Relations between Hydrochloric Acid Secretion and Electrical Phenomena in Frog Gastric Mucosa

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For over a hundred years the existence of a potential difference (p.d.) across the stomach wall has been recognized, and there has been sporadic speculation as to the relation, if any, between this p.d. and the secretory activity of the gastric mucosa. The p.d. was first recorded by Donné (1834) who, discussing these 'courants électriques' asked 'sont-ils capables de rapprocher, de séparer les molécules l'une de l'autre, et de donner naissance à des corps nouveaux, comme cela arrive dans le règne inorganique?' This question still remains unanswered in spite of a large amount of work by numerous investigators. The early experiments, using primitive techniques and uncontrolled conditions, have been reviewed by Biedermann (1895). Later work, some with more refined techniques, but still using the stomach *in situ*, have been discussed by Rehm (1944) and by Rehm & Hokin (1947). In the present investigations isolated frog gastric mucosa was chosen as the experimental material because it can secrete hydrochloric acid *in vitro*, under conditions capable of controlled variations, and because it is possible to obtain information about its metabolic activity (Davies, Longmuir & Crane, 1947). These investigations into a possible correlation between electric properties and metabolism were initiated in 1945, after it had become clear that the hydrogen ions of the acid could not be produced directly by the fermentation or oxidative degradation of any carbon compound (Davies, 1948; Davies *et al.* 1947).

This paper deals with the techniques and apparatus used, and presents the results of experiments which show that there is a general correlation between acid secretion and the p.d. across the mucosa, the resistance of the latter and its ability to produce electric power externally. This is taken to indicate that the elaboration of hydrochloric acid is an electrochemical phenomenon. A hypothesis of acid production is presented which is in accordance with the known biochemical and biophysical observations. Accounts of investigations into the effects of direct electric currents on acid production and the effects of metabolic activators and inhibitors will be given in subsequent papers.

A part of the present work has been communicated to the Biochemical Society (Crane, Davies & Longmuir, 1946).

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EXPERIMENTAL

Material. Frogs (*Rana temporaria temporaria* L.) were used in these experiments. They were captured locally, the

Fig. 1a, Perspex holder for supporting mucosa; b, clamp for Type I and Type II vessels; c, Type I vessel for measuring p.d. across gastric mucosa and amounts of acid secreted. N = nutrient vessel; S = secretory vessel.

web tattooed for identification, and were housed in a concrete froggery at 15–17°. An excess of cockroaches or worms was provided for food.

Preparation and mounting of tissue. The gastric mucosa was washed and isolated according to the method of Davies (1948) and the tube of mucosa then cut along the lesser curvature. Holders consisting of a pair of perspex sheets 1.5 mm. thick and 4 or 5 cm. square with a central hole were used for supporting the mucosa (see Fig. 1*a*). The holes, from 3 to 12 mm. diameter, were surrounded on the inner sides by concentric grooves in the perspex which gripped the tissue. Rubber gaskets fixed on the outer sides prevented leakage at the joints with the glass vessels described below. The mucosa was laid across the hole between the two sheets and firmly clamped in position by tightening the four corner screws. In order to insulate it electrically the edges and screws of the holder were dipped in melted dental wax.

Each saturated KCl bridge consisted of two 15 cm. lengths of 2 mm. bore glass tubing connected by 30 cm. of bicycle-valve rubber tubing. The glass tubes were constricted at each end, and the lower end contained a tapered glass stopper 1 cm. long ground into the tube. The narrow end of the stopper protruded a few millimetres beyond the tube (Fig. 1*c*). These bridges were designed because agar bridges, soaked strings and filter papers proved unsatisfactory. They were flexible and easily flushed, refilled and cleaned, and their leak was not more than 10^{-4} ml./hr. with an excess pressure of 1 cm. of saturated KCl solution. Their resistance was about 50,000 Ω .

The bridge from each vessel led to a test tube containing a reference electrode at 25.0°, and the p.d. between the two

Fig. 2. Type II vessel for measuring pH of secretory solution, p.d. and resistance of mucosa, and for passing current through it. Oblique side-tubes for locating bridges attached at SS. ZZ = zinc-zinc acetate electrodes.

Vessels. The above unit was placed between the ground flanges of two glass vessels, which were then held together by a brass clamp with rubber-lined jaws (Fig. 1*b*). Several kinds of vessels were developed which were of two main types: Type I for measuring the p.d. across the mucosa and the amounts of acid secreted, and Type II for measuring additional quantities. Type I vessels are comparable with those of Delrue (1930) and of Gray, Adkison & Zelle (1940) and are shown in Fig. 1*c*. The nutrient vessel *N* contained about 36 ml. of nutrient solution (see p. 323) gassed with 5% $\text{CO}_2 + 95\% \text{O}_2$ (v/v). The secretory vessel *S* contained about 14 ml. of 0.12M-NaCl solution gassed with O_2 . There was an excess pressure of about 1 cm. of solution on the nutrient side. The capillary draining tube at the foot of each vessel was closed by a glass rod attached by rubber tubing. Each bung supported by means of small rubber collars a funnel, a glass bubbler and one end of a saturated KCl bridge. The bubbler was a narrow glass tube delivering a fine stream of either O_2 or 5% $\text{CO}_2 + 95\% \text{O}_2$ previously wetted and warmed to 25.0°.

electrodes was measured by a potentiometer described later (see also Fig. 3). In early experiments Ag-AgCl electrodes (Brown, 1934) in 0.1M-KCl solution were used. Later, calomel electrodes were used in saturated KCl solution, the level of which was kept slightly above that in the vessels and storage test tube. The end *A* of each bridge was kept in this tube containing 0.12M-NaCl solution at 25.0°, except during measurements. With this precaution diffusion potentials were satisfactorily eliminated. Checks were frequently made that the p.d. between the two reference electrodes was zero with both bridges connected by the same solution, and showed calomel electrodes to be completely reliable and more satisfactory than Ag-AgCl electrodes.

Type II vessels (Fig. 2) were designed for the measurement of the pH of the secretory solution, the p.d. and resistance of the mucosa, and for passing current through it by means of the non-polarizable zinc-zinc acetate electrodes ZZ. These were made by rubbing saturated Zn amalgam on to disks of pure cleaned Zn. They had a constant resistance for current densities up to about 50 ma./cm.²

Before each experiment the horizontal tubes were filled with four layers of 5% agar, the first made with saturated zinc acetate solution and the other three with physiological salt solution (see p. 323) or 0.12M-NaCl solution. With this method no Zn was ever detected in the vessel solutions with 8-hydroxyquinoline or sodium diethyldithiocarbamate, and mucosae behaved normally. If all the saline agar was poured in together, so much zinc acetate dissolved in it that a toxic quantity subsequently reached the mucosa.

Bubblers were similar to those in Type I vessels. Bridges were similar, but for more accurate resistance measurements some of the Type II vessels were provided with side tubes inclined at 30° to the vertical (not shown in Fig. 2) which

mounted in the bath was 20–30 min. This period was found to be well within the safety limit for normal subsequent behaviour.

