can combine with the enzyme. It was apparently decarboxylated very slowly by acetone-dried preparations of *Strep. faecalis* R, and it caused slight decreases in the rates of decarboxylation of tyrosine by these preparations; but these effects may have been within the limits of experimental error.

As a result of the experiments of Epps (1944) it is evident that the tyrosine decarboxylase of *Strep. faecalis* attacks both tyrosine and 3:4-dihydroxyphenylalanine. The experiments described in the present paper have not been done with pure preparations of the enzyme, but the evidence presented suggests that *m*-hydroxyphenylalanine is in fact a substrate of tyrosine decarboxylase. It is evident that the replacement of the para hydroxyl group of tyrosine by a hydroxyl group in the meta position relative to the side chain produces a substance which is still decarboxylated by *Strep. faecalis*; on the other hand, our experiments with *o*-hydroxyphenylalanine have shown that the rate of decarboxylation of this compound by tyrosine decarboxylase is very low.

SUMMARY

1. Acetone-dried preparations of *Strep. faecalis* R decarboxylate *m*-hydroxyphenylalanine at about 30% of the rate at which they decarboxylate tyrosine. The reaction appears to be specific for the L-form of *m*-hydroxyphenylalanine, which is decarboxylated quantitatively to *m*-hydroxyphenylethylamine. Washed suspensions of intact resting cells of the organism have no detectable action on *m*-hydroxyphenylalanine.

2. Dried preparations of *Strep. faecalis* R do not decarboxylate *o*-hydroxyphenylalanine at a significant rate, nor has this amino-acid any detectable affinity for tyrosine decarboxylase.

3. Neither dried preparations nor intact cells of *Strep. faecalis* R have any significant action on 2:5-dihydroxyphenylalanine.

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REFERENCES

- Barger, G. & Dale, H. H. (1910). *J. Physiol.* **41**, 19.
 Bellamy, W. D. & Gunsalus, I. C. (1945). *J. Bact.* **50**, 95.
 Blaschko, H. (1939). *J. Physiol.* **96**, 50 P.
 Blaschko, H. (1949). *Biochem. J.* **44**, 268.
 Blaschko, H., Holton, P. & Sloane-Stanley, G. H. (1949). *J. Physiol.* In the Press.
 Blaschko, H. & Sloane-Stanley, G. H. (1948). *Biochem. J.* **42**, iii.
 Epps, H. M. R. (1944). *Biochem. J.* **38**, 242.
 Gale, E. F. (1940). *Biochem. J.* **34**, 846.
 Gunsalus, I. C. & Bellamy, W. D. (1944). *J. biol. Chem.* **155**, 357.
 Landy, M. & Dicken, D. M. (1942). *J. Lab. clin. Med.* **27**, 1086.
 Umbreit, W. W., Bellamy, W. D. & Gunsalus, I. C. (1945). *Arch. Biochem.* **7**, 185.

The Effects of Applied Pressure on Secretion by Isolated Amphibian Gastric Mucosa

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Ludwig (1851) has shown that secretion by the sub-maxillary gland can occur against a pressure greater than the arterial blood pressure, but no evidence is available about the relation between pressure and secretion in the stomach. The apparatus described in this paper was developed to study simultaneously the rates of fluid secretion and respiration by isolated gastric mucosa. The results show that the secretion of hydrochloric acid can occur against pressure. This supports the view that the hydrostatic pressure of the blood is not an essential part of the mechanism of secretion of the solution of hydrochloric acid by the oxyntic cell.

EXPERIMENTAL

Apparatus

The apparatus used is shown in Figs. 1 and 2. It consists of a manometer *A*, a vessel *B*, and a hollow stopper *S*. *A* is a constant pressure manometer (modified from Dixon, 1943). Like a Warburg manometer it consists of a U-tube provided with a rubber reservoir *R*₁ containing modified Brodie solution. A graduated 0.5 ml. pipette *P* is attached to its right-hand limb; a rubber reservoir *R*₂ containing mercury is attached to the end of the pipette. The vessel *B* is a conical cup (volume c. 27 ml.) with a central ground socket *J*₁ and two side arms *C*₁ and *C*₂. The vessel is attached to the manometer by the ground joint *J*₂ on the side arm *C*₁. The side arm *C*₂, which is not shown in Fig. 2, has a tap

stopper. Instead of the usual centre well a side well W is fused to the wall of the vessel. The stopper S fits into the socket J_1 . The hollow base of S dips into the nutrient saline in B , and communicates with a graduated horizontal capillary side tube G which can be closed by tap T . The stopper S is in turn closed by a tap stopper V , which must have a wide hole to allow easy flow of the excess liquid paraffin (see Methods).

