owing to its higher molarity. The amylopectin which accompanies it into the infusion, amylopectin I, gave much lower blue values with iodine than the other preparations, and indeed resembled in this respect the best preparation otherwise obtained. It can scarcely be representative of the amylopectin of potato starch, and presumably consists of the smaller or less complex molecules.

It is worthy of note that infusion of potato starch for 15 min. at 63°, followed by a further 15 min. at 45–50° before centrifuging, led to the separation of fractions of both amylopectin and amylose, which differ from the main portions of these substances.

SUMMARY

1. β-Amylase splits maltose from the amylopectin constituent of potato starch about 20 times as rapidly as from the amylose.

2. Its reactions with both amylose and amylopectin conform to the Michaelis and Menten formulations, the values of K_m (expressed as percentage) being about twice as great for amylose as for amylopectin.

3. The hydrolysis of an artificial mixture of 4 parts of amylopectin and 1 part of amylose follows the same course as the hydrolysis of starch.

4. The results support the accepted view that starch paste contains these ingredients in unmodified form.

5. From structural considerations it might be expected that amylopectin would be hydrolysed even more rapidly than was found to be the case.

6. The rate of hydrolysis of amylopectin is to some extent influenced by its mode of preparation.

7. The amylose which diffuses from slightly swollen potato starch granules at 63° is hydrolysed much faster than normal potato amylose, while the amylopectin which accompanies it gives an unusually low blue value with iodine.

8. The amylose of Lintner starches is hydrolysed much faster than normal amylose, it having presumably been split in the acid treatment and its molarity in solutions correspondingly raised.

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On the Mechanism of Secretion of Ions by Gastric Mucosa and by Other Tissues

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In this paper the theory of gastric secretion, discussed by Crane, Davies & Longmuir (1948*a*), is extended and certain new evidence is presented.

All the explanations of ionic secretion so far proposed have been more or less incomplete. Franck & Mayer (1947) have examined the general mechanisms required for an 'osmotic diffusion pump'; Rosenberg (1948) has discussed the thermodynamic relationships which must hold for secretory processes; Stiehler & Flexner (1938) related the secretion of chloride into the cerebrospinal fluid to maintained differences of electrical potential and of redox potential across the choroid plexus; Hollander (1943) proposed that the separation of gastric hydrochloric acid is due to differential permeability to ions. None of these authors have related secretion to the activity of particular physico-chemical systems within the cell.

More specific proposals have been made by Crane *et al.* (1948*a*) and Crane & Davies (1948), who have also reviewed a number of other suggestions of the chemical mechanism involved. They distinguished

two types of mechanism. In the first of these, also suggested by Robertson & Wilkins (1948*a*, *b*) to account for secretion by plant cells, an oxidation-reduction system (such as Fe^{++}-Fe^{+++}) produces hydrogen ion (H^+), by oxidation of substrate hydrogen, and OH^- in equivalent amount by reduction of O_2; this mechanism cannot, however, produce more than four H^+ per O_2 used in oxidation and is insufficient to account for the large yields of acid by gastric mucosa. A second type of mechanism was therefore proposed which should transport H^+, derived from water, at the expense of 'metabolic energy'. Recently, Patterson & Stetten (1949) have proposed a mechanism of gastric secretion which is essentially the first mechanism of Crane *et al.* (1948*a*).

The two mechanisms mentioned above are now further examined, in the light of past evidence and of new evidence, presented here, concerning the permeability of gastric mucosa. This evidence was obtained by measuring the potential difference between pairs of electrodes, of three different types, in electrical contact with the solutions bathing the two sides of the mucosa. Specific proposals are made as to the way in which the 'metabolic energy', for the second mechanism, is produced and applied to secretion; the thermodynamic status of each mechanism, and of their interrelationship, is discussed; the electrochemical properties of gastric mucosa are accounted for in terms of the secretory process and of the permeability of the mucosa to ions. The conclusions reached are applied to other types of ionic secretion and to the question of energy-coupling in cellular processes.

EXPERIMENTAL

Methods

The materials, apparatus and methods used in these experiments were similar to those previously described by Davies (1948*a*, *b*) and by Crane *et al.* (1948*a*, *b*).

Frog (*Rana temporaria temporaria* L.) gastric mucosa was mounted in a Perspex holder held between two chambers at 25·0°. The Type II vessels of Crane *et al.* (1948*a*) were used. The potential differences between electrodes were measured with a battery-operated Marconi pH meter and potentiometer, Type TF. 511 C: this instrument discriminates better than 0·02 pH or 1 mV. and is accurate to 0·03 pH or 2 mV. Three types of electrode were used: saturated calomel electrodes (Marconi type TM. 3887 A), which were connected to the 'nutrient' and 'secretory' solutions by saturated KCl bridges with internal ground-glass joints (Crane *et al.* 1948*a*); glass electrodes (Marconi type TM. 3888 A), dipped directly into the two solutions; and silver-silver chloride electrodes of the type described by Brown (1934) dipped directly into the two solutions.

Direct measurements were made of the potential differences between pairs of electrodes, except in the case of the glass electrodes, whose combined resistance was too high to allow satisfactory measurements to be made: therefore the differences were taken of the readings obtained between glass in the secretory solution and calomel in the secretory solution, and between glass in the nutrient solution and calomel in the secretory solution. In the course of each experiment, all readings were frequently repeated to ensure that no drifting of the electrodes or deterioration of the mucosa was occurring. All changes of composition were made in the secretory solution, the nutrient solution being, throughout the experiments, frog-bicarbonate Ringer. The concentration of Cl^- in the secretory solution was varied by replacing NaCl with $NaNO_3$, so that the concentration of Na^+ was kept constant. The pH of the secretory solution was varied by the presence or absence of bicarbonate in the secretory solution and by gassing it with 5% CO_2–95% O_2 or with O_2.

