the oxyntic cells in the adult.

In conclusion it appears that the stomach of the rabbit foetus during the last ten days of gestation is highly active in a physiological sense. The predominant function is the active absorption of sodium ions resulting in a passive absorption of salts and water down an osmotic gradient. During the last seven days of gestation an active secretion of HCl is superimposed

on the absorptive process resulting in a slight lowering of pH of the gastric contents. The active absorption of Na^+ appears to be associated with the presence of the non differentiated cells and the secretion of HCl with the oxyntic cells: this latter finding gives considerable support to the classical theory. It seems likely in view of the present findings that H^+ and Cl^- are secreted by the same cells.

Outline of future work.

The assumption that the short-circuit current is equal to the net transfers of Na^+, Cl^- and H^+ needs to be tested by simultaneous measurement of these fluxes along with the short-circuit current. Na^+ and Cl^- fluxes should be measured using isotopes and the H^+ ion flux measured directly by potentiometric titration. The non-specific anion transport should be investigated in more detail along the same lines.

The inter-relationships between Na^+, H^+ and Cl^- transport should be investigated in a quantitative manner.

The nature of the p.d. across the two cell types in the mucosa should be investigated in further detail, using micro-electrodes if possible to give the most direct measurements. The possible existence of redox potentials should be considered.

The action of drugs should be investigated in further detail. As well as the drugs already used, the effects of

acetylcholine and the cardiac glycosides should be determined since these have been used on other active transport systems.

Analysis of the complex impedance of the gastric mucosa at various gestation ages may help in discovering further properties of the transport mechanisms.

The transport processes should be investigated in relation to the composition of the extra cellular fluid with respect to Ca^{++}, HCO_3^-, pH, $H_2PO_4^-$, HPO_4^{--}.

Metabolic studies should be carried out in an attempt to determine the energy sources for the transport processes. These processes, in the first instance, should be studied in relation to the pO_2 of the extracellular fluid using an oxygen cathode. At a later stage the actions of metabolic poisons should be determined.

Absolute permeability constants of the cell membranes of the foetal gastric mucosa to molecules of known sizes, lipid solubilities and hydration energies should be determined in order to elucidate the nature of the pathways available for penetration.

Experiments should be designed to determine quantitatively the role of the foetal gastric mucosa in determining the salt and water balance between mother, foetus and extrafoetal compartments.

Summary.

1. An outline of the history of the electrolyte physiology of the stomach and other relevant organs has been presented.

2. A method for studying in vitro the net transfers of electrolytes across the gastric mucosa of the rabbit foetus has been described.

3. A difference of electrical potential was found across the isolated gastric mucosa at all ages studied; from 20 to 31 days (full term). The mucosal side was negative with respect to the serosal side and the potential difference was dependent on the presence of Na^+ in the mucosal solution.

4. There was a net absorption of Na^+, Cl^- and water when the mucosal solution was bicarbonate Ringer's solution or 154mM - NaCl. Na^+ moved against its gradient of electrochemical potential whilst Cl^- moved down its gradient of electrochemical potential. Net transfers of water were passive and tended to equalize osmotic pressures.

5. From the 23rd day onwards there was a net secretion of titratable acid on the mucosal side. It is known that the oxyntic cells appear on the 23rd day in the rabbit foetus.

6. When choline replaced Na^+ on the mucosal side there was an increase in volume and amount of HCl from the 23rd day onwards. H^+ and Cl^- were transported against their gradients of electrochemical potential. Under these conditions there was also a net transfer of Na^+ down its gradient of electrochemical potential.

7. Net movements of K^+ only took place down its gradient of electrochemical potential, irrespective of the anatomical direction of the gradient.

8. A method has been described for measuring the potential difference and short-circuit current of an isolated piece of the foetal stomach.

9. The transport numbers of the principal ions crossing the cell membranes of oxyntic and non differentiated cells were measured.

10. A sodium 'pump' was postulated to exist in association with the membrane on the serosal side of the non differentiated cells.

11. A chloride pump was postulated to exist on the serosal side of the oxyntic cells. A hydrogen ion pump was also considered to be present in these cells.