Fig. 1. Constant pressure manometer. R_1 , reservoir for Brodie solution; R_2 , reservoir for mercury, outer diameter of tubing 0.75 cm.; P , mercury pipette, bore 0.18 cm., capacity 0.5 ml., graduation 1 division = 5 μ l. (length 20 cm.). U-tube graduated in mm. (bore 0.11 cm.).

Theory of the apparatus

Dixon (1943) described a differential constant pressure manometer which had some similarities to the present apparatus, but the theory of the system when used for CO_2 measurements was not given.

Case 1. Gas space filled with 100% O_2 , CO_2 absorbed by NaOH. The amount of O_2 absorbed is given by the readings of pipette P , multiplied by a factor correcting it to N.T.P.

Case 2. Gas space filled with 5% CO_2 + 95% N_2 . CO_2 is evolved and the partial pressures of both CO_2 and N_2 change. Since the solubility of CO_2 is high ($\alpha = 0.74$ at 25°), the amount dissolved in the liquid changes appreciably. The solubility of N_2 , however, is small ($\alpha = 0.015$ at 25°), and

since the partial pressure changes during an experiment are not more than 0.3%, the changes in the amount of N_2 dissolved in the liquid can be safely neglected. The simplified theory of the system for measuring the anaerobic glycolysis is given below.

Case 3. Gas space filled with 5% CO_2 + 95% O_2 . O_2 is absorbed and respiratory CO_2 is evolved. In a non-secreting mucosa the CO_2 output nearly balances the O_2 uptake so that the manometric readings show a very small resultant uptake of gas. In a secreting mucosa 1 mol. of CO_2 is absorbed to form a bicarbonate ion for each molecule of HCl produced. The increase in the metabolic rate of the tissue during secretion is usually about 30%, and, as was shown by Davies (1948), no significant change occurs in the overall uptake as measured on Warburg manometers. The

Fig. 2. Vessel B , hollow stopper S , with graduated side tube G , and part of manometer A . Capacity of B , 25 ml.; capacity of side arms C_1 and C_2 (C_2 is not shown), 1 ml. Outer diameter of G , 0.6 cm.; bore 0.1 cm., length 15 cm., graduation 1 division = 1 μ l. The mucosa is tied to the base of S (diameter 0.9 cm.).

rate of uptake of the CO_2 absorbed by the mucosa during secretion of acid can, therefore, be used to estimate the rate of secretion of HCl (see Davies, 1948). The solubility of O_2 is low ($\alpha = 0.028$ at 25°), and so the simplified theory of the system, when used for measuring the uptake of CO_2 due to acid secretion, is the same as in Case 2.

Let x = the amount of gas evolved (μ l. at N.T.P.);

v = the observed change in the readings of the mercury pipette (μ l.);

V_g = the volume of the gas space in the vessel and manometer (μ l.);

V_l = the volume of the liquid in the vessel (μ l.);

T = the absolute temperature;

P = the initial pressure in the vessel (i.e. the barometric pressure $\pm$ the pressure maintained in the manometer, mm. Brodie solution);

P_0 = the normal pressure (10,000 mm. Brodie solution);

p = the vapour pressure of water at temperature T (mm. Brodie solution);

α = Bunsen's absorption coefficient (expressed as ml. gas/ml. liquid when the partial pressure of the gas is equal to P_0) at N.T.P.

c = the initial percentage of the gas under consideration in the gas mixture of the cylinder; and

c' = the final percentage of this gas in the gas space.