Saline medium. The solution used during the washing and preparation of the mucosa was similar to the physiological salt solution of Krebs & Henseleit (1932) diluted by 0.29 vol. of water, except that it had a HCO_3^- content of 0.004M and, under working conditions, a pH of 7.4. The nutrient solution was that of Krebs & Henseleit diluted by 0.29 vol. of water and containing 0.2% glucose. In aerobic experiments it was gassed with 5% CO_2 + 95% O_2 , in anaerobic experiments with 5% CO_2 + 95% N_2 . Analyses showed that our cylinders of this mixture contained 0.3–0.5% O_2 .

Fig. 3. Diagram of circuits for measuring p.d. and passing current through mucosa.

located the bridges accurately. This allowed precise determination of the correction due to the resistance of the solution. In experiments where a low correction was desirable, large Type II vessels were used, the horizontal tube having a diameter of 2.4 cm., nutrient and secretory volumes 25 ml. Where a low secretory volume was needed to give a higher acid concentration, vessels of 1.2 cm. diameter were used; the nutrient volume was 7 ml. and secretory volume 4–6 ml. The glass electrode (Marconi TM. 388A) was mounted in the secretory vessel, on the far side of the bridge and bubbler from the mucosa. It was connected to a Marconi pH meter Type TF. 5511C (see Fig. 2), and a saturated KCl bridge connected the secretory solution to a calomel reference electrode at 25.0°. The p.d. was measured as in Type I vessels.

Both types of vessel were immersed in a water bath at 25.0°, which also contained the reference electrodes, storage tube and gas manifolds. The temperature was kept uniform by water circulated through a warmed flask by air from a small motor blower; a second air stream stirred the water in the bath. In order to prevent the possibility of interference with electrical measurements electrical heating was avoided. The time elapsing between the pithing of the frog and the commencement of the experiment with the vessels

Measurement of p.d. and resistance. When measuring the p.d. across the mucosa it is desirable that virtually no current pass through it. The p.d. recorded is then the e.m.f. of the mucosa, and the mucosa is unaffected by the measurement. Two potentiometers, both null instruments, were used: in early experiments a Cambridge Electrometer Valve Potentiometer 44236, and later a battery-operated Marconi pH meter and potentiometer Type TF 511C. The negative (secretory) side of the mucosa was connected to the grid of a valve and the positive (nutrient) side to the cathode circuit. The valve in each of these instruments has a very high grid-cathode resistance, at least $10^9 \Omega$, so that the current through the mucosa was entirely negligible. The p.d. was determined to ± 1 mV. except in very early experiments when the accuracy was ± 2 mV. The resistance of the mucosa was determined by measuring the p.d. between the two saturated KCl bridges when a small current was passed through the membrane by means of the two zinc-zinc acetate electrodes. By measuring the p.d. between points near the mucosa the resistance correction was kept small, and by passing the current between electrodes covering the whole cross section of the tubes, uniform current flow was obtained. A potential divider provided currents from 0.01 to 10 ma. (see Fig. 3), which were measured by multiple range meters

with ranges from 0.1 ma. upwards. Calibration showed them to be accurate to 1% (2% for lowest range). The value of the correction for the resistance of the solutions was calculated and also determined experimentally for each pair of vessels.

Estimation of acid secreted. In early experiments the secretory solution was periodically removed and its acid content determined manometrically. In later experiments when a pH meter was available, the pH of the secretory solution was also measured *in situ* as described above. In the first method, a 6 ml. sample of the secretory solution consisting of 0.12M-NaCl together with any secretion, also 0.12M, was placed in the main compartment of a Warburg cup, and 0.5 ml. of a solution containing 0.02M-NaHCO₃ and 0.10M-NaCl was placed in the side arm. After equilibration with 5% CO₂+95% O₂ at 25° the solutions were mixed and the output of CO₂ was observed. This determination, to about pH 6, was compared with those from control solutions of NaCl+HCl of total strength 0.12M, but of varying acidity. This procedure is necessary because it has been found that CO₂ is less soluble in acid than in neutral solutions of equivalent concentrations (Davies & Longmuir, 1947). The 'isotonic' NaHCO₃ was used to keep the blanks as low as possible.

The dry weight of the mucosa was obtained by removing the tissue quantitatively from the hole in the perspex holder, washing in distilled water and drying overnight at 110°. The Q_{HCl} was then calculated, and is given in μ l./mg. dry wt./hr.

RESULTS

Electrical properties of non-secreting gastric mucosa

Potential difference. There was a p.d. across all of 150 frog gastric mucosae, the secretory side being in every case negative (in an external circuit) with respect to the nutrient side. Each experiment lasted between 6 and 12 hr. after the vessels containing the mucosa were mounted in the water bath, this instant being taken as zero time. During the first 0.5 hr. the p.d. between the secretory and nutrient sides usually increased in magnitude (Figs. 6-8). We have found that after slight mechanical injury to either side of the mucosa the p.d. was temporarily reduced in magnitude by 2-15 mV. (eight experiments). The initial rise is thus probably due to the decrease of injury potentials resulting from dissection and mounting, and we are not primarily concerned with it in these experiments. It occurred in seventy-two out of eighty-two experiments; the average initial p.d. of the secretory side was -20 mV. and the average maximum value -32 mV. After this peak the p.d. dropped slightly and remained rather steady for many hours in an untreated mucosa unless acid secretion occurred.

In ninety-four experiments the steady p.d. varied between -7 and -50 mV., with a mean value of -30 mV. and a standard deviation of 9 mV. There was no significant difference between males (69) and females (25), nor between fasting frogs and those actively digesting at the time of pithing. Nor was any correlation apparent with the weight of the

frog. There was, however, a difference between summer and winter frogs, in spite of the fact that the latter were kept warm and accepted food. From March to June 1946 the mean p.d. across thirty-seven mucosae was -35 mV. and the standard deviation 7 mV.; from September to February the mean p.d. across fifty-seven mucosae was -27 mV. and the standard deviation 9 mV. Mucosae of frogs which had been in captivity over a month usually gave rather a low p.d., and were in general less active than those of freshly caught frogs. To overcome this difficulty frogs are now kept out of doors under natural conditions.

Resistance. The resistance of 1 cm.² of thirty-nine mucosae varied between 90 and 310 Ω -cm.²; the mean value was 210 Ω -cm.² and the standard deviation 60 Ω -cm.² Taking the mean thickness as 0.25 mm., these results give for the mean specific resistance of frog gastric mucosa 8×10^3 Ω -cm. and the standard deviation 3×10^3 Ω -cm. It should be noted, however, that in all cases the area used was that of the hole in the perspex holder. Since the mucosa is essentially non-planar, these areas are minimum values, and represent between 0.5 and 1.0 times the true area. For this reason the unit of mucosa used in power calculations was not 1 cm.² but 1 mg. dry weight.