12. The short-circuit current of the foetal gastric mucosa was measured and the actions of drugs on it were determined.

13. It is submitted that the work presented in this thesis has contributed to knowledge of the general physiology of the stomach and to the physiology of the foetus.

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Appendix i.

Variation of composition of gastric contents with gestation age. Mean values are shown, with the range in brackets.

Age (days)	Cl (m.equiv/l)	Na (m.equiv/l)	K (m.equiv/l)	pH	osmolality (m.osmol/kg.water)
21	90	135	-	7.8	-
22	122	150	-	7.4	-
23	102	150	8.0	7.4	336
24	-	125	-	7.6	-
26	100 (94 - 105)	-	-	7.0	-
27	73 (29 - 100)	71 (15 - 142)	6.8	6.1 (5.2 - 7.0)	-
28	47 (22 - 73)	47 (10 - 116)	15.3 (13.2 - 19.2)	6.5 (4.0-5.8)	320 (306 -305)
29	22	20 (15 - 25)	-	5.0 (4.5-5.5)	-
30	47 (26 - 49)	15 (10 - 26)	9.4 (3.4 - 15.5)	4.8 (4.7-5.0)	347 (332 - 363)
31	39 (37 - 41)	15 (10 - 19)	12.3 (11.7 -12.5)	5.1 (5.0 -5.2)	342 (327 - 358)

Appendix A4.

Variation of composition of amniotic fluid with foetal age.

Age (days)	Cl (m.equiv/l)	Na (m.equiv/l)	Osmolality (m.osmol/kg.water).
22	-	145	342
23	-	-	-
24	-	125	-
25	100	150	-
26	110	147.5	360
27	110	148	333
28	100	145	-
29	-	125	325
30	115	-	-

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Absorption of Amniotic Fluid in the Gut of Fœtal Sheep

SWALLOWING movements are known to be made by the fœtus *in utero*, but the ability of the gut to absorb material from the amniotic fluid has received little attention. This latter aspect has been investigated in fœtal sheep over the age-range 80–145 days (full term).

Under spinal anæsthesia and sedation with sodium thiopentone the fœtus was exposed by Cæsarian section and its abdomen opened. Electrical potential differences were measured between gut contents and fœtal extracellular fluid at various levels, using a Vibron 33*B* electrometer, calomel electrodes and 3 *M* potassium chloride in agar salt bridges. Representative segments of the gut were used to study the absorption of amniotic fluid to which polyethylene glycol, mol. wt. 3,300–4,000, was added to serve as a volume marker[1]. The segments used were abomasum, jejunum, ileum and colon; approximately 8 cm. lengths of these latter three segments were used. Each segment, isolated by ligation, with intact blood supply, was flushed and filled with the labelled amniotic fluid and then left unexposed for 1–3 hr. The initial volume was calculated from the final weight of the contents and the change in concentrations of polyethylene glycol.

All significant potential differences (greater than ± 3 mV.) showed the gut lumen to be electrically negative with respect to the fœtal extracellular fluid at all ages studied. The abomasal potential difference was 14 mV. at 80 days, 21 mV. at 107 days and 26–30 mV. after 120 days. The potential difference across the small intestine was close to zero prior to 120 days, after which values of 4–10 mV. were obtained. The potential difference across the colon was close to zero at 80 days. From 120 days the transcolon potential-difference was 4–24 mV. These values may be compared with those obtained in the adult which were of the same sign: abomasum 40 mV., small intestine 10–14 mV., colon 15 mV.