Case 1. When 100% O₂ is used and CO₂ is absorbed by NaOH

$$x = v \frac{273}{T} \frac{P-p}{P_0},$$

$$x = vk_1.$$

Cases 2 and 3. Gas space filled with 5% CO₂ + 95% N₂, or 5% CO₂ + 95% O₂. Since the total amount of gas in the apparatus equals amount of gas in gas phase plus amount of gas in solution, the initial amount of gas equals

$$V_g \frac{c}{100} \frac{273}{T} \frac{P-p}{P_0} + V_F \alpha \frac{c}{100} \frac{P-p}{P_0}, \quad (i)$$

and the final amount of gas equals

$$(V_g + v) \frac{c'}{100} \frac{273}{T} \frac{P-p}{P_0} + V_F \alpha \frac{c'}{100} \frac{P-p}{P_0}. \quad (ii)$$

Hence by subtraction,

$$x = \frac{c'}{100} \frac{P-p}{P_0} \left[(V_g + v) \frac{273}{T} + V_F \alpha \right] - \frac{c}{100} \frac{P-p}{P_0} \left[V_g \frac{273}{T} + V_F \alpha \right]. \quad (iii)$$

Since v = the increase in the volume of the gas space of the manometer at constant pressure due to the evolution of gas,

$$v = \frac{c'}{100} (V_g + v) - \frac{c}{100} V_g, \quad (iv)$$

and

$$c' = 100 \frac{v + \frac{c}{100} V_g}{V_g + v}. \quad (v)$$

By substitution of (v) in (iii),

$$x = v \frac{273}{T} \frac{P-p}{P_0} + v \frac{V_F \alpha}{V_g + v} \frac{P-p}{P_0} \left(1 - \frac{c}{100} \right). \quad (vi)$$

v is small compared with V_g and an error of less than 0.2% is made if v is neglected in the denominator of the second term of (vi) and the equation used in the form

$$x = v \left[\frac{273}{T} \frac{P-p}{P_0} + \frac{V_F \alpha}{V_g} \frac{P-p}{P_0} \left(1 - \frac{c}{100} \right) \right], \quad (vii)$$

or $x = vk_2$.

In most of our experiments in which gas mixtures containing 5% CO₂ were used, k_2 was 1.02–1.04 depending on V_F .

The constant pressure manometers were compared with the standard Warburg manometers. The evolution of CO₂ from NaHCO₃ solution by adding a known amount of standard acid solution and the uptake of O₂ by a yeast suspension agreed in both types of apparatus.

Saline media

The media were prepared from the stock solutions used for making the physiological saline of Krebs & Henseleit (1932): (1) a basal saline isotonic with frog serum (modified from Gray, Adkison & Zelle, 1940), prepared by adding to 2000 ml. of doubly distilled water 79 ml. NaCl (18%), 81 ml. KCl (1.15%), 34.7 ml. CaCl₂ (0.11M), 12 ml. KH₂PO₄ (0.2M), 15.8 ml. MgSO₄·7H₂O (3.82%), 47.3 ml. NaHCO₃ (1.3%); (2) 0.12M-NaHCO₃ solution; (3) 0.080M-phosphate

buffer, pH 7.4. During experiments in 100% O₂ a phosphate saline was used which was prepared by adding 1 vol. of solution (3) to 10 vol. of solution (1). CO₂ was absorbed by NaOH in the side well of the flask. For experiments in 5% CO₂ and 95% O₂ a bicarbonate saline was prepared by adding 16 vol. of solution (2) to 100 vol. of solution (1). This bicarbonate saline was also used in anaerobic experiments in 5% CO₂ and 95% N₂. Traces of O₂ in the gas mixture were removed by a stick of yellow phosphorus in the side well. All nutrient salines contained 0.2% glucose.

Material

Frogs (*Rana temporaria temporaria* L.) and toads (*Bufo bufo bufo* L.) were used. They were captured locally and housed in an outdoor froggery under natural conditions. An excess of worms and insects was provided for food. Similar results were obtained with both species, but toad gastric mucosa is less fragile and was preferred. The animal was pitthed and the stomach isolated and washed with frog saline. The muscle layer was removed and the tube of mucosa cut open along the lesser curvature (Davies, 1948). The open sheet was then tied, with the secretory side upwards, to the open base of the stopper S and the excess of tissue cut away. In early experiments the mucosa was stretched tightly over the end of the stopper, but was found to tear easily under the stress of applied pressure. This was prevented by laying the mucosa loosely over the end of the stopper before tying; it could then withstand pressures much greater than 300 mm. (Fig. 3), but large

Fig. 3. Effect of tightness of membrane on stress. Let h = total pressure on secretory side of mucosa, x = resultant stress in mucosa, θ = angle of stress vector to horizontal. From the triangle of forces $h/x = \sin \theta$. When $\theta = 5^\circ$, $x = 11.5 h$; when $\theta = 30^\circ$, $x = 2.0 h$.

fluctuations occurred in the readings. The continuous slight adjustments of the pressure in the manometer caused visible contractions of the muscularis mucosae. It was found, however, that 10^{-3} M-atropine in the nutrient saline stopped these movements, but did not prevent good secretory responses to histamine. This concentration of atropine was therefore used in all later experiments.