For both p.d. and resistance the standard deviation was about 30% of the mean value. No significant variation of the resistance for unit area or for 1 mg. dry weight was observed with any of the variables mentioned in the last paragraph but one, nor with the area of mucosa used. Mucosae with a low specific resistance, however, never attained such high rates of acid secretion as some with high specific resistance (fifteen mucosae), and the magnitude of the specific resistance seemed to give rather a good indication of the 'state of health' of a mucosa at any particular time. The initial rise in p.d. was accompanied by a rise in resistance representing about 20% of its steady value (fifteen experiments). Injury to the mucosa reduced both p.d. and resistance. Death was accompanied by a rapid decrease of the p.d. to zero, and of the resistance to a small fraction of the live value. For mucosae killed by hydrochloric acid or hydrogen cyanide the dead resistance was 10-30% of the live resistance; for mucosae killed by heat (30 min. at 60°) not more than 5%. An interesting observation is the effect of iodoacetate. During 1-2 hr. after its addition to 0.0015M the p.d. was abolished and the resistance rose to about twice its normal value. Subsequently, the p.d. remained zero and the resistance dropped in 0.5 hr. to the dead value.

Electrical power output. An important observation is the constancy of the resistance for currents below about 1 ma./cm.² This was true for applied currents both enhancing and opposing the natural p.d.,

and also for currents maintained by the e.m.f. of the mucosa itself when no other source of power was included in the circuit. Results for one mucosa are shown in Fig. 4. As in all other similar experiments, the points obtained with an applied current, and those obtained when the mucosa itself provided the power, lie on the same straight line within experimental error; the mucosa obeyed Ohm's Law. Moreover, when the two sides were connected electrically (through the solution, agar, electrode and galvanometer resistances) a current was maintained without appreciable polarization. The greatest current observed when the mucosa was the only source of electrical power in the circuit was 0.07 ma. This occurred when the resistance in the circuit apart from the mucosa ($120\ \Omega$) was $460\ \Omega$. The electrical power output of the mucosa was then $(0.07)^2 \times 580\ \mu\text{W.}$, or $2.8\ \mu\text{W.}$ The effective area of the mucosa was $1.8\ \text{cm.}^2$ allowing for bellying, and

Electrical properties of some other parts of the gastro-intestinal tract

Various neighbouring parts of the digestive tract of the frog were examined. Across oesophageal, pyloric, duodenal and ileal mucosa (fourteen experiments) no p.d. was found comparable in magnitude with that across mucosa from the corpus of the stomach. The highest p.d. observed was that across the pyloric mucosa, which in eight experiments gave an average of $-5\ \text{mV.}$ The other membranes had a mean p.d. of $-2\ \text{mV.}$ (six experiments). In all cases, as in the corpus, the secretory side was negative with respect to the nutrient side. The resistance of oesophageal, pyloric, duodenal, ileal and rectal mucosa was determined in six experiments only: all values were within the range found for the corpus. This is also true of their specific resistance and resistance for 1 mg. dry weight.

Fig. 4. Relation between p.d. across a frog gastric mucosa and current through it.
 x—x with mucosa as only source of power; •—• with external source of power.

its dry weight $10.8\ \text{mg.}$ The greatest observed power output was thus $1.6\ \mu\text{W./cm.}^2$ or $0.26\ \mu\text{W./mg.}$ dry weight. The magnitude of the external resistance precluded the observation of a higher power output.

However, when sheets of mucosa were immersed in a beaker of nutrient saline solution at 25.0° the p.d. across them, measured between two points close to them, was maintained without polarization at about one fifth of the open circuit value, so that the power developed was $11\ \mu\text{W.}$ This suggests that if the resistance external to the mucosa could be reduced to zero, the total electrical power, (p.d. across mucosa) 2 /resistance of mucosa, would be developed. In the case considered above this maximum power would be $40^2/120\ \mu\text{W.}$, i.e. $13\ \mu\text{W.}$ This is also equal to (intercept on current axis) $^2 \times$ resistance of mucosa = $(0.33^2 \times 120)\ \mu\text{W.}$ The maximum power is thus $7\ \mu\text{W./cm.}^2$ or $1.2\ \mu\text{W./mg.}$ dry weight.

Experiments with three rectal mucosae gave steady p.d. values of -8 , -12 and $-27\ \text{mV.}$ The muscle layer dissected off from frog gastric mucosa was also investigated: the p.d. across it was zero in all of five experiments. The resistance of unit area was measured in three cases, giving a mean of $70\ \Omega\text{-cm.}^2$ Since the thickness was about $0.5\ \text{mm.}$ the specific resistance was $1400\ \Omega\text{-cm.}$, considerably lower than that of the gastric mucosa.

Experiments in the absence of oxygen

Experiments on nine mucosae have shown that an adequate supply of oxygen is necessary to maintain both acid secretion and the p.d. across the membrane. Their mean p.d. with an oxygen pressure of 1 atm. was $-27\ \text{mV.}$; this was reduced to $-3\ \text{mV.}$ when the oxygen was replaced by nitrogen (containing from 0.3 to 0.5 % oxygen), and in four cases the p.d. fell to zero. The abolition of the p.d. was

reversible, and recovery occurred in *all* cases on supplying oxygen, even after several hours of anaerobiosis. The whole cycle could then be repeated using the same mucosa. When the experiment was begun under anaerobic conditions, there was no initial rise in p.d., which instead fell rapidly to a very low value as the oxygen in the tissue became depleted. Fig. 5 gives a typical curve for two complete cycles.

Fig. 5. Effect of anaerobiosis on p.d. across a frog gastric mucosa.

The resistance of the tissue was increased in the absence of oxygen in both of two experiments by about 50%. It fell again to approximately its previous value on subsequent oxygenation. The secretion of acid by the mucosa was inhibited concomitantly with the decrease in p.d., and in no case was any hydrochloric acid produced anaerobically.

Secretion of acid by frog gastric mucosa

In dealing with the secretion of acid by frog gastric mucosa it was found that the seasonal variations in the behaviour of frogs both presented problems and gave a wide range of material from which to choose. In late spring and summer, about half the mucosae secreted acid spontaneously, and after the addition of histamine ($5 \times 10^{-5} M$) to the nutrient solution most others secreted acid. Histamine also often increased the rate of acid secretion of spontaneously secreting mucosae. The Q_{HCl} had values up to 8.0. In winter, fewer mucosae secreted acid and none did so spontaneously. The average rate was lower than in summer, and the maximum Q_{HCl} observed was 4.6. There was a period in spring, however, when summer conditions were not yet fully established, and almost no mucosa secreted spontaneously, but all did so after the addition of histamine, whenever this was added (usually between 1 and 7 hr. experimental time, Figs. 6–8). When spontaneous secretion occurred, it most often started at about 2 hr. experimental time. In some winter and spring mucosae

secretion stopped on the removal of histamine, and secretion could subsequently be reinitiated by the addition of more histamine.

We wish to introduce the term 'secretive' for a mucosa capable of secreting acid, either spontaneously or during stimulation, whether this mucosa is actually secreting or not. The term 'non-secretive' is used for a resistant mucosa, i.e. one which cannot be made to secrete acid even by the addition of histamine.

No acid secretion was detected in oesophageal, pyloric, duodenal, ileal or rectal mucosa (eight experiments).