Control experiments on single segments showed that the recovery of polyethylene glycol using a turbidimetric method[2] was of the order of 60–80 per cent over the experimental period. Calculations based on an assumed recovery of 60 per cent showed a net absorption of water, sodium and fructose from all segments at all ages studied. The amount of

water absorbed from any segment was greater than could be accounted for by the hypotonicity[3] of the amniotic fluid. Sodium absorption occurred against a gradient of concentration and electrical potential. Absorption of fructose took place down a concentration gradient (140–500 mgm./100 ml. in amniotic fluid, 80–120 mgm./100 ml. in fœtal plasma), the fructose concentration in the gut decreasing. The results obtained for water absorption were extrapolated to the whole gut. From this it was computed that the 100–110 day fœtus absorbed fluid at a rate of 120 ml. per day. At 120 days this was about one litre, falling to 500 ml./day at term. The total volume of fluid absorbed from 80 days to term is about 32 litres, which is of the same order of magnitude as the volume of urine produced during this time (about 40 litres[4]). At 100 days fructose absorption occurred such that 6·7–10·7 per cent of the fructose introduced into the small intestine was absorbed in 1 hr., 12·6 per cent/hr. being absorbed from the colon. At 107 days the rate of fructose absorption was 9·0 per cent/hr. from the abomasum, 26–40 per cent/hr. from the small intestine and 46–47 per cent/hr. from the colon.

The results suggest that absorptive powers of the gut are retained in this species from at least 80 days. The gut is capable of absorbing a considerable volume of amniotic fluid with active sodium absorption probably accounting for much of the water absorption. Fructose absorption occurs at a high rate, and this may be of some nutritional significance to the fœtus. The fœtal abomasum appears to bear a functional similarity to the stomach of the fœtal rabbit in its capacity to absorb sodium actively[5].

This work was aided by an M.R.C. grant to Prof. A. St. G. Huggett and a grant to one of us (G. H. W.) from the Central Research Fund, University of London. We thank the Shell Chemical Co., Ltd., for donating the polyethylene glycol.

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Appendix IV.

Application of the constant field equation to two membrane systems in parallel.

Consider a membrane of specified area separating two electrolyte solutions and let this membrane contain two areas $A\alpha$ and $A\beta$ of different permeability properties.

The current $j\alpha$ passing from side 1 to side 2 across $A\alpha$ is given by

$$j\alpha = \frac{Z^2EF}{d} A\alpha \left[\frac{\lambda^+_\alpha - \lambda^-_\alpha \exp(-ZEF/RT)}{1 - \exp(-ZEF/RT)} \right] \quad \ldots\ldots\ldots(1)$$

and the current passing from side 1 to side 2 across $A\beta$ is given by

$$j\beta = \frac{Z^2EF}{d} A\beta \left[\frac{\lambda^+_\beta - \lambda^-_\beta \exp(-ZEF/RT)}{1 - \exp(-ZEF/RT)} \right] \quad \ldots\ldots\ldots(2)$$

where Z is the valency of the ions passing the membrane (assumed identical for all ions)

E is the electrical potential of side 1 with respect to side 2

F " " Faraday.

d " " thickness of the two membranes (assumed to be identica

R " " gas constant ($8.7\ \text{erg.mol}^{-1}$ A bs)

T " " absolute temperature

$$\lambda^+_\alpha = \underset{\text{side 1 cations}}{\sum^{i} U_i^\alpha C_i^+ \ (1)} + \underset{\text{side 2 anions}}{\sum^{i} U_i^\alpha C_i^- \ (2)}$$

$$\lambda^-_\alpha = \underset{\text{side 2 cations}}{\sum^{i} U_i^\alpha C_i^+ \ (2)} + \underset{\text{side 1 anions}}{\sum^{i} U_i^\alpha C_i^- \ (1)}$$

where U_i^{α} is the mobility of the ion species i through the membrane area $A\alpha$ and C_i is the concentration of ion species i.