In the second experiment, only the total concentration of NaCl in the secretory solution was varied, other factors remaining constant; this was designed to test the effect of the concentration of Na^+ on the potential differences.

Results

The results are not given in full but an abstract of the conditions and readings are given in the tables. Table 1 summarizes the results of the first experiment. Under the headings of 'AgCl-AgCl' and

Table 1. *Potential differences, measured across resting mucosa, with variation of* [Cl^-] *and* pH *of the secretory solution*

(Nutrient solution [Cl^-] = 0·095 M; pH is 7·4 throughout.)

Secretory solution		Potential difference (electrode in nutrient solution = 0 mV.) mV.				
			AgCl-AgCl		Glass-glass	
[Cl^-] *M*	pH	Calomel-calomel	Observed	Calculated	Observed	Calculated
0·095	7·4	−22	−20	−20	—	−20
	7·50	−19, −20	−15	−20	−23, −24	−26
	7·81	—	—	—	−40, −39	−44
	7·89	—	—	—	−41	−49
	8·40	−18, −19	−18	−20	−77, −79	−79
0·012	5·25–5·5	−22	+30	+33	+93	+98
	5·55	−22	+29	+33	+90	+89
	5·60	−22	+34	+33	+84	+86
0·0012	5·50	−22	+91, +87, +90	+92	+88	+92

'glass-glass' are given the observed differences of potential and also those calculated by the equations:

$$\text{P.D.} = 59\cdot1 \log \frac{[\text{Cl}^-]\ (\text{non-secretory side})}{[\text{Cl}^-]\ (\text{secretory side})} - 20\ \text{mV.},$$

and

$$\text{P.D.} = 59\cdot1\ [\text{pH (non-secretory side)} - \text{pH (secretory)}] - 20\ \text{mV.}$$

The agreement throughout between the observed and predicted values is reasonably close.

Table 2. *Potential differences, measured across resting mucosa, with variation of* $[Na^+]$ *of the secretory solution*

(Nutrient solution: $[Cl^-]=0\cdot095$ M; $[Na^+]=0\cdot11$ M; $[K^+]=0\cdot0045$ M.)

Secretory solution		Potential difference mV.			
			AgCl-AgCl		
$[Na^+]$ M	$[Cl^-]$ M	Calomel-calomel	Observed	Expected	Difference
0·12	0·12	−43	−47	−49	—
0·012	0·012	−44, −40, −38	+ 5	+12	− 7
0·0012	0·0012	−50	+32	+71	−39
0·12	0·12	−30	−34	−36	—

Table 2 summarizes the results of an experiment designed to measure the effect of changing the concentration of Na^+ in the secretory solution on the 'asymmetry' potential of the mucosa. It had been noted in previous experiments that the potential difference measured between calomel electrodes tends to become more negative if $[Na^+]$ is reduced as well as $[Cl^-]$ in the secretory solution. The column 'AgCl-AgCl expected' gives the potential difference which should have been obtained between silver chloride electrodes, were the only controlling factor (as in Table 1) the value of $[Cl^-]$ in the secretory solution: the column 'Difference' gives the difference of the observed value from this expected value. In both cases, this difference is negative, by 9 mV. and by $(39-7)=32$ mV. for the two intervals of concentration of Na^+. It is possible, in view of the changing state of the mucosa, revealed by the change of calomel-calomel and silver chloride-silver chloride potential differences on return to 0·12 M-sodium chloride, that the values of these differences are too high, but it seems clear that an increasingly negative 'asymmetry potential' is obtained as the concentration of Na^+ on the secretory side is lowered.

In an earlier experiment, the sodium chloride solution on the secretory side was replaced successively by isotonic sodium nitrate, sodium sulphate, potassium chloride, calcium chloride and phosphate buffer (pH 7·3): the potential difference between calomel-potassium chloride electrodes was in each case the same, within a few mV.; the potential between calomel-potassium chloride electrodes was also independent of changes in the pH of the nutrient solution.

THEORETICAL

General conditions required for the secretion of ions

'Secretion' of ions here implies the net movement of electrolyte from lower to higher chemical potential.

A single type of ion cannot be secreted alone to any detectable extent, since it is necessary that electrical neutrality should be maintained on both sides of the secreting structure, by the equivalent movement either of ions of opposite sign in the same direction or of ions of the same sign in the opposite direction.

To effect secretion, it is necessary that a constraint of some sort should be applied to at least one of the ions transported and the ion to which such a constraint is applied may be said to undergo 'primary' secretion. The consequent movement of other ions, essentially electrolytic in nature (to maintain electrical neutrality) may be termed 'secondary'. The primary secretion cannot be simply electrolytic, in a wholly aqueous medium; for the charge separation needed to cause electrolytic migration to occur must itself be a result of the previous transport of ions.* Thus neither the maintenance of a difference of redox potential in a metallic system, nor a Donnan distribution across a membrane, can lead to continuous secretion (cf. Hollander, 1943; footnote to p. 417).

Primary secretion must therefore involve the generation of the ion from a precursor in which it is chemically bound, either *de novo*, or following the previous formation of the precursor from the free ion and a carrier substance at some other site. In the former case, the chemical energy required may be resident in the precursor; in the latter it must be made available either at the formation or breakdown of the precursor, or both. These two mechanisms are illustrated in Fig. 1.

It follows that primary secretion can involve only such ions as are capable of being bound chemically,

* Even 'electron transfer', as through a ferrous-ferric system, is equivalent to the transport of ions, since one positive ionic charge necessarily appears for each electron lost.

and this is likely to exclude Cl^-, Na^+, K^+ and probably Ca^{++} from primary secretion. Examples of substances and processes which might play a part in primary secretion are: (1) H^+, by reduction to hydrogen atoms and their combination with a carrier; (2) phosphate, by esterification; (3) NH_4^+, by amination or amide formation as ammonia; (4) HCO_3^- by combination of carbon dioxide by carboxylation or formation of a carbamino compound. The last three may also involve, indirectly, the transport of H^+.