Relation between potential difference, resistance and acid secretion

The commencement of acid secretion, whether spontaneous or induced by histamine, was accompanied by a drop in the p.d. across the gastric mucosa. This drop also accompanied a histamine-induced increase in the rate of secretion of a mucosa

Fig. 6. P.d. and Q_{HCl} of a frog gastric mucosa. Spontaneous secretion and effect of histamine.

already secreting spontaneously. (In Fig. 6 spontaneous secretion occurred during the initial rise in p.d., which masked the effect.) This decrease in magnitude of the p.d. was usually higher for high rates of secretion than for low ones, being of the order of 10 mV. for a Q_{HCl} increase of 2, or 17 mV. for

a Q_{HCl} increase of 6. The effect was reversed when secretion stopped, either spontaneously (Fig. 6) or on removal of histamine (Fig. 8), or by the addition of thiocyanate (0.012M) to the nutrient solution, which inhibited acid secretion (Fig. 7).

Fig. 7. Effect of histamine and thiocyanate on p.d. and Q_{HCl} of a frog gastric mucosa.

Fig. 8. Effect of addition and removal of histamine on p.d. of a (winter) frog gastric mucosa.

In summer, acid secretion sometimes commenced spontaneously before experimental time 0.5 hr., and in some cases it continued for periods up to 11 hr.

Fig. 6 gives results for a spontaneously secreting mucosa in April, which also responded to histamine and secreted for 9 hr., the rate falling off after the third hour. The maximum Q_{HCl} (over 1 hr.) was 7.6 and the total amount of acid secreted 435 μl . Fig. 7 shows results for a secretive mucosa in March, which did not secrete acid until histamine was added at experimental time 1.5 hr. In this mucosa, acid secretion was almost completely inhibited after 1.5 hr. by the addition of 0.012M-thiocyanate which does not inhibit respiration (Davies & Turner, 1948), and the p.d. rose at the same time. Fig. 8 refers to a mucosa in December which responded twice to histamine, the effect being reversed both times when the histamine was removed. In this experiment nutrient saline was used on both sides of the mucosa, and the acid was not estimated.

The variation of the resistance of the mucosa when acid secretion commenced (or was stimulated by histamine) was followed in six experiments, four of which were concerned with the actual onset of secretion. In all cases the resistance rose while the p.d. fell, and the fractional rise in resistance (x) increased both with the fractional fall in p.d. (y) and with the actual fall. In three cases x and y were both below 15%, in two cases both were between 30 and 40% and in the remaining case $x = 20\%$ and $y = 11\%$. x and y were thus of the same order of magnitude in each case.

Diffusion potentials

The possibility was investigated that the change in p.d. across the mucosa at the onset of acid secretion is partly a diffusion potential due to acid in the tubules of the mucosa. Two types of experiment were carried out. (a) The 0.12M-sodium chloride on the secretory side of a non-secreting mucosa was replaced by 0.12M-hydrochloric acid, and after more than 10 min. this was replaced by 0.12M-sodium chloride, the p.d. changes being watched carefully during the second change-over. The 0.12M-hydrochloric acid left in the gastric crypts by this treatment is likely to take some minutes to diffuse completely into the new solution, and a p.d. level during this interval comparable with the p.d. after the onset of secretion would support the hypothesis stated above. No such temporary drop was observed. (b) A model was set up,

Hg, Hg_2Cl_2 | sat. KCl | 0.12M-NaCl | cellophan |
sintered glass disk | 0.12M-NaCl | sat. KCl | Hg_2Cl_2 , Hg,
to represent the system

Hg, Hg_2Cl_2 | sat. KCl | nutrient solution |
muscularis mucosae | glandularis mucosae |
secretory solution | sat. KCl | Hg_2Cl_2 , Hg.

When the 'secretory' sodium chloride was replaced by 0.12M-hydrochloric acid the p.d. changed immediately from 0 to -20 mV. and rose to -32 mV.

during the next 5 min. while the acid diffused through the sintered glass to the cellophan. After 1.5 hr. the p.d. had fallen to -29 mV. When the hydrochloric acid was replaced by sodium chloride, the p.d. fell within 5 sec. to -2 mV., and never changed sign, as would have been expected if the acid in the 'tubules' of the sintered glass caused asymmetrical diffusion potentials. In both these experiments care was taken to avoid momentary diffusion potentials at the bridges by rinsing the end in saturated potassium chloride solution before each p.d. measurement in a new solution.

It was important to know whether, at the pH values of the secretory solution obtained with secreting mucosae, diffusion potentials were produced which affected the measured p.d. In six experiments hydrochloric acid solutions of pH 4, 3 and 2 were used consecutively on the secretory side of a non-secreting mucosa in place of $0.12M$ -sodium chloride. In no case did a solution of pH 4 or 3 produce any change in p.d. greater than 2 mV., whether or not it was made isotonic by the addition of sodium chloride. Hydrochloric acid ($0.01M$) produced no greater effect on mucosae when added within several hours of mounting, but in some cases increased the magnitude of the p.d. by up to 20 mV. when added after 6 hr. experimental time. These hydrochloric acid solutions had no effect on the p.d. of a secreting mucosa.

In our experiments the pH of the secretory solution never fell below 3; moreover, when the secretory solution of a secreting mucosa was replaced by fresh sodium chloride solution, there was no observable change in the measured p.d. The secretory solution was always replaced before its pH fell below 3, and in view of all the above evidence it seems impossible to account for the p.d. changes during secretion on the basis of simple diffusion potentials.

DISCUSSION

Sign of potential difference across gastric mucosa

Previous workers, including Rosenthal (1865), Bohlen (1894), Biedermann (1895), Mond (1927), Rehm (1943, 1944) and Teorell & Wersäll (1945), found with the stomachs of a variety of animals that in an external circuit the secretory surface was at a negative potential with respect to the serosa, and that the p.d. diminished with the onset of acid secretion. Delrue (1935) claimed opposite signs for the p.d. and its change on secretion; it is probable that he was considering the direction of internal rather than external currents.

Injury potentials

The rapid increase in the magnitude of the p.d. across the mucosa after mounting is probably caused by the decrease of injury potentials which are in the

opposite direction to the natural p.d. The accompanying increase in resistance is not great enough to account, by the reduction of local currents through the damaged edges as the tissue becomes stabilized, for more than a small fraction of the p.d. rise. Injury potentials of dog stomach have been discussed by Rehm (1944).

Relationship between potential difference, resistance and acid secretion

When acid secretion commenced, either spontaneously or after histamine, the magnitude of the p.d. fell by an amount greater for high than for low rates of acid secretion. The experiments on diffusion potentials support the view that not more than a small part, if any, of this fall can be due to a diffusion potential caused by the presence of hydrochloric acid either in the secretory solution or in the gastric tubules. It is clear from the data of Rehm (1944) and Rehm & Hokin (1947) that the pH in the secretory chamber attached to an actively secreting dog's stomach could fall from above 5 to 2 without any change in the p.d. across the stomach wall. Teorell & Wersäll (1945) recorded only small changes in the p.d. across secreting isolated frog gastric mucosa when the pH of the secretory solution was changed from 4 to 2 by the addition of hydrochloric acid. They also noted that the secretory surface was 'insensitive towards the ionic composition of the solutions bathing it'. We also have found that wide variations in the ionic composition of the secretory solution had little effect on the p.d. across the mucosa. Similar results have been obtained by Rice & Ross (1947), who have reviewed the literature on this subject.