Similarly, for the area $A\beta$ we have:

$$\lambda^{+}_{\beta} = \sum^{i} U_i^{\beta} C_i^{+} \quad (1) \quad + \quad \sum^{i} U_i^{\beta} C_i^{-} \quad (2)$$

$$\lambda^{-}_{\beta} = \sum^{i} U_i^{\beta} C_i^{+} \quad (2) \quad + \quad \sum^{i} U_i^{\beta} C_i^{-} \quad (1)$$

When no external current is passing and electroneutrality is preserved,

$$j\alpha + j\beta = 0$$

if current passes only through $A\alpha$ and $A\beta$. Equating the right hand sides of equations (1) and (2) and rearranging gives

$$E = \frac{RT}{ZF} \cdot \ln \left[\frac{A\alpha \lambda^{-}_{\alpha} + A\beta \lambda^{-}_{\beta}}{A\alpha \lambda^{+}_{\alpha} + A\beta \lambda^{+}_{\beta}} \right] \qquad \ldots\ldots(3)$$

If $\lambda^{-}_{\alpha} \simeq \lambda^{-}_{\beta}$, $\lambda^{+}_{\alpha} \simeq \lambda^{+}_{\beta}$ and $A\alpha \gg A\beta$

the contributions of λ^{+}_{β} and λ^{-}_{β} become negligible and equation (3) reduces to the familiar form

$$E = \frac{RT}{ZF} \cdot \ln \left(\frac{\lambda^{-}_{\alpha}}{\lambda^{+}_{\alpha}} \right) \qquad \ldots\ldots\ldots\ldots\ldots\ldots(4)$$

i.e. the p.d. is independent of the presence of the area $A\beta$

It is assumed that

$$A\alpha = \sum a\alpha$$

and $$A\beta = \sum a\beta$$

where $a\alpha$ and $a\beta$ are microscopic areas to which the quantities λ^{+}_{α}, λ^{-}_{α} and λ^{+}_{β}, λ^{-}_{β} apply respectively. These microscopic areas are considered to be distributed evenly over

the whole of the membrane in question.

In this thesis it is assumed that the distribution of non differentiated cells and oxyntic cells in the foetal gastric mucosa satisfies the requirement described above.

The area of one side of a given piece of gastric mucosa due to non differentiated cells is considered equivalent to A_α in equation (3) and A_β is considered equivalent to the surface area due to oxyntic cells. From the histological work of Menzies (1958) it is then assumed that $A_\alpha \gg A_\beta$. Equation (4) is then considered to describe that part of the p.d. resulting from diffusion potentials across the membranes of the non differentiated cells.

If in the absence of Na^+ on the mucosal side all p.d.s associated with the non differentiated cells vanish, (see results section) as a result of which A_α is functionally considered to vanish, the observed p.d. is then associated with the oxyntic cells and the contribution of diffusion potentials is given by

$$E = \frac{RT}{ZF} \cdot \ln \left(\frac{\lambda_\beta^-}{\lambda_\beta^+} \right).$$

Appendix v.

[*From the Proceedings of the Physiological Society*, 20–21 February 1959.]
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Continuous recording of short-circuit current through frog skin.

By G. H. Wright. *Department of Physiology, St Mary's Hospital Medical School, London, W.* 2

Since there is a p.d. across living frog skin (inside positive with respect to outside) even when there are identical solutions on each side of the skin, it must be possible to draw current from it by connecting the two sides through an external circuit (Francis, 1933). If reversible electrodes of very low impedance were available the whole of this current could be tapped off and measured. Such a current is defined as the short-circuit current of the skin (Ussing & Zerahn, 1951). Since no such electrodes are available the short-circuit current has been measured by passing a counter current through the skin in a direction opposite to that of the active current until the p.d. across the skin is equal to zero: the counter current is then equal to the short-circuit current (Ussing & Zerahn, 1951). In the past, the counter current has been obtained by tapping off current from a high tension source by means of a potentiometer which is operated manually or by a servo motor actuated by the d.c. output of the d.c. millivoltmeter used to measure the p.d. across the skin (Mullins, 1958). However, rapid changes in short-circuit current cannot be measured accurately by these means since mechanically moving parts are involved.

This demonstration shows how the short-circuit current may be measured continuously without the aid of any mechanically moving parts.

The p.d. across the skin is measured by means of a 'Vibron' Model 33B millivoltmeter manufactured by Electronic Instruments Ltd. This instrument converts the d.c. input into an alternating p.d. which is then amplified by an a.c. amplifying circuit, the output being finally fed through a phase-sensitive rectifier and meter. By means of two test sockets provided in the back of the instrument an alternating p.d. proportional to the d.c. input can be tapped off and amplified by a simple error amplifier. The output of the error amplifier is isolated by means of a transformer, rectified by a double diode, smoothed and fed back through the skin in opposite phase to the active current.