Methods

The secretory surface of the mucosa was covered with 0.2 ml. of 0.12M-NaCl pipetted into stopper S . The stopper and part of the graduated side tube G were then filled with liquid paraffin. Tap T at the end of G was shut and the tap stopper V was inserted gently to avoid stretching the mucosa. S was then placed into the main compartment of vessel B which contained sufficient nutrient saline (4–5 ml.) to cover the mucosa. The unit was attached to the manometer, filled with an appropriate gas mixture, placed in a

water bath at 25.0° and shaken in a horizontal plane. An identical unit in which the mucosa was replaced by a rubber or plastic membrane was used as a thermobarometer. After equilibration the pressure inside the manometer was adjusted to the desired value, stopper *V* turned off and tap *T* opened.

The pressure acting on the mucosa was the difference of two components: (*a*) the pressure of the gas in the manometer, regulated by means of the mercury in *P* (Fig. 1); (*b*) the hydrostatic pressure of the column of liquid in stopper *S* less the hydrostatic pressure of the nutrient medium in the cup *B*. When (*a*) balanced (*b*) the resultant pressure was zero. When (*a*) was greater than (*b*), pressure was exerted in the direction of normal secretion (from the nutrient to the secretory side of the mucosa); the resultant pressure (*a*)–(*b*) was positive and was prefixed with a positive sign. When (*a*) was less than (*b*) the resultant pressure was opposed to the direction of normal secretion and was given a negative sign. All pressures recorded in this paper are in mm. Brodie solution (Dixon, 1943). Since the gas exchanges of the tissue and the transport of liquid across the mucosa altered the pressure in the manometer, the mercury in *P* had to be adjusted continuously in order to keep the pressure on the mucosa constant at any desired value. This value could be altered at will several times during an experiment. The possible range of pressures was from –300 to +300.

The mucosa secreted either spontaneously, or following the addition from the side arm of histamine to 5×10^{-5} M. These secretions displaced the liquid paraffin, the movement of which along the graduated capillary tube *G* was measured. At the same time measurements of the gaseous exchanges were made with pipette *P*.

Three types of experiment were performed. (1) The rates of respiration (Q_{O_2}) and fluid secretion (q_{H_2O}) were measured in phosphate saline gassed with 100% O_2 ; (2) the rate of acid secretion (Q_{HCl}) and the q_{H_2O} in bicarbonate saline gassed with 5% CO_2 + 95% O_2 ; (3) the rate of anaerobic glycolysis ($Q_{CO_2}^N$) and the q_{H_2O} in bicarbonate saline gassed with 5% CO_2 + 95% N_2 . For each set of measurements one mucosa was used.

After the experiment the mucosa was removed in two portions by cutting round the silk ligature with a sharp razor, washed in distilled water and dried overnight at 110°. The dry weight of the circular portion was used in the calculation of the Q_{HCl} , and q_{H_2O} , while the Q_{O_2} and $Q_{CO_2}^N$ were calculated using the sum of the dry weight of the two portions. The values of the Q_{HCl} , Q_{O_2} and $Q_{CO_2}^N$ are in $\mu\text{l./mg. dry wt./hr.}$ (HCl being considered as a gas at N.T.P.); the q_{H_2O} is defined as $\mu\text{l. transported fluid/mg. dry wt./hr.}$

Calculation of results

Since the secretion of liquid into stopper *S* during any period resulted in a loss of liquid from the cup *B*, allowance had to be made for this reduction of liquid volume when calculating the gas exchanges. Thus, when the change of volume in the mercury pipette *P* was $-10 \mu\text{l./15 min.}$, while the increase in the volume of liquid in the graduated capillary tube *G* was $+2 \mu\text{l./15 min.}$, the uptake of gas was actually $-10 + 2 = -8 \mu\text{l./15 min.}$ This was multiplied by a constant which depended on the gas used, to give the volume of gas at N.T.P. (see Theory of the apparatus). Specimen protocols are given at the end of the paper.