The rise in resistance which accompanies the p.d. drop at the onset of acid secretion, and seems to be quantitatively related to it, suggests that the electrical structure of gastric mucosa is changed when acid secretion commences, and that the p.d. fall is due to more fundamental changes than superimposed diffusion potentials. While many experimental procedures have been found which change both the resistance of live frog gastric mucosa and the p.d. across it, none was found which affected only one of these. Teorell & Wersäll (1945) recorded a rise in both p.d. and resistance of isolated frog gastric mucosa between the time of mounting and the commencement of acid secretion about 1 hr. later. They did not, however, differentiate between the injury effects due to mounting and changes accompanying the onset of secretion.

When acid secretion finished, either spontaneously or as a result of the addition of thiocyanate ($0.012M$) the magnitude of the p.d. rose again towards its previous value. Davies & Turner (1948) found that at this concentration thiocyanate had no effect on the rate of oxygen uptake of isolated frog gastric

mucosa, and that it neither prevented nor abolished the increased rate of oxygen uptake due to 5×10^{-5} M-histamine. In one experiment, when histamine was added to the nutrient solution of a secretive mucosa which already contained 0.01 M-thiocyanate, the magnitude of the p.d. increased by 6 mV.

On the other hand, when both the cyanide-sensitive respiration and acid secretion were abolished by 0.001 M-cyanide, the p.d. fell to zero and the resistance fell to 10–30% of the original value. During anaerobiosis both respiration and secretion were also absent, but, whereas the p.d. fell rapidly to zero, the resistance increased by about 50%. On subsequent oxygenation the p.d. and resistance returned to their previous values even after several hours of anaerobiosis. Both Rehm & Hokin (1947) and Rice & Ross (1947) found that the p.d. across a dog's stomach wall was dependent upon an adequate oxygen supply to the tissue. No workers have recorded acid secretion in the absence of respiration or a p.d. across the mucosa.

Our results further show that the dead/live resistance ratio for isolated frog gastric mucosa may be considerably greater than for other active biological material for which information is available. Cole (1947) gives the value for the dead/live membrane resistance of cells as a fraction of 1%. Blinks (1930) found that the dead/live resistance for *Valonia ventricosa* cells was 1 or 2%. The highly active oxyntic cells in gastric mucosa may, however, have a different dead/live resistance ratio from the whole tissue.

Potential difference and acid secretion of other regions of the gastro-intestinal tract

The p.d. across the oesophageal, pyloric, duodenal and ileal walls, and across the muscle layers dissected off the corpus of the stomach, was found to be small or zero and unaffected by histamine. It is of interest that of these regions only the acid-secreting mucosa had a high p.d., and that the p.d. was affected by histamine only if it was secretive.

Experiments on rectal mucosa are being extended, and will be discussed in a later paper. It should be borne in mind in connexion with the appreciable p.d. of this tissue (mean of three experiments – 16 mV.), that frog gastric mucosa, frog skin (which shows an even higher p.d.) and frog rectal mucosa are all tissues concerned actively with water transport. The last two membranes transport an approximately neutral solution in the same direction as their external positive p.d., whilst gastric mucosa secretes an acid solution in the opposite direction to its external positive p.d.

In frogs, pepsin is largely or entirely secreted by the oesophagus (see Friedman, 1937). In Friedman's (1934) experiments with frogs the brain was destroyed and 'one third of the way up from the pylorus the

stomach was divided to form a pyloric portion and a cardiac portion'. He found that when either the cardiac or pyloric part was stimulated with glass beads, acid was formed only in the pyloric region. No figures were published to support this claim. Using the technique described above, we have shown beyond any doubt that the corpus of the stomach produces acid, and have also failed to detect any acid secreted by the pyloric mucosa of a stomach whose corpus secreted acid. Friedman (1934) used *Rana esculenta*, and we have confirmed that in this species too acid is secreted by the corpus region of the stomach.

Electric power produced by isolated frog gastric mucosa

Donné (1834) first raised the question as to whether the electric currents observed in the stomach could do useful chemical and mechanical work. Brücke (1859) suggested that the electromotive forces in gastric mucosa may enable it to send an acid fluid towards the secretory surface and a basic fluid in the opposite direction. In 1872 Engelmann published experiments which are of interest in connexion with these suggestions. He studied the secretion of the glands in frog skin by drying the skin and observing microscopically when it became wet again. Although he did no quantitative work he showed that the glands secreted only when an e.m.f. could be detected, and that various agents which abolished the e.m.f. also abolished secretion. He suggested that the glands in the skin produced their secretion by electroendosmosis (Engelmann, 1872). Since then there has been much speculation, but until recently very little experimental evidence has been published, and this is more suggestive than conclusive. Some of the work has been reviewed by Keller & Pisha (1947).

Recently evidence has accumulated which supports the hypothesis that electrical energy is utilized in the production of acid by the stomach. It has been found that the rate of acid secretion of gastric mucosa can be increased by passing through it an electric current which enhances the natural p.d., and decreased by a current in the opposite direction (Rehm, 1945 (dogs); Crane *et al.* 1946; Crane & Davies, 1947a (frogs)). It has also been found that gastric mucosa can maintain an electrical power output without appreciable polarization (Rehm, 1943; Crane *et al.* 1946). The greatest observed power output of the frog gastric mucosa considered above (p. 325 and Fig. 4) was $0.25 \mu\text{W./mg. dry weight}$, and the power output expected with zero external resistance was $1.2 \mu\text{W./mg. dry weight}$. Davies & Longmuir (1947) found the average Q_{O_2} of fifty non-secreting isolated frog gastric mucosa to be 2.1. If we take as a first approximation the usual value of $0.005 \text{ cal./}\mu\text{l. oxygen}$ for the oxidation of glucose (Brody, 1945), the average rate at which energy is

liberated in non-secreting frog gastric mucosa by glucose oxidation is 0.0050×2.1 cal./hr./mg. dry weight, or $12 \mu\text{W./mg. dry weight}$ ($1 \text{ cal./hr.} = 1.16 \times 10^3 \mu\text{W.}$). Using this value for the mucosa discussed above, the greatest electrical-power output observed represents $0.25/12$, i.e. 2% of the metabolic power, which with zero external resistance would be increased to $1.2/12$, i.e. 10%.

mately 10%) in maintaining an electric current. This refers to a non-secreting mucosa and, since the p.d. is lower and the resistance higher during acid secretion, less power is then available to drive a current round an external circuit. This may be because electric power is being used internally to produce acid. The maximum electric power output of the mucosa considered earlier (p. 325), which was