The skin is mounted in an apparatus similar to that described by Ussing & Zerahn (1951), only constructed of glass. Calomel electrodes are used for p.d. measurements and Ag–AgCl electrodes for passage of current.

It is found that, using this apparatus, a p.d. of 80 mV across 2·5 cm^2 of frog skin can be reduced to less than 0·5 mV, the output current of the error amplifier then being about 200 μA.

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Hypoxic death in the foetal sheep. By H. G. Britton, D. A. Nixon and G. H. Wright. *Department of Physiology, St Mary's Hospital Medical School, London, W.2*

Hypoxia has been induced in foetal sheep of ages 120–140 days by the administration of 5% O_2 in nitrogen to the mother anaesthetized with sodium thiopentone. This procedure differs from that of Dawes, Mott & Shelly (1959), who produced hypoxia by umbilical occlusion. Foetal and maternal blood pressures were recorded and samples of foetal and maternal blood were taken for estimation of glucose, fructose, lactate, pH, plasma potassium and bicarbonate. After foetal death tissues were removed, frozen in liquid nitrogen and analysed for lactate and glycogen.

When the hypoxia was maintained until foetal death, which took place in about 28 min, a rapid continuous rise in the blood lactate (up to about 150 mg/100 ml.) occurred and a marked acidosis developed. The plasma potassium also increased but did not exceed 5·8 m-equiv/l. in any experiment. There was usually a small fall in the pCO_2. The mean ventricular glycogen at death was 0·27 g/100 g moist tissue and the mean ventricular lactate concentration was 290 mg/100 ml., compared with control values of 1·3 g/100 g and 100 mg/100 ml. respectively.

When the blood lactate was experimentally elevated to approximately 100 mg/100 ml. by the administration of L-lactic acid, acute hypoxia produced a similar increase in blood lactate to that observed previously and the survival time was approximately the same.

To examine recovery from hypoxia, experiments were carried out on twins in which an initial bout of 15 min hypoxia was given followed by a recovery period of $5\frac{1}{2}$ hr. One foetus was then removed and the other foetus subjected to hypoxia to death. The total survival time (i.e. initial + terminal bout) to hypoxia was not increased; nor was there any return of the blood lactate or pH to normal during the recovery period. However, the cardiac glycogen of the foetus removed before the second hypoxia had been restored to normal. The second hypoxia killed the remaining foetus without gross depletion of cardiac glycogen, but there was a sharp rise in the blood lactate. The terminal lactate concentrations in the second foetus were about 230 mg/100 ml. in the blood and 400 mg/100 ml. in the heart.

These experiments confirm Dawes *et al.* (1959) that a gross depletion of cardiac glycogen takes place in acute hypoxia. The experiment in which the lactic acid level was artificially raised suggests that the blood lactate is not a limiting factor under these conditions. In the recovery experiments, however, gross cardiac glycogen depletion did not occur, and foetal death must have been due to other factors. The blood and cardiac lactate values in the

[P. T. O.

recovery experiments were extremely high and may have been the cause of death but the severe acidosis and the rise in the plasma potassium may have also contributed.

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Aerobic energy production and the stimulation of active sodium transfer across isolated frog skin by neurohypophysial extract.

By G. H. Wright. *Department of Physiology, St Mary's Hospital Medical School, London, W.2*

The increase in active sodium transport by isolated frog skin following administration of a maximal dose of neurohypophysial extract varies considerably among different skins of the same variety.

Leaf & Renshaw (1957) showed that neurohypophysial extract failed to stimulate active sodium transport in anoxic skins. They concluded the action of neurohypophysial hormones on ion transport is 'somehow associated with their ability to increase the availability of aerobic energy sources for operation of the ion transport mechanism'.