RESULTS

Water transport and pressure. Application of pressures of approximately ± 50 mm. did not force liquid across a membrane of aerobic gastric mucosa which was not secreting. Mucosae secreting either spontaneously or after stimulation with histamine transported liquid from the nutrient to the secretory side only. The rate was not influenced by the direction or magnitude of the applied pressures within the limits of about ± 50 mm. (Fig. 4). The average maximum q_{H_2O} calculated for twenty-four secreting

Fig. 4. Effect of pressure on Q_{HCl} and Q_{O_2} . Histamine was added to a concentration of 5×10^{-5} M at arrows marked *H*. The applied pressures are given in mm. Brodie solution.

mucosae was between 1.0 and 4.0, average 2.3, standard deviation 0.9. The average maximum Q_{HCl} in ten secreting mucosae was from 2.8 to 7.0, average 4.8, standard deviation 1.3. The average maximum Q_{O_2} in twelve secreting mucosae was from 1.9 to 4.8, average 3.2, standard deviation 1.0. The pressures in these experiments ranged from –80 to +50 mm. The highest opposing pressure so far used was –120 mm. and acid was secreted by the mucosa. The figure of 18 cu.mm. secretion/mg. dry wt./hr. stated by Davies & Turner (1948) has been found to be in error because of a leak due to a fault in tap *V* of the stopper *S*. This fault has now been corrected. In some experiments neutral red was added to the nutrient saline to a final concentration of 10^{-4} M. It was transported from the nutrient to the secretory side against opposing pressures (cf. Bradford & Davies, 1948). No water transport or HCl secretion occurred in mucosae under anaerobic conditions when the resultant pressure was zero. When the

Table 1. *Effect of pressure on transport of fluid across frog gastric mucosa*

(+ Indicates direction of natural secretion, i.e. from nutrient to secretory side; – the direction opposed to natural secretion.)

Mucosa no.	Date	Gas mixture	Experimental time	Resultant pressure (mm. Brodie solution)	q_{H_2O} (μ l./mg. dry wt./hr.)	Remarks
1	1. x. 47	5 % CO ₂ + 95 % N ₂	0.30–1.45	– 10	– 0.4	$Q_{CO_2}^N = 1.6$
			1.50–3.00	– 30	– 0.9	
			3.05–4.00	+ 20	+ 0.9	
			4.05–5.00	+ 40	+ 1.35	
2	21. vi. 48	5 % CO ₂ + 95 % N ₂	1.58–2.23	– 30	– 5.2	$Q_{CO_2}^N = 2.0$
			2.28–3.18	0	0	
3	9. vi. 48	100 % O ₂	1.38–3.08	– 35	+ 0.92	Histamine added to 5×10^{-5} M at 0 hr. 53 min.
			3.23–4.23	– 65	+ 0.46	
4	15. viii. 47	5 % CO ₂ + 95 % O ₂	1.00–1.30	– 42	+ 1.64	Histamine added to 5×10^{-5} M at 1.00 hr.
			1.40–3.05	– 12	+ 1.0	
			3.10–4.40	+ 18	+ 1.64	
			4.50–5.30	+ 33	+ 1.36	

pressure was increased above, or reduced below, zero a neutral fluid was forced across the mucosa in the direction of the applied pressure. Typical results are given in Table 1.

Fig. 5 gives the results of two experiments done simultaneously on two pieces, *A* and *B*, of one large mucosa which had been divided along the greater and lesser curvatures. The two portions of mucosa from the corpus were tied over the end of two different stoppers. *A* was used to measure the q_{H_2O} and Q_{O_2} . The nutrient solution was phosphate saline gassed with 100 % O₂. CO₂ was absorbed by sodium hydroxide in the side well. *B* was placed in bicarbonate saline gassed with 5 % CO₂ and 95 % O₂ and measurements made of the q_{H_2O} and Q_{HCl} . As described on p. 378, in a non-secreting mucosa the CO₂ output nearly balances the O₂ uptake, but in a secreting mucosa one molecule of CO₂ is absorbed to form bicarbonate for each molecule of HCl produced. The rate of uptake of this CO₂ can, therefore, be used to estimate the rate of secretion of HCl. A comparison of the two pieces of mucosa showed that the Q_{O_2} , Q_{HCl} and q_{H_2O} increased simultaneously after stimulation with 5×10^{-5} M-histamine. Both *A* and *B* secreted against a pressure of –30 maintained throughout the experiments. The molarity of the HCl in the secretion was calculated from the q_{H_2O} and Q_{HCl} , and was found to be between 0.09 and 0.10.