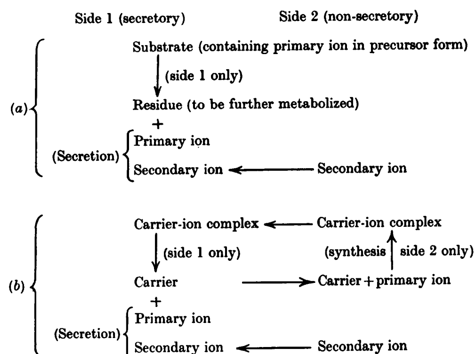


Fig. 1. General schemes showing the mechanisms of secretion (*a*) when the primary ion is derived from a substrate, and (*b*) when the primary ion is initially uncombined.

The ion which undergoes primary secretion need not, in the final state, undergo net secretion, since it may be replaced, after undergoing primary secretion, by exchange with another ion of the same sign: this may be regarded as a 'tertiary process'. In this way it is possible for an electrolyte to be secreted without either of its ions undergoing primary secretion. For example, the primary secretion of H^+, with secondary secretion of Cl^-, followed by tertiary exchange of H^+ and Na^+ (across a structure impermeable to Cl^-), would result in the net secretion of sodium chloride.

Some selective permeability of the structure across which secretion is to take place must always be postulated; it must be relatively impermeable to the primary ion in its free state, so that a rapid backward leakage does not occur, and selectively permeable to the secondary ion.

GASTRIC SECRETION

Summary of evidence

This summary is derived from evidence presented in this paper, and by Davies (1948*a*, *b*), Crane *et al.* (1948*a*, *b*) and Crane & Davies (1948).

The main experimental facts which any theory must account for are:

(1) The mucosa can secrete acid continuously for long periods, doing secretory work at a rate of the order of 10,000 cal./g. mol. of HCl.

(2) The secretory process depends upon respiration and is interfered with by any agent which inhibits respiration.

(3) The value of the ratio of g. mol. of HCl secreted to g. mol. of extra O_2 used during secretion ($q_{HCl}/|q_{O_2}|$) may be as high as 11 and such a value may be maintained for a considerable period of time.

(4) For each mol. HCl secreted, there is normally an uptake, on the non-secreting side of the mucosa, of one mol. CO_2, leading to the formation of one mol. HCO_3^-. This process probably requires the activity of carbonic anhydrase (Davies & Roughton, 1948; Davies & Edelman, 1948).

(5) (*a*) The resting mucosa can maintain a potential difference between identical electrodes in identical solutions on the two sides of the mucosa for long periods, even when current is allowed to pass freely between the electrodes in an external circuit. Thus the resting mucosa is capable of the continuous performance of electrical work. (*b*) The potential difference between two calomel electrodes across the resting mucosa is constant and is usually 20–30 mV., the secretory side being negative in the external circuit. The potential difference between two silver-silver chloride or between two glass electrodes, varies systematically with the concentration of Cl^- and H^+, respectively, in the secretory solution, but in both cases there is included an asymmetry potential of the same sign and magnitude as is observed with calomel electrodes. The magnitude of this asymmetry potential varies with the concentration of alkali-metal cation on the secretory side.

(6) Passage of current through the mucosa between zinc-zinc acetate electrodes increases or decreases the secretion of acid by a secreting mucosa according as the applied current augments or diminishes the spontaneous potential measured between calomel electrodes on either side of the mucosa.

(7) Histamine can cause the change between resting and secreting states to occur. When secretion begins, there is an increase of oxygen uptake and a decrease of the asymmetry potential.

Mechanisms of secretion

It is simplest to assume that H^+ is the primary ion and that Cl^- is secreted secondarily. One possible mechanism (mechanism 1) for primary secretion of H^+, by oxidation of the hydrogen of substrate, has already been mentioned; although this is quantitatively inadequate, there is no reason to suppose that it does not occur, but it must be supplemented by another (mechanism 2).

Mechanism 1. The essentials of this mechanism are shown in Fig. 2. A redox system is required which can transfer electrons across a structure which

separates two regions of the cell: this system oxidizes hydrogen of substrates to H^+ and reduces an equivalent amount of oxygen to OH^-, these processes occurring on opposite sides of the structure which is impermeable to H^+, OH^- and to HCO_3^- formed from it. The substance oxidized by this system is likely not to be the ultimate substrate of the cell, but rather a

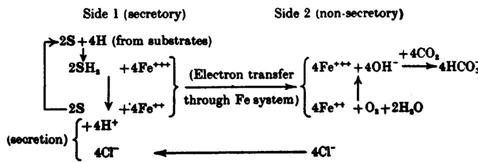
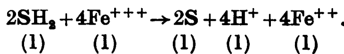


Fig. 2. Scheme of the secretion of HCl by mechanism 1, where the hydrogen is derived from the substrate.

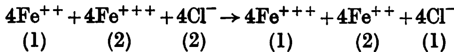
carrier SH_2, such as a flavoprotein; the most likely redox system is cytochrome *c* and cytochrome oxidase. Thus, the reactions are as follows, the subscripts indicating in which region they occur:

Oxidation of SH_2 on side 1:



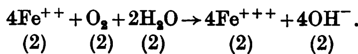
$$\underset{(1)}{2SH_2} + \underset{(1)}{4Fe^{+++}} \rightarrow \underset{(1)}{2S} + \underset{(1)}{4H^+} + \underset{(1)}{4Fe^{++}}.$$

Oxido-reduction between sides 1 and 2, with transport of Cl^- to maintain electrical neutrality:



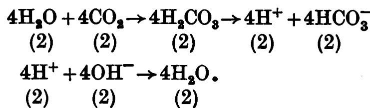
$$\underset{(1)}{4Fe^{++}} + \underset{(2)}{4Fe^{+++}} + \underset{(2)}{4Cl^-} \rightarrow \underset{(1)}{4Fe^{+++}} + \underset{(2)}{4Fe^{++}} + \underset{(1)}{4Cl^-}$$

Reduction of O_2 on side 2:



$$\underset{(2)}{4Fe^{++}} + \underset{(2)}{O_2} + \underset{(2)}{2H_2O} \rightarrow \underset{(2)}{4Fe^{+++}} + \underset{(2)}{4OH^-}.$$

Reaction of OH^- with CO_2: the reaction of OH^- with CO_2 takes place in the cytoplasm of the acid-secreting cell, according to the reactions (Davies & Roughton, 1948):

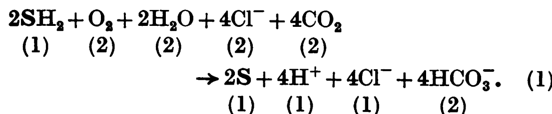


$$\underset{(2)}{4H_2O} + \underset{(2)}{4CO_2} \rightarrow \underset{(2)}{4H_2CO_3} \rightarrow \underset{(2)}{4H^+} + \underset{(2)}{4HCO_3^-}$$

$$\underset{(2)}{4H^+} + \underset{(2)}{4OH^-} \rightarrow \underset{(2)}{4H_2O}.$$

Once a steady state of secretion and of exchange of HCO_3^- with Cl^- of the nutrient fluid (i.e. with blood *in vivo*) is established, these reactions occur *effectively* at the pH of the nutrient fluid.

The overall reaction is:



$$\underset{(1)}{2SH_2} + \underset{(2)}{O_2} + \underset{(2)}{2H_2O} + \underset{(2)}{4Cl^-} + \underset{(2)}{4CO_2} \rightarrow \underset{(1)}{2S} + \underset{(1)}{4H^+} + \underset{(1)}{4Cl^-} + \underset{(2)}{4HCO_3^-}. \quad (1)$$

Since H^+ and OH^- must be produced exclusively on opposite sides of the structure, it follows that the cytochrome system must be oxidizable by oxygen only on side 2, and that SH_2 must be oxidizable by the cytochrome system only on side 1. Equation (1) shows that mechanism 1 cannot produce more than $4H^+$ per O_2, whatever the source of the hydrogen.

Mechanism 2. Since mechanism 1 exploits fully the power of the oxidative system to form H^+ from the hydrogen of substrates, and since no anion other than OH^- (or HCO_3^-) is formed, it follows (Crane *et al.* 1948*a*) that any second mechanism must secrete preformed H^+, that is, H^+ derived from hydrogen atoms in water by ionization. It is again simplest to assume the primary secretion of H^+. The energy of dehydrogenation reactions is not likely to be directly available, but can be made available in the form of unstable phosphate compounds, such as creatine phosphate (see Ogston & Smithies, 1948).

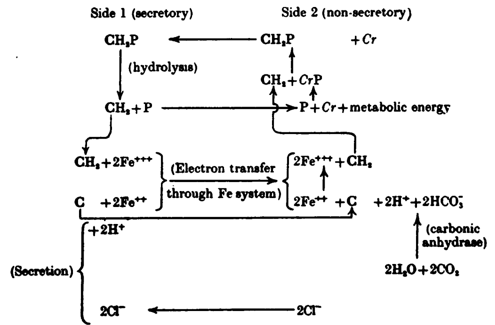
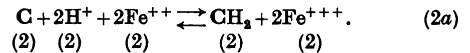


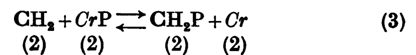
Fig. 3. Scheme of the secretion of HCl by mechanism 2, where the hydrogen is ultimately derived from water.

The essentials of mechanism 2 are shown in Fig. 3. A carrier substance C undergoes the reaction, on side 2:



$$\underset{(2)}{C} + \underset{(2)}{2H^+} + \underset{(2)}{2Fe^{++}} \rightleftharpoons \underset{(2)}{CH_2} + \underset{(2)}{2Fe^{+++}}. \quad (2a)$$

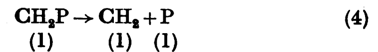
If, as is likely, the standard redox potential of the carrier system is considerably lower than that of the ferric system, no considerable amount of CH_2 can be formed by reaction (2*a*). However, phosphorylation of CH_2 by creatine phosphate (*Cr*P), on side 2:



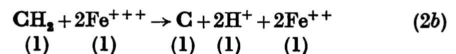
$$\underset{(2)}{CH_2} + \underset{(2)}{CrP} \rightleftharpoons \underset{(2)}{CH_2P} + \underset{(2)}{Cr} \quad (3)$$

can cause considerable accumulation of CH_2P, provided that the free energy of breakdown of *Cr*P is sufficiently greater (more negative) than that of CH_2P.

The accumulation of CH_2P on side 2 causes it to diffuse across the separating structure to side 1. Here it loses phosphate with liberation of CH_2, which then undergoes reaction (2*a*) in the reverse direction.

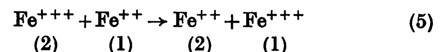


$$\underset{(1)}{CH_2P} \rightarrow \underset{(1)}{CH_2} + \underset{(1)}{P} \quad (4)$$



$$\underset{(1)}{CH_2} + \underset{(1)}{2Fe^{+++}} \rightarrow \underset{(1)}{C} + \underset{(1)}{2H^+} + \underset{(1)}{2Fe^{++}} \quad (2b)$$

C and phosphate diffuse back to side 2. The ferric system transfers electrons across the structure:



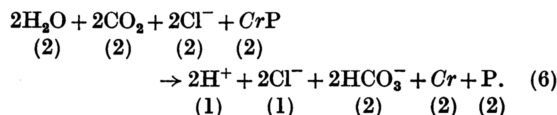
$$\underset{(2)}{Fe^{+++}} + \underset{(1)}{Fe^{++}} \rightarrow \underset{(2)}{Fe^{++}} + \underset{(1)}{Fe^{+++}} \quad (5)$$

and Cl^- diffuses from side 2 to side 1.