Table 1. *Production of maintained electric power by various living tissues*

Author	Material	Temperature (° C.)	Approximate p.d. across membrane (mV.)	Greatest observed current density in active membrane (ma./cm. ²)	Greatest observed electrical power output of active membrane ($\mu\text{W./cm.}^2$)	Greatest observed electrical power output of active membrane ($\mu\text{W./mg. dry wt.}$)	Electrical power Metabolic power (Author's figure) (%)
Blinks (1933)	Halicystis cell	25-30	70	0.01 ma./cell	0.7 $\mu\text{W./cell}$	—	—
Francis (1933)	Isolated frog skin	20	—	0.015	2.5	0.5	5-10
Stapp (1941)	Isolated frog skin	25	40	0.02	0.8	0.15	1-2.5
Rehm (1943)	Dog stomach	39	60	0.15	8.9	0.34	2-4.5
Crane <i>et al.</i> (this paper)	Isolated frog gastric mucosa	25	40	0.04	1.6	0.26	2

At least five workers besides ourselves have determined the electrical-power output maintained by living tissue, and some of the results are collected in Table 1. (The specialized tissue of electric organs of some fishes is excluded from the present discussion; the current varies rapidly and widely during a train of discharges.) All the results in Table 1 refer to experiments in which the two sides of the membrane were connected electrically through a circuit of fairly low resistance, and the current densities cited were maintained without appreciable polarization. Throughout, the power produced was limited by the external resistance, which differed in each case, and a higher power would be expected with zero external resistance. The last column gives an approximate value of the ratio: greatest observed electrical power output/estimated rate at which metabolic energy is liberated (the efficiency of the process). Whilst some of the figures in this table are based on incomplete data, it is of interest that the efficiencies are of the same order of magnitude. Lund & Stapp (1947) have recently published a series of results for frog skin, which give efficiencies (electrical power/metabolic power) of 1-2%.

From a comparison of the structure, the p.d. and resistance of nearby regions of the gastro-intestinal tract, and from the relations previously described between the electrical properties of gastric mucosa and acid secretion, it seems likely that most, if not all, of the p.d. across this tissue is due to the oxyntic cells, but that their resistance is only a small part of the whole.

It is remarkable that a gastric mucosa can expend such a large fraction of its metabolic energy (approx-

not secreting acid, could support a Q_{HCl} of the order of 2, i.e. about one quarter of the maximum Q_{HCl} observed in frog gastric mucosa. The relationship between the electrical energy and the energy used in secretion will be discussed elsewhere. It should be noted that more metabolic energy is available to the oxyntic cells during acid secretion because the rate of respiration is increased (Davies, 1948).

A SUGGESTED ELECTRON-CYCLE MECHANISM OF HYDROCHLORIC ACID PRODUCTION

Golgi (1893) found that the intracellular canaliculi form a network just below the surface of the oxyntic cells (shown schematically in Fig. 9b). Bradford & Davies (1948), using indicators, demonstrated that these canaliculi contained acid during acid secretion, thus showing that the acid is formed in the region between the canaliculi and the external cell wall (pericanalicular zone).

Chemical processes involved

The fundamental overall reaction concerned in hydrochloric acid production (Davies, 1948) is

and evidence has been presented in this paper and previously (Rehm, 1943, 1945; Crane *et al.* 1946; Davies *et al.* 1947; Crane & Davies, 1947a) which supports the theory that some of the energy available from metabolism within the oxyntic cells is utilized in an electrochemical process which results in the net separation of the electrically charged H^+ and OH^- ions.

We suggest that the cyclic mechanism described in the following paragraph, and shown in Fig. 10, is

According to our hypothesis two coupled systems of enzymes are involved (a) to transport H atoms

Fig. 9a.

Fig. 9b.

Fig. 9. a, Diagram of gastric tubule showing lines of current flow across and in the mucosa due to its own e.m.f. Three oxyntic cells shown. b, Diagram of single oxyntic cell enlarged from a showing network of canaliculi. Sample cross-section of pericanalicular zone enclosed in rectangle.

Fig. 10. Suggested electron-cycle mechanism of HCl production in the pericanalicular zone of oxyntic cells (enlarged from Fig. 9b). This schematic diagram is not drawn to scale; the H atom and electron transport systems probably occupy only a small fraction of the space between the canalicular and cell walls.

operative. The overall reaction is that which occurs when the well-known cell

(see MacDougall, 1939) is reversed by a source of power (Davies *et al.* 1947).

(electrons + protons) across the pericanalicular zone, and (b) to return the electrons to the starting point near the cell wall. Energy derived from aerobic metabolism drives a system composed of a series of dehydrogenating enzymes (a) whose prosthetic groups (such as coenzymes I and II) send H atoms

through the pericanalicular zone, thus maintaining a very low H atom concentration at the side nearest the cell wall and a very high H atom concentration at the canalicular wall. The electrons are returned by a coupled cytochrome or cytochrome-like system (b) (e.g. $\text{Fe}^{++}\text{-Fe}^{+++}$) oriented across the zone. A unit cycle can now be described. A H^+ ion formed from water near the cell wall oxidizes a Fe^{++} to a Fe^{+++} ion, and by accepting the electron becomes a H atom. The atom is transported to the region of high H concentration at the canalicular wall, this endergonic process being coupled with energy-giving reactions. The H atom then reduces a Fe^{+++} to a Fe^{++} ion; the resultant H^+ ion passes into the canaliculus together with the Cl^- ion released by reduction of the Fe^{+++} ion. The electron from the H atom is returned to the region of low H concentration near the cell wall by the potential of the $\text{Fe}^{++}\text{-Fe}^{+++}$ system, and passes round the cycle again. Valency requirements are satisfied by the passage of a Cl^- ion across the system in the opposite direction to the electron (cf. Lundegårdh, 1945). The overall result for each electron cycle is thus reaction (1) with the transport of one Cl^- ion.

Previous work has shown that the residual OH^- ions from the water react (via the cytoplasmic buffers) and are converted by carbon dioxide from internal and external sources into HCO_3^- ions (Davies, 1948; Davies & Longmuir, 1948), this process being catalyzed by carbonic anhydrase (Davies & Roughton, 1948). It is clear, therefore, that this cyclic mechanism involves no *net* transfer of charge through the acid-secreting oxyntic cells or across the mucosa: at the cell wall Cl^- ions exchange with HCO_3^- ions, and at the canalicular wall H^+ and Cl^- ions are secreted (cf. Davenport, 1946).

Effect of histamine. The action of histamine could be explained by the assumption that it takes part as a link in the H atom transport system of the electron cycle by the tautomerism or resonance of its iminazole ring. A similar role for histidine has been suggested as part of the mechanism of cytochrome *c* activity (Theorell, 1947). The work of Hallenbeck, Code & Gregory (1947) supports the view that agents stimulating hydrochloric acid secretion act finally through histamine (cf. Babkin, 1944).

Crane & Davies (1947*b*) have calculated that in order to form 1 mol. of hydrochloric acid under the conditions obtaining in frog oxyntic cells, the system must be supplied with about 1.0×10^4 cal. of free energy (a few hundred calories more are required in mammalian oxyntic cells). Lipmann (1941) and Meyerhof (1944) have calculated the free energy released by the hydrolysis of high-energy phosphate bonds. For ATP they found c. 10,000 and 12,000 cal./mol., respectively. It is interesting that the splitting of one such bond might thus be able to energize one unit electron cycle and thus produce one H^+ ion.