It was decided in view of the above finding to investigate the relation between the magnitude of the response to a maximal dose of neurohypophysial extract (Pituitrin; Parke, Davis and Co. Ltd, Batch no. LY 616A) and the aerobiosis of the skin.

The experiments were carried out during the months of May, June and July, using frogs kept in captivity for 3 or 4 months. Active Na^+ transport was measured on the short-circuit current principle (Ussing & Zerahn, 1951) by means of a continuous recording technique (Wright, 1959). The Ringer's solution used had the following composition (mM): NaCl 115, $CaCl_2$ 1·4, $KHCO_3$ 2·5. The pH of this solution was adjusted to 7·8.

After a 2 hr period of equilibration the skins were subjected to total anoxia for about 30 min, then quickly restored to aerobic conditions. About 30 min later the Pituitrin was administered.

Measurement showed a highly significant positive correlation ($r = 0{\cdot}763$, $P < 0{\cdot}001$) between the magnitude of the response to Pituitrin and the magnitude of the aerobic component of the short-circuit current (defined here as the difference in values of the steady short-circuit current under aerobic and anaerobic conditions). The equation of the regression line is $y = 0{\cdot}787x + 4{\cdot}4$.

From these results it is concluded that the degree of response of the ion-transport mechanism to a maximal dose of neurohypophysial extract is dependent upon the functional state of the aerobic energy source at the time of administration of the extract.

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Absorption and secretion of electrolytes in the stomach of the rabbit foetus

By G. H. Wright. *Department of Physiology, St Mary's Hospital Medical School, London, W.2*

The experiments were carried out *in vitro* on stomachs from 19 to 31 days gestation age. A glass cannula was tied into the oesophageal stump and the duodenal stump was tied off. The stomachs were filled with the experimental solutions through the cannula. The stomachs were then immersed in bicarbonate-Ringer at 35° C and pH 7·4. The experiments lasted from 3–6 hr.

Net changes in volume of gastric contents were measured as well as changes in amount of Na, Cl and H, which was measured as titratable acid. The electrical potential difference (p.d.) between the gastric contents and the bathing solution was also measured.

When the stomachs were filled with Ringer's solution there was found to be a p.d. across the gastric epithelium of 15–30 mV (lumen negative). This p.d. was unaltered in sign and magnitude if the internal Ringer's solution was replaced by 150 mM-NaCl. When the internal Na was replaced by choline the p.d. fell in a manner dependent upon gestation age. Thus up to 23 days the p.d. fell to zero when choline replaced Na. However, after 23 days the p.d. only fell to about 30% of its initial value. These effects were quite reversible.

When the internal solution was 150 mM-NaCl there was a decrease in volume of gastric contents and amount of Na and Cl. This occurred at all ages studied. When choline replaced Na there was no volume change observed in stomachs up to 23 days, although a decrease in amount of Cl took place due to passage of this ion down its electrochemical potential gradient. From 23 days onwards there was an increase in volume of contents and amount of H and Cl when choline replaced Na inside. With 150 mM-NaCl inside there was still an increase in amount of titratable acid although Na, Cl and volume of contents decreased.

It was found that all net transfers of Na out of the stomachs occurred against the gradient of electrochemical potential for this ion. Similarly, all net transfers of Cl into the stomachs under conditions of no Na inside took place against the electrochemical potential gradient.

When net water transfer is plotted against net solute transfer a straight line of slope 332 m-osmole/kg water passing through zero is obtained for stomachs of all ages studied.

Thus from 19 to 23 days the foetal stomach is solely absorptive in func-

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tion, the primary process being active outward transfer of Na at a rate of about 0·8 μ equiv/hr at 23 days and 18 μ equiv/hr at 29 days. After 23 days HCl secretion is superimposed on the absorptive process, active inward Cl transfer being involved at a rate of about 1·6 μ equiv/hr (at 29 days), All water transfer is accounted for by solute transfer.

Menzies (1958) has shown that the only cell type present up to 23 days is an undifferentiated columnar cell, the oxyntic cells appearing on the 23rd day onwards. Thus active Na absorption must be associated with the former cell type and HCl secretion with the latter.

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REFERENCE

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