The time lapse between mixing and the response to histamine varied from 1 min. to 2 hr. and the responses were of varying intensity and duration (Davies, 1948). When the secretory phase was over, the volume of the secretion did not increase and the q_{H_2O} and Q_{HCl} fell to zero. The Q_{O_2} returned to its basal value. It was also found that mucosae that had stopped secreting were not influenced by pressures of the order of ± 50 mm. of water.

A difficulty was encountered during experiments

with actively acid-secreting mucosae in phosphate saline. These often ulcerated because there were insufficient supplies of CO₂ to neutralize the alkali

Fig. 5. Effect of histamine and thiocyanate on Q_{HCl} , Q_{O_2} and q_{H_2O} . The curves ii, iii and iv result from an experiment in 100 % O₂ on the anterior portion *A*, curve i from an experiment in 5 % CO₂ and 95 % O₂ on the posterior portion *B*, of the same gastric mucosa. The secretion curves of *B* were similar to those of *A* (iii and iv) and are not given. Both *A* and *B* were secreting against a resultant pressure of –30 mm. Brodie solution. Histamine was added to a concentration of 5×10^{-5} M at arrows marked *H*; thiocyanate to a concentration of 10^{-2} M at arrows marked *T*.

formed in the oxyntic cell concomitantly with the acid (cf. Davies & Longmuir, 1948).

Effect of thiocyanate. Thiocyanate is known to inhibit acid secretion *in vivo* (Davenport, 1940) and *in vitro* (Crane, Davies & Longmuir, 1946). It was observed that 10^{-2} M-thiocyanate also reduced the volume of the secretions (Fig. 5, i and iii). Secretion of acid was usually stopped for 30–45 min. The mucosa appeared to lose its relative impermeability in either direction. With opposing pressures some water and acid passed back through the mucosa into the cup, and this resulted in an output of CO_2 if bicarbonate saline was used as the medium (Fig. 5, i).

Fig. 6. Effect of histamine and thiocyanate on Q_{O_2} . Sheets of frog gastric mucosa were incubated at 25° in phosphate saline gassed with 100% O_2 in Warburg cups with 2 side arms. Histamine was added to a concentration of 5×10^{-5} M at arrows marked H, thiocyanate to a concentration of 10^{-2} M at arrows marked T.

This movement of liquid and CO_2 output were greater for greater opposing pressures. After 30–45 min., however, the mucosa recovered and resumed secretion of water and acid, though at lower rates. Neither the basal Q_{O_2} , nor the increase in the Q_{O_2} following histamine stimulation, was affected by 10^{-2} M-thiocyanate (Fig. 6), whereas the Q_{HCl} was inhibited by 60–95% of its value during secretion.

DISCUSSION

Pressure and secretion. Ludwig (1851) inserted a cannula into the duct of the submaxillary gland and showed that the secretory pressure could exceed the arterial blood pressure. The apparatus described in this paper makes it possible to study the relation between secretion and pressure in isolated sheets of tissue as opposed to glands with large ducts. This type of experiment is not usually possible in the intact mammal because pressures applied to the gastric mucosa, for instance, cause collapse of the blood capillaries and the tissue stops secreting, probably because of the resulting anoxia.

The experiments described in this paper show that

under aerobic conditions secretion by isolated amphibian gastric mucosa can occur against pressure, and that the rate of secretion is not increased by pressures applied in the direction of the natural secretion. This result bears on the statements by Davenport & Fisher (1940) that 'since the osmotic pressures of blood and gastric juice are the same the mol fraction of water in each is the same. Consequently, no reversible work is done when water passes from the blood to the secretion, and no reversible work is expended. A very small amount of irreversible work is done on the water in overcoming viscosity when water moves at a finite rate. The energy necessary to perform this work is doubtless derived from the difference in hydrostatic pressure between the blood and the gastric juice', and by Gray (1943) that '... it is conceivable that the filtration pressure of the blood does supply the energy required to move water against the mechanical resistance offered by the tissues and cell membranes'. Since secretion can occur, not only in the absence of blood pressure but even against resultant pressures, it is now certain that blood pressure is not necessary to move water through the tissues during acid secretion by gastric mucosa.