Electrical processes involved

Potential differences in oxyntic cells. The postulated electric circuits within the mucosa are shown schematically in Fig. 11*a* and *b*, which represent a sample cross-section of the mucosa containing a single secretory unit in the pericanalicular zone of an oxyntic cell. E is the e.m.f. which maintains the p.d. measured in our experiments.

Fig. 11 *a*.

Fig. 11 *b*.

Fig. 11. Suggested electric circuit diagram within sample cross-section of gastric mucosa. Enclosed portion represents the pericanalicular zone of the single oxyntic cell considered. *a*, Non-secreting mucosa (showing external current). *b*, Acid-secreting mucosa. E = e.m.f. of pericanalicular zone. R_i = internal resistance of pericanalicular zone. R_s = mucosal resistance in series with R_i . R_p = resistance of rest of sample cross-section of mucosa.

Since the pH difference across the pericanalicular zone of a frog oxyntic cell secreting 0.12M-hydrochloric acid is c. 6.3 (calculated from Davies & Roughton, 1948), the p.d. between the two sides of this zone, measured with respect to a reference electrode, must be about 370 mV. This p.d. is measurable by electrodes sensitive to H^+ ions only (e.g. glass electrodes). Since the p.d. occurs across a distance not greater than 1μ (Fig. 9*b*), the electric field is at least 3700 V./cm. between the canaliculus (positive) and the cell wall (negative). If the hypothesis presented above be true, this p.d. due to the pH difference during acid secretion must be more than compensated by that due to the difference

in H atom concentration. Assuming that the H atoms are in equilibrium with hydrogen molecules, then electrodes sensitive to both H_2 and H^+ ions (i.e. hydrogen electrodes), if used together with a reference electrode, would detect a p.d. in the opposite direction, from cell wall (positive) to canaliculus (negative). On this hypothesis the ratio of the equivalent hydrogen pressures must be more than $10^2 \times 10^3 = 10^{12-6}$ (see MacDougall, 1939). Such ratios certainly occur within living cells (Hewitt, 1936; Ball, 1942).

There has been controversy (see Korr, 1939) as to whether redox potentials can be the direct source of bioelectric potentials measurable with non-metallic, i.e. calomel, electrodes. This has been resolved by Korr (1939), who pointed out that the redox potential of the following system can be observed by calomel electrodes

and that if a system able to transfer electrons existed in the cell (as we postulate for the oxyntic cell) then redox potentials could be manifested as bioelectric potentials. Stiehler & Flexner (1938), using indicators, measured the difference in redox potential in the choroid plexus and found it to be 230 mV. with epithelium positive to stroma. Similar results were obtained by Friedenwald & Stiehler (1938) for the ciliary body. It is of interest that, on the basis of these and other results, they suggested that these redox potentials can do secretory and electrical work.

Production of current by tissue. The production of electric power by living tissue is a major problem in itself. If the secretory units in the pericanalicular zone of the oxyntic cells function by the mechanism proposed above, they do not produce a current when actually secreting acid, but can do so when there is no net production of H^+ , OH^- (and hence HCO_3^-) ions. In this case the H transport system is modified and the H^+ ions, instead of passing into the canaliculus, return and react with OH^- ions to re-form water. This part of the hypothesis is very similar to the 'osmotic diffusion pump' theory published by Franck & Mayer (1947).

The transport of Cl^- ions by the non-acid-secreting cell involves a net transfer of charge, the circuit being completed by conducting pathways inside and outside the mucosa. It can be observed as an electric current through a circuit external to the mucosa, in which the negative sign of the secretory side is determined by the morphological structure of the

tissue (Fig. 9a). Experiments are in progress in an attempt to determine whether the current consists of Cl^- ions alone, as in the system

or whether cations move in the opposite direction. In this latter case the mucosa must be able to move cations such as H^+ , Na^+ , etc., from the secretory to the nutrient side.

Potential differences observed. In our present technique we measure the p.d. across the whole mucosa, with saturated calomel electrodes connected to the nutrient and secretory solutions by saturated potassium chloride bridges. It seems

likely that the average value of 30 mV. observed across non-secreting mucosa is less than the average p.d. across the pericanalicular zones theoretically observable with calomel electrodes, because of conducting pathways within the mucosa (see Fig. 11a). For reasons already given, we consider it probable that the redox potential of the Fe^{++} - Fe^{+++} or similar system which transfers the electrons is the source of this p.d. across the pericanalicular zone. Each secretory unit contributes to the p.d. measured, and there are probably of the order of 10^{13} secretory units in each cm^2 of frog gastric mucosa (Crane & Davies, 1947b). This figure is based on the known structure and activity of the tissue, and on an assumed turnover number of 10^3 H^+ ions/secretory unit/min. (see McIlwain, 1946). As each secretory unit commences to secrete acid (Fig. 11b), then according to the hypothesis there is no longer any net transfer of charge through it, i.e. it is unable to produce a current. Since fewer non-secreting units are now available to maintain the p.d. across the mucosa, whilst the other conducting pathways remain, the p.d. observed externally should fall as more and more secretory units start to produce acid. The experimental results show that such a fall in p.d. occurs. As previously suggested, in the secreting unit the internal electron current, the magnitude of which is affected by the increased metabolic activity of the oxyntic cell, is effective in elaborating the acid secretion, but for reasons given above does not produce a p.d. across the mucosa.

Changes in electric resistance. In non-secreting units the production of a Cl^- ion is believed to be concomitant with the transfer of an electron across the $\text{Fe}^{++}\text{-Fe}^{+++}$ system. Since during the remainder of the electron cycle there is no net production of H^+ ions, the process is likely to have a low energy requirement and be easily increased or decreased by externally applied electric p.d., i.e. the non-acid-secreting unit will have a low electric resistance. As has been discussed above, the acid-secreting unit produces hydrochloric acid but not current as a result of the cyclic movement of electrons. The same externally applied electric p.d. can now send very little current through the secreting unit, because the Cl^- ions forming the current must be forced across the $\text{Fe}^{++}\text{-Fe}^{+++}$ system independently of, and in addition to, the electron cycles. Thus the observation of an increase of electric resistance of the mucosa on secretion is in accordance with the hypothesis.

Effect of applied currents. The increase in the rate of acid secretion by the action of applied direct electric currents enhancing the natural p.d. and the decrease by opposing currents (Crane *et al.* 1946; Davies *et al.* 1947; Crane & Davies, 1947a; Rehm, 1945) has been mentioned earlier. On the basis of our hypothesis an applied p.d. from cell wall (positive) to canaliculus (negative) could act in two ways, by assisting the return of electrons in the $\text{Fe}^{++}\text{-Fe}^{+++}$ or similar system, and by assisting the removal of newly formed H^+ ions from the canalicular end of the H transport system, thus speeding up their formation according to the law of mass action. It seems likely that the effect of the applied p.d. on the Cl^- ions would be approximately balanced by its effect on the HCO_3^- ions travelling in the opposite direction. An opposing current would have an effect on the system opposite to that of an enhancing current. A quantitative account of the effects of applied current will be given in a subsequent paper.