The immediate source of the H^+ ions removed from side 2 can be any or all of the acid components

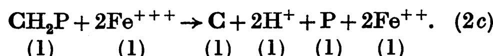
of the cytoplasmic buffer system (e.g. water, protein, H_2CO_3, $H_2PO_4^-$, etc.). Once a steady state of secretion is established, the uptake of carbon dioxide and formation of HCO_3^- balance the removal of H^+, whose ultimate source is therefore water. The formation of HCO_3^-, as in mechanism 1, occurs effectively at the pH of the nutrient fluid (blood).

Thus the total reaction is:



$$\underset{(2)}{2H_2O} + \underset{(2)}{2CO_2} + \underset{(2)}{2Cl^-} + \underset{(2)}{CrP} \rightarrow \underset{(1)}{2H^+} + \underset{(1)}{2Cl^-} + \underset{(2)}{2HCO_3^-} + \underset{(2)}{Cr} + \underset{(2)}{P}. \quad (6)$$

In this scheme it must be postulated that the separating structure is relatively impermeable to CH_2, as compared with CH_2P and C; otherwise reaction (2*b*) could occur on side 2 instead of on side 1. An alternative postulate would be that CH_2P does not simply hydrolyse on side 1, but that the enzyme on side 1 which catalyses the reverse of reaction (2*a*) is specific for CH_2P, so that,



$$\underset{(1)}{CH_2P} + \underset{(1)}{2Fe^{+++}} \rightarrow \underset{(1)}{C} + \underset{(1)}{2H^+} + \underset{(1)}{P} + \underset{(1)}{2Fe^{++}}. \quad (2c)$$

In either case, CH_2P must be degraded relatively slowly on side 2.

Thermodynamic relationships

In this section the quantitative data used are those quoted by Ogston & Smithies (1948), unless reference is made to other sources.

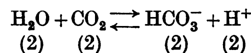
General. The free energy required to secrete hydrochloric acid at a concentration isotonic with blood is of the order of 10,000 cal./mol. (Crane & Davies, 1948). The free energy of oxidation of glucose by atmospheric oxygen under physiological conditions is about −58,000 cal./atom of oxygen. Thus the thermodynamic maximum value of $q_{HCl}/|q_{O_2}|$ would be about 12.

Ogston & Smithies (1948) have concluded that the total free energy of oxidation of substrates is divisible into two parts according to the oxido-reduction mechanism which produces it and its position in the scale of redox potentials; the free energy liberated in the transfer of hydrogen to the cytochrome system ($E'_h < 0{\cdot}26$ V.) can lead to the phosphorylation of creatine, while that available from oxidation within the cytochrome system (E'_h between $+0{\cdot}26$ and $+0{\cdot}82$ V.) is not known to lead to phosphorylation. This division fits in well with the two postulated mechanisms of secretion.

Mechanism 1. The free energy available from an oxido-reduction process is measured by the interval of redox potential over which the process occurs; an interval of 1 V. corresponding with the evolution of 23,100 cal./g. electron. In this case it is supposed that oxidation occurs between atmospheric oxygen at $+0{\cdot}82$ V. (the potential of the oxygen electrode at pH 7·0) and cytochrome *c*, at its standard redox potential, $E'_h = +0{\cdot}26$ V. at pH 7·0: this interval of 0·56 V. is equivalent to an evolution of 12,900 cal./g. electron, that is, per g.ion of H^+ and this is amply sufficient for the secretory process.

Mechanism 2. The immediate source of energy is the hydrolysis of creatine phosphate: its standard free energy at 37° is −16,700 cal./mol. and this is unlikely to be very different at 25°; but at a concentration of phosphate of 14 mM., and assuming a ratio of $CrP/Cr = 1$, the actual free energy becomes −19,000 cal./mol.*

Assuming that, in reaction (6), the reaction



$$\underset{(2)}{H_2O} + \underset{(2)}{CO_2} \rightleftarrows \underset{(2)}{HCO_3^-} + \underset{(2)}{H^+}$$

is at equilibrium and neglecting activity coefficients since the secretion is isotonic with blood, the free energy change in reaction (6) is

$$\Delta F_{(6)} = RT \ln \frac{[H^+_{(1)}]\,[Cl^-_{(1)}]}{[H^+_{(2)}]\,[Cl^-_{(2)}]} - \frac{1}{2} \times 19{,}000 \text{ cal./mol. HCl}$$

$= 0$ for secretion of HCl at the maximum possible concentration.

Taking $[H^+_{(2)}]$ for the nutrient fluid $= 10^{-7\cdot3}$M and $[Cl^-_{(2)}] = 10^{-1}$M, this gives at 37°

$$[H^+_{(1)}] = [Cl^-_{(1)}] = 10^{-0\cdot79} \text{ or pH} = 0{\cdot}79,$$

which is equivalent to 0·162M-HCl. This value depends on the values assumed for the pH of the nutrient fluid and for the concentration of phosphate; the value used for the latter is the resting concentration of frog muscle.

Thus the hydrolysis of 1 mol. of *Cr*P is capable of providing enough free energy to secrete 2 mol. of HCl at the order of concentration at which it is produced by oxyntic cells (Babkin, 1944).

The total free energy of oxidation of glucose, less the amount expended by mechanism 1, is 32,000 cal./atom of oxygen. The maximum P/O ratio for phosphorylation of creatine would thus be 32,000/19,000 = 1·7 and the maximum value of $q_{HCl}/|q_{O_2}|$ would be $4 \times 1{\cdot}7 = 6{\cdot}8$.