Under anaerobic conditions applied pressures caused a seepage of water through the mucosa in the direction of the pressures. This did not occur in the presence of O_2 , and shows that aerobic metabolism maintains the membrane in a water-tight condition.

The effects of thiocyanate. The thiocyanate ion is known to inhibit gastric secretion (Davenport, 1940), and, although it can inhibit carbonic anhydrase, the reduction of the Q_{HCl} must depend on other processes (Feldberg, Keilin & Mann, 1940). Since 10^{-2} M-thiocyanate caused 60–95% inhibition of the Q_{HCl} , but did not inhibit the increase in the Q_{O_2} following histamine, nor prevent this increase when added before histamine, it seems possible that thiocyanate inhibits H^+ ion production by interfering with the metabolic chain at some point past that at which histamine acts.

The immediate effect of the thiocyanate was to allow the mucosa to let through water in response to applied pressures in a similar way to that under anaerobic conditions. If there was a pressure opposing secretion then some of the hydrochloric acid which had been secreted earlier could seep back through the mucosa. This early phase after the addition of 10^{-2} M-thiocyanate ended in 30–45 min., after which secretion continued at lower but steady rates.

It has not so far been possible to separate experimentally the secretion of acid and water by oxyntic cells, and the evidence at present available supports the view that there is a very close connexion between the mechanisms by which the acid and the water of the histamine-stimulated gastric juice are secreted by oxyntic cells.

SPECIMEN PROTOCOLS

(See calculation of results, p. 380)

(1) *Respiration and fluid secretion*

19 April 1948. Nutrient medium: phosphate saline containing 0.2% glucose and 0.001 M-atropine. Gas: 100% O₂, CO₂ absorbed by NaOH. Histamine added from side arm at 90 min. (concentration in medium after mixing 5×10^{-5} M). Temperature of bath 25.0°. Barometric pressure 751 mm. Hg. Resultant pressure applied to mucosa -15 mm. Brodie solution. Dry weight of mucosa 18.5 mg.; dry weight of secreting portion 11.7 mg.

Time (min.)	Change of manometer readings (μ l.)	Change of side tube readings (μ l.)	Change of gas volume		Q_{O_2} (μ l./mg. dry wt./hr.)	q_{H_2O} (μ l./mg. dry wt./hr.)
			At experimental temp. and pressure (μ l.)	At N.T.P. (μ l.)		
0 (in bath)						
30-60	-14	+2	-12	-10	-1.1	+0.3
90	-15	+2	-13	-11	-1.2	+0.3
90 (histamine added)						
120	-20	+4	-16	-14	-1.5	+0.7
150	-26	+7	-19	-17	-1.8	+1.2
180	-25	+8	-17	-15	-1.6	+1.4

(2) *Hydrochloric acid and fluid secretion*

25 June 1948. Nutrient medium: bicarbonate saline containing 0.2% glucose and 0.001 M-atropine. Gas: 5% CO₂ + 95% O₂. Histamine added from side arm at 45 min. (concentration in medium after mixing 5×10^{-5} M). Temperature of bath 25.0°. Barometric pressure 760 mm. Hg. Resultant pressure applied to mucosa -35 mm. Brodie solution. Dry weight of mucosa 7.9 mg.; dry weight of secreting portion 5.6 mg. $k_{CO_2} = 1.04$.

Time (min.)	Change of manometer readings (μ l.)	Change of side tube readings (μ l.)	Change of gas volume at experimental temp. and pressure (μ l.)	Change of gas volume due to HCl secretion		Q_{HCl} (μ l./mg. dry wt./hr.)	q_{H_2O} (μ l./mg. dry wt./hr.)
				At experi- mental temp. and pressure (μ l.)	At N.T.P. (μ l.)		
0 (in bath)							
30-45	-1.5	+0.5	-1*	0	0	0	+0.4
45 (histamine added)							
60	-9	+3	-6	-5	-5.2	+3.7	+2.2
75	-8	+3	-5	-4	-4.2	+3.0	+2.2
90	-10	+4	-6	-5	-5.2	+3.7	+2.9
105	-8	+3	-5	-4	-4.2	+3.0	+2.2
120	-7	+2	-5	-4	-4.2	+3.0	+1.4

* The small uptake of gas before addition of histamine is the resultant of the respiratory exchanges of the mucosa, and is subtracted from the volume changes occurring during each period after the addition of histamine, to give the changes of gas volume due to HCl secretion (see Theory of apparatus, Case 3.)