An inorganic system which can produce hydrochloric acid by an electron-cycle mechanism. A 'model' of the oxyntic cell has been made which can carry out many of the reactions believed to occur in the living cell.

The system contains two units A and B. Unit A consists of two beakers of dilute NaCl solution (1 and 2) connected by a NaCl bridge and an inverted U tube, containing H_2 , covering two bent Pt black electrodes. A stream of CO_2 bubbles through solution 1. The pH of each solution is measured with glass electrodes and the whole unit is placed on a small trolley which can move to and fro and, by producing waves, cause the NaCl solutions and the H_2 to cover the Pt electrodes alternately. Unit B consists of two beakers, one of FeCl_2 (3) and one of FeCl_3 (4) connected by an inverted U tube packed with the chloride of an anion exchange resin (De-acidite B) which can pass a

current consisting virtually of Cl^- ions only. Pt black electrodes connect the solutions with those of unit A. A battery can be connected across unit B.

Fig. 12. Diagram of an inorganic system which can produce HCl by an electron-cycle mechanism.

The reactions in unit A are:

Na^+ ions migrate from 2 to 1 and Cl^- ions from 1 to 2. In unit B the reactions are:

Cl^- ions migrate from 4 to 3.

The fundamental overall electron cycle is thus

In this model the reactions stop when the p.d. due to the pH differences in unit A counterbalances the redox potential of unit B. It seems probable that in the oxyntic cell this process is driven by the differences in hydrogen atom concentration maintained across the secreting unit: it is technically easier in this model to drive the process by the 'electron pressure' difference across the $\text{Fe}^{++}\text{-Fe}^{+++}$ system which can be recharged with a battery. The maximum concentration of hydrochloric acid obtainable by passing current through unit A is about twice the concentration of the salts initially present (Na^+ ions migrate to 1, Cl^- ions to 2). Starting from physiological saline solutions, this unit can produce

acid at any concentration claimed for the primary acidity of oxyntic cell secretion (see Babkin, 1944). This is taken to support the electron-cycle mechanism of hydrochloric acid production.

Phenomena accounted for by the electron-cycle mechanism. This electron-cycle mechanism thus fits the following experimental observations on gastric mucosa: the secretion of H^+ , Cl^- and HCO_3^- ions, the relations between Q_{HCl} , Q_{O_2} and Q_{CO_2} , and the action of histamine and of carbonic anhydrase; the p.d. across the mucosa and its sign, the decrease of p.d. and increase of resistance on secretion, and the correlation between both Q_{HCl} and resistance changes with p.d. changes; the maintenance of an external current by the mucosa, and the effect of enhancing and opposing currents on acid secretion.

SUMMARY

1. The potential difference (p.d.), resistance, electrical power production and rate of acid secretion of isolated frog gastric mucosa were measured with an apparatus which is described.

2. The secretory side of frog gastric mucosa was negative with respect to the nutrient side in an external circuit. During acid secretion the p.d. was decreased and the resistance increased.

3. Anaerobically the resistance increased and both p.d. and acid secretion were abolished.

4 The mean p.d. across mucosa from the corpus of the stomach was -30 mV.; rectal mucosa -16 mV.; pyloric mucosa -5 mV.; oesophageal, duodenal and ileal mucosa -2 mV.

5. The rise of p.d. after mounting the gastric mucosa was probably caused by the decay of injury potentials. Experiments suggest that the fall in p.d. on secretion was not due to diffusion potentials between the solutions bathing the mucosa.

6. Unstimulated frog gastric mucosae could send a current round an external circuit, producing a maintained electrical power output of the order of $1 \mu W./mg.$ dry weight without appreciable polarization. About 10% of the metabolic energy was thereby canalized into electrical energy. This is taken to support the theory that secreting mucosae produce hydrochloric acid by an electrochemical mechanism.

7. Based on the foregoing and other results, an electron-cycle mechanism of acid secretion is suggested, in which H atom and electron transport systems are coupled to produce a net separation of H^+ and OH^- ions in the pericanalicular zone of the oxyntic cells. The energy required is obtained from aerobic metabolism.

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The Effect of Electric Current on HCl Secretion by Isolated Frog Gastric Mucosa

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Hoffmann (1889) was one of the first workers to maintain, on the basis of animal experiments, that stomach secretion was increased by direct current. Many confused and contradictory results have since been published. Claims have been made that direct current affects the motor but not the secretory activity of the human stomach (Schawerin, 1937), the motor and secretory activity (Kirstner, Gorinstein & Rudoy, 1931), and that it has no effect at all (Goldschmidt, 1895). All this early work can be criticized as insufficiently controlled. The secondary effects due to vagal stimulation, when the currents were large enough, were not separated from any direct action on the oxyntic cells.

Recently, Rehm (1945) has shown by controlled experiments on the stomach of a live dog that current passed through the wall of a secreting stomach from mucosa to serosa decreased the rate of secretion of hydrochloric acid, but that current passed from serosa to mucosa increased the rate of acid secretion. In a non-secreting stomach, acid production was not initiated by passing electric current through it in either direction.

These results became available to us during our investigation into the possibility that gastric mucosa could utilize electrical energy presented to it from external sources. Since acid secretion in frog gastric mucosa is accompanied by a change in the electrical potential difference across it, it seemed possible that the rate of secretion might be affected when this p.d. is changed by passing a current through the mucosa.

The experiments described in this paper show that currents enhancing the natural p.d. of isolated frog gastric mucosa increased the rate of acid secretion and sometimes even initiated it. Currents in the opposite direction decreased the rate of acid

secretion. The effect was specific to secretive gastric mucosa, i.e. mucosa which is capable of secreting hydrochloric acid (either spontaneously or by stimulation) whether or not it is actually secreting (Crane & Davies, 1947). The significance of the experimental results is discussed.

A part of the present work was communicated to the Biochemical Society (Crane, Davies & Longmuir, 1946).

EXPERIMENTAL

The materials, apparatus and methods used during these experiments were similar to those previously described by Crane, Davies & Longmuir (1948). Three species of frog (*Rana temporaria temporaria* L., *R. esculenta* L. and *R. esculenta ridibunda* Pallas) and toads (*Bufo bufo bufo* L.) were used.

Frog or toad gastric mucosa was mounted in a perspex holder held between two chambers at 25°. The Q_{HCl} of the mucosa (in μ l./mg. dry wt./hr.) was estimated from changes in pH of the secretory solution measured *in situ*. In most experiments it was also found by changing the secretory solution and estimating the acid content of the samples thus obtained either manometrically by the addition of a bicarbonate solution, or by electrometric titration using a glass electrode and 0.01N-NaOH delivered from a Conway burette.

Owing to the buffering capacity of the relatively large amount of mucus secreted by frog gastric mucosa, the HCl concentration determined from the pH of the secretory solution may be considerably less than the total concentration determined manometrically or by electrometric titration (cf. Davies & Longmuir, 1948). In cases where only a pH determination *in situ* was made the value of the acid concentration was corrected for this buffering effect.

The p.d. across the mucosa was measured with a battery-operated Marconi pH meter and potentiometer Type TF 511 C, using saturated calomel electrodes and saturated KCl bridges with internal ground-glass joints, which dipped