Some light may be thrown on the components of mechanism 2 by considering its kinetic requirements in relation to thermodynamic quantities. Thus, for the reactions on side 2 to proceed rapidly, the steady-state ratios $[CH_2P]/[C]$ and $[Fe^{+++}]/[Fe^{++}]$ should not differ greatly from unity. If the system on side 2 is further assumed to be near equilibrium, under the conditions stated, then the sum of the free energies of reactions (2*a*) and (3) is zero: this sum is given by the sum of the standard free energies of the phosphorylation processes (free phosphate is not involved in these reactions) and of the oxido-reduction

* Ogston & Smithies (1948), in considering the probable amount of phosphorylation accompanying the oxidation of pyruvate, used the value −16,700 for the free energy of hydrolysis of *Cr*P. Had they taken the concentration of phosphate into consideration, their predicted values of P/O ratio would have been lower still.

processes. Assuming that the standard free energy of hydrolysis of CH_2P is -3000 cal. (i.e. that this is a 'low energy' phosphate compound) and that $\Delta E_0'$ is the difference of the standard redox potentials of the ferric and the $C\text{-}CH_2$ systems, then

$$2F\Delta E_0' - 16{,}700 + 3000 = 0$$

(F is the Faraday equivalent of electric charge) so that $\Delta E_0' = 0{\cdot}30$ V. This value would suggest that either cytochrome b ($E_0' = -0{\cdot}04$ V.: Ball, 1938) and malate-oxaloacetate ($E_0' = -0{\cdot}17$ V.) or cytochrome c ($E_0' = +0{\cdot}26$ V.) and fumarate-succinate ($E_0' = 0$) might suitably act as the ferrous-ferric carrier combination.

Combination of mechanisms 1 *and* 2

Mechanisms 1 and 2 might conceivably act together in three different ways.

(1) In series, such that one supplies H^+ to be further concentrated by the other. The value of $q_{HCl}/|q_{O_2}|$ of this arrangement would be limited to 4, but a concentration of H^+ of the order of 10^{12}-fold could be produced. There is no reason to think that this arrangement occurs in nature. Two secreting structures separating three regions of the cell would be required.

(2) In series, but back-to-back, so that one mechanism supplies H^+ to the secretory side of the other; this is identical with the 'in parallel' arrangement (3), if escape of H^+ from the cell cannot occur. This is an interesting possibility, because, each mechanism being reversible, mechanism 1 could drive mechanism 2 backwards, leading to phosphorylation of creatine and this might explain how oxidation in the cytochrome system at high E_h' could bring about phosphorylation (Ogston & Smithies, 1948). Friedkin & Lehninger (1948) have shown that phosphorylation can accompany the oxidation of dihydrocoenzyme I by cytochrome c, in a liver-particulate system: it is doubtful, however, whether such a degree of organization as would be required by our mechanism could have survived in such a preparation.

(3) In parallel, each mechanism contributing additively to the secretion of H^+. It has been shown that each mechanism is thermodynamically adequate. The sum of their maximum yields ($4 + 6{\cdot}8$) is of the same order as the observed maximum of $q_{HCl}/|q_{O_2}|$. There are, however, certain further limitations on the distribution and reaction of components between the regions of the cell, if the two mechanisms are to work successfully side by side.

(i) The oxidation of hydrogen (i.e. of SH_2) must occur only on side 1 and the reduction of oxygen only on side 2.

(ii) The phosphorylation of creatine, resulting from the transfer of hydrogen from substrate to flavoprotein (**S**), can occur anywhere in the cell, provided that *Cr*P can transfer phosphate to the carrier **C** (mechanism 2) only on side 2.

(iii) The ferrous-ferric system of mechanism 2 must not be accessible to oxidation by O_2 and so must not be coupled with the ferrous-ferric system of mechanism 1: otherwise its redox potential would tend to rise too high to allow sufficient formation of CH_2 on side 2. This consideration would perhaps exclude the cytochrome c-fumarate-succinate system from acting in mechanism 2.

Resting and secreting states

From the facts that the oxygen utilization of resting mucosa is fairly high and that the resting mucosa is capable of developing continuous electrical work in an external circuit (up to 10% of its total metabolic energy), we assume that the processes which lead to secretion are still active in the resting state, but do not actually lead to secretion. Potential measurements (see below) show that the resting mucosa, as a whole, is effectively impermeable both to H^+ and to Cl^- and the impermeability of the outer membrane of the cell would be a sufficient cause for non-secretion. If the secretory mechanism were still active in the resting state, the result would be to produce an accumulation of HCl within the region of the cell called 'side 1' (Figs. 2, 3). It is reasonable to suppose that the structure across which secretion takes place is not completely impermeable to H^+, so that there would be a continuous backward leakage of H^+ from side 1 to side 2 and the metabolic energy of the cell would be required to maintain a steady-state excess of HCl on side 1.

The onset of secretion must involve the cell outer membrane becoming permeable to H^+ and to Cl^- and the acquisition of this permeability would alone be sufficient to cause secretion to occur. At the same time, the loss of HCl from side 1 of the interior of the cell removes a constraint on the oxidative mechanisms, so that an increase of oxygen uptake would ensue automatically. It is possible, also, that the permeability of the secretory structure to H^+ and to Na^+ (see below) might be reduced at the same time, which would increase the efficiency of secretion; Cope, Cohn & Brenizer (1943) have shown that radioactive Na^+ is absorbed more rapidly by the resting than by the secretory stomach. It seems possible, therefore, that the action of histamine is to affect the permeability of cellular structures and so to allow H^+ to pass out of the cell (cf. Crane *et al.* 1948*a*).

It is of interest, in this connexion, that thiocyanate, in concentrations of 10^{-3} to 10^{-2} M, inhibits both the uptake of iodide by the thyroid (Franklin, Chaikoff & Lerner, 1944) and the secretion of acid by the gastric mucosa (Davenport, 1940; Crane *et al.* 1948*a*); it is possible that both actions are due to the prevention of halide ions from entering cells.