(3) *Anaerobic glycolysis and fluid transport*

21 June 1948. Nutrient medium: bicarbonate saline containing 0.2% glucose. Gas: 5% CO₂ + 95% N₂. Temperature of bath 25.0°. Barometric pressure 759 mm. Hg. Resultant pressure applied to mucosa +10 mm. Brodie solution. Dry weight of mucosa 6.5 mg., dry weight of secreting portion 3.8 mg. $k_{CO_2} = 1.04$.

Time (min.)	Change of manometer readings (μ l.)	Change of side tube readings (μ l.)	Change of gas volume		$Q_{CO_2}^{N_2}$ (μ l./mg. dry wt./hr.)	q_{H_2O} (μ l./mg. dry wt./hr.)
			At experimental temp. and pressure (μ l.)	At N.T.P. (μ l.)		
0 (in bath)						
60-75	+3	+0.5	+3.5	+3.6	+2.2	+0.5
90	+5	+0.5	+5.5	+5.7	+3.5	+0.5
105	+4	+0.5	+4.5	+4.7	+2.9	+0.5

SUMMARY

1. An apparatus is described which has been used to study simultaneously the rates of fluid secretion and of respiration by isolated gastric mucosa. It consists of a constant pressure manometer, a modified Warburg cup and a stopper with a graduated capillary side tube (Figs. 1 and 2). External pressure can be applied to the mucosa and be kept constant or varied at will.

2. Secretion occurred against opposing pressures of at least -120 mm. Brodie solution, and was, within the limits of ± 50 mm. Brodie solution, independent of the magnitude and direction of the applied pressures. Neutral red was transported across the mucosa against such pressures.

3. On stimulation with 5×10^{-5} M-histamine the rates of secretion and of respiration increased concomitantly.

4. In anaerobic experiments neutral fluid was

forced across the mucosa in the direction of the applied pressure. This did not occur with non-secreting mucosae under aerobic conditions.

5. Thiocyanate (10^{-2} M) did not affect the basal Q_{O_2} or the increase of the Q_{O_2} which followed stimulation by 5×10^{-5} M-histamine, but strongly inhibited the rate of hydrochloric acid secretion. Thiocyanate often caused passage of water across the mucosa in the direction of the external pressure for up to 1 hr. before secretion of acid and liquid was resumed at lower but steady rates.

6. These results show that the energy for the movement of water across acid-secreting gastric mucosa is derived not from the difference in hydrostatic pressure between the blood and the gastric juice, but from the aerobic metabolism of the oxyntic cells.

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REFERENCES

- Bradford, N. M. & Davies, R. E. (1948). *Biochem. J.* **42**, xii.
 Crane, E. E., Davies, R. E. & Longmuir, N. M. (1946). *Biochem. J.* **40**, xxxvi.
 Davenport, H. W. (1940). *Amer. J. Physiol.* **129**, 505.
 Davenport, H. W. & Fisher, R. B. (1940). *Amer. J. Physiol.* **131**, 165.
 Davies, R. E. (1948). *Biochem. J.* **42**, 609.
 Davies, R. E. & Longmuir, N. M. (1948). *Biochem. J.* **42**, 621.
 Davies, R. E. & Ternner, C. (1948). *Biochem. J.* **42**, xiii.
 Dixon, M. (1943). *Manometric Methods*, 2nd ed. Cambridge: University Press.
 Feldberg, W., Keilin, D. & Mann, T. (1940). *Nature, Lond.*, **146**, 651.
 Gray, J. S. (1943). *Gastroenterology*, **1**, 390.
 Gray, J. S., Adkison, J. L. & Zelle, K. (1940). *Amer. J. Physiol.* **130**, 327.
 Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
 Ludwig, C. (1851). *Z. ration. Med.* **1**, 255.