POTENTIAL MEASUREMENTS

General

When two electrodes are present in any system and a potential difference between them can be observed, this potential difference represents the electrical work which is

done when a unit of electric charge is allowed to pass between the electrodes. This work must be derived from changes of free energy taking place within the system. When the whole system is reversible, the amount of electrical work can be equated to this change of free energy, but where irreversible changes take place as, for example, through free diffusion at a junction between two ionic solutions, this exact relationship does not necessarily hold good. Also, changes which do not affect the electrodes can occur without contributing to the electromotive force of the cell.

In attempting to interpret potential measurements, it is important to use this approach whenever it is possible to do so, and not to treat the electromotive force of a cell as the sum of a number of 'single potentials' (e.g. potentials of electrode against solution, diffusion or membrane potentials). It has been repeatedly pointed out that such single potentials can neither be measured nor defined and, though an argument based on their use may often lead to the right conclusion, it cannot be relied upon to do so.

Unfortunately, it is the common practice to measure potentials with electrode systems which introduce unnecessary liquid junctions: the ionic transport across such junctions can usually not be calculated, so that it becomes necessary to use the 'single potential' approach.

Valuable information can be obtained about systems which contain membranes selectively permeable to different ions, by the use of simple reversible electrodes. The general conditions which govern the potential difference between a pair of such electrodes are as follows.*

(1) If two phases are separated by a membrane impermeable to all ions and one electrode is in each phase, no definable or reproducible potential is observed.

(2) If the membrane is permeable to some ion or ions and the two phases are at equilibrium with respect to those ions, then (*a*) zero difference of potential is found with electrodes reversible to any ion to which the membrane is permeable; (*b*) a finite and reproducible potential difference is observed with electrodes reversible to an ion to which the membrane is impermeable; as a special case, the potential difference is zero if this ion is distributed as it would be at equilibrium, were the membrane permeable to it. In this case, the potential difference measures the free energy of transfer of electrolyte from one phase to the other, which results from the reaction of the reversible ion at the electrodes.

(3) If two phases are not at equilibrium with respect to ions which can move from one phase to the other, and if passage of current between a pair of electrodes, one in each phase, facilitates the transfer of ions with respect to which the disequilibrium exists, then a reproducible potential is observed. The magnitude of this potential depends upon the mobilities of all ions which are transported when current passes between the electrodes, and can be calculated only in simple cases. (This case (3) is equivalent to 2 (*b*), if the transport number of the ion to which the electrodes are reversible is zero.)

The considerations outlined above do not indicate the sign of the potential difference; to determine this it is necessary to discover in which direction current must pass between the pair of electrodes used so as to diminish the free energy of the system.

* The conditions under which a potential difference is observed between a pair of electrodes in two phases in membrane equilibrium were given incorrectly by Ogston (1947, p. 234) and are here corrected.

Resting gastric mucosa

When resting frog gastric mucosa has identical solutions on its two sides, there is found to be a potential difference between a pair of silver chloride or glass or calomel electrodes of about 30 mV., the secretory side being negative in an external circuit. If the concentration of Cl^- on the secretory side is changed, the potential difference between a pair of silver chloride electrodes is independent of pH and is closely given by

$$\frac{2{\cdot}303\,RT}{F}\log\frac{[Cl^-]\text{ nutrient}}{[Cl^-]\text{ secretory}}-0{\cdot}03\text{ V.}$$

This shows that the resting mucosa is effectively impermeable to Cl^-: the result of passage of 1 g. ion equivalent of electricity between the electrodes is to transfer 1 g.ion of Cl^- from one side to the other and there is an equivalent transport of Na^+ through the membrane, the concentration of Na^+ being the same on both sides. Similarly, the potential difference between a pair of glass electrodes is independent of the concentration of Cl^- and is given by

$$\frac{2{\cdot}303\,RT}{F}\log\frac{[H^+]\text{ secretory}}{[H^+]\text{ nutrient}}-0{\cdot}03\text{ V.}$$

It follows that the resting mucosa is also effectively impermeable to H^+.

Patterson & Stetten (1949) measured independently the potential difference across acid-secreting rat gastric mucosa, with glass electrodes and stated: 'It was repeatedly observed that the pH difference, as computed from the total potential difference, and as calculated from individual determinations of pH on both sides of the membrane, agreed within 0·2 pH unit.' This shows that the potential difference between calomel electrodes which was not determined (Stetten, 1949) could not have been more than 12 mV. Such low values of the potential difference between calomel electrodes are usually found with partially anoxic or damaged isolated mammalian gastric mucosa (Davies, 1948*b*).

The variations of potential differences between these pairs of electrodes with composition of the secretory solution represent changes of the free energy of ionic transport between the outer solutions. The constant 'asymmetry potential' of about -30 mV. must represent a process occurring within the mucosa independently of changes in the composition of the secretory solution.

The effective impermeability of the whole mucosa to H^+ and Cl^- is likely to be due to its external membrane. This membrane is permeable to Na^+ and so will behave as a pair of reversible sodium electrodes with respect to the interior of the cell: formation or removal of H^+ or Cl^- by the external electrodes can result only in a corresponding movement of Na^+ into and out of the cell. In the case where the external

solutions are identical, the whole electromotive force of the system (-30 mV.) must therefore arise from changes in an electrolytic cell of this type (Fig. 4).

Now, if this represented simply a Donnan distribution of Na^+ and Cl^-, as a result of the presence of a fixed excess of H^+ on side 1, then no difference of potential could arise between the pair of sodium electrodes. But if H^+ can be transferred backwards (whether through the secretory mechanism or by 'leakage' through the separating structure), then the passage of current between the electrodes will affect this transfer; the cell is not in membrane equilibrium but is a 'concentration cell with liquid junction', whose composition is continuously maintained by the secretory mechanism. Equilibrium can be approached by transfer of H^+ from side 1 to side 2: evidently the addition of Na^+ to side 1 and its withdrawal from side 2 facilitates this transfer of H^+. Thus the condition is satisfied that passage of current between the external electrodes, such that that on the secretory is negative, facilitates the approach to equilibrium. Since this is not a reversible system, and since the values of the mobilities of the ions concerned are not known, no quantitative prediction of the magnitude of this e.m.f. can be made: but it is within the limits commonly observed in 'diffusion potentials' (cf. the values observed by Crane *et al.* 1948*a*, across a cellophan membrane).

Side 1 (secretory) — Side 2 (non-secretory)

(Secretory mechanism)

$H^+ \xrightarrow{>} H^+$

Na:Na^+ — $Cl^- \xrightarrow{>} Cl^-$ — Na^+:Na

$Na^+ \xleftarrow{<} Na^+$

Fig. 4. Diagram of the resting oxyntic cell, regarded as a 'concentration cell, with liquid junction', using reversible sodium electrodes. The sign $>$ indicates differences of concentration between the two sides, and the arrows the directions of movement of ions.

The system becomes more complicated if K^+ or Ca^{++} is substituted for Na^+ on the secretory side. The unchanged potential difference observed between calomel electrodes presumably means that these ions are closely similar to Na^+ with respect to their rate of migration through the cellular structure; the mucosa has, in fact, been found to be easily permeable by a variety of cations, including those of dyes (Visscher, 1942; Höber, 1945).

Reduction of the concentration of Na^+ in the secretory solution has been shown to increase the magnitude of the asymmetry potential. This is explained if the concentration of Na^+ within the oxyntic cells is thereby lowered, for this will decrease the transport number of Na^+ and increase that of H^+, resulting in a more negative 'diffusion potential' arising within the cells. Such a reduction of the concentration of Na^+ within the cells would necessarily imply that the cells are somewhat permeable to some anions; but this is not inconsistent with the conclusion, reached above, that the mucosa is effectively impermeable to H^+ and to Cl^-, since this means only that it is far less permeable to these ions than to Na^+ or K^+.

Secreting gastric mucosa

When the mucosa passes into the secreting state, the outer walls of the cells must become permeable to H^+ and to Cl^-. Since the increase of H^+ in the outer fluid is normally accompanied by an equivalent increase of Cl^-, the process of tertiary exchange of H^+ against Na^+ must be relatively slow. But it is not necessary that there should be a decrease of permeability to Na^+; the relatively high electrical resistance of the resting mucosa shows that this is never very great. The rate of back-diffusion of H^+ through the secreting structure likewise need not be great. The removal of hydrochloric acid from the interior of the cell will tend to reduce the 'diffusion potential' across the secreting structure, so that, as is observed, the potential differences between external calomel electrodes should fall.

The effect of an external source of current on the rate of secretion of active mucosa is probably simply electrolytic in nature: with the secretory electrode negative, the system will behave as a Hittorff cell, provided that the transport number of H^+ through the mucosa is greater than 0·5, and will concentrate hydrochloric acid on the secretory side. The failure to obtain electrolytic formation of acid using resting mucosa and other membranes (Crane *et al.* 1948*b*) was probably due to their low permeability to H^+.

Other types of ionic secretion

Although the evidence about other types of ionic secretion is less complete, it seems likely that all will show features in common. (For reviews see Ussing, 1949*b*; Davies, 1948*b*.) Thus where a neutral salt is secreted it is possible, in most cases, to assume that an ion such as H^+, NH_4^+, HCO_3^- or $H_2PO_4^-$ may undergo primary secretion and may then effect a tertiary exchange with another cation or anion, as the case may be (cf. Kinsey, 1949; Davson, Duke-Elder, Maurice, Ross & Woodin, 1949; Lundegårdh, 1945; Robertson & Wilkins, 1948*a*, *b*; Hoagland, 1948). This hypothesis makes no demand upon unknown sorts of chemical process, though it does imply a mosaic arrangement or an alternating function of the structures concerned. Similarly, it is possible to modify the interpretation put by Ussing (1949*a*) upon his experiments with frog skin by assuming that the transport of Na^+ is secondary to that of H^+, rather than the reverse.

The secretion of acid and the absorption of bicarbonate by the tubules of the kidney (Pitts, 1945; Pitts & Lotspeich, 1946*a*, *b*; Pitts, Ayer & Schiess, 1948) could occur by the gastric mechanism, a greater flexibility with regard to anions being due either to a variable secondary secretion or to a variable tertiary exchange. The secretion of bicarbonate by the pancreas is likely to depend on the gastric mechanism working in reverse (Davies, 1949).

The transport of ions between the interior and exterior of cells, across a single-cell membrane, appears to be the simplest case. Conway & O'Malley (1946) showed that yeast may secrete H^+ into the external medium in exchange for K^+ and the 'acidic region' which they postulate, and the membrane separating this from the rest of the cell, might well correspond with the 'side 1' and the 'secreting structure' of our model. In other cases, as where Na^+ appears to be actively extruded, there is insufficient evidence to decide whether its primary secretion must be assumed.

SUMMARY

1. Evidence is presented of the differences of potential observed between pairs of calomel, silver chloride and glass electrodes when one of a pair is in the 'nutrient' solution, the other in the 'secretory' solution, of isolated, non-secreting gastric mucosa of the frog. The values of these potential differences and their variation with composition of the secretory solution have been interpreted in terms of the permeability of the mucosa to ions.

2. An extended theory of the chemical and physicochemical mechanism of gastric secretion has been developed, in the light of present knowledge of the chemistry and thermodynamics of cellular processes. This is shown to be consistent with the evidence available concerning gastric secretion.

3. In the light of this theory, some other cases of ionic secretion are discussed.

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