7. Glycogenolysis in diaphragm was favoured by potassium but unaffected by insulin. The order of magnitude of glycogenolysis was the same in 'normal' and 'diabetic' diaphragm.

8. Metabolic changes of tissues *in vitro* may become manifest only in a correspondingly changed

medium and may be undetectable in an indifferent salt medium.

This investigation was aided by a grant of the Dazian Foundation for Medical Research and by the Sam and H. Ansel Moskowitz Fund for research in diabetes.

REFERENCES

- Bendall, J. R. & Lehmann, H. (1941). *Nature, Lond.*, **148**, 538.
- Boyer, P. D., Lardy, H. A. & Phillips, P. H. (1942). *J. biol. Chem.* **146**, 673.
- Boyer, P. D., Lardy, H. A. & Phillips, P. H. (1943). *J. biol. Chem.* **149**, 529.
- Cori, C. F. & Cori, G. T. (1936). *J. biol. Chem.* **116**, 119.
- Cross, M. C. A. & Holmes, E. (1937). *Brit. J. exp. Path.* **18**, 370.
- Dixon, M. (1943). *Manometric Methods*. New York: Macmillan.
- Fenn, W. O. (1940). *Physiol. Rev.* **20**, 377.
- Gemmell, C. L. (1940). *Johns Hopk. Hosp. Bull.* **66**, 232.
- Gemmell, C. L. (1941). *Johns Hopk. Hosp. Bull.* **68**, 329.
- Gemmell, C. L. & Hamann, L. Jr. (1941). *Johns Hopk. Hosp. Bull.* **68**, 50.
- Gey, G. O. & Gey, M. K. (1936). *Amer. J. Cancer*, **27**, 45.
- Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
- Hahn, L. & Hevesy, G. (1941). *Acta physiol. Scand.* **2**, 51.
- Hastings, A. B. & Buchanan, J. M. (1942). *Proc. nat. Acad. Sci., Wash.*, **28**, 478.
- Hechter, O., Levine, R. & Soskin, S. (1941). *Proc. Soc. exp. Biol., N.Y.*, **46**, 390.
- Hegnauer, A. H. & Cori, G. T. (1934). *J. biol. Chem.* **105**, 691.
- Kanner, O. (1946). *Science*, **104**, 253.
- Kaplan, N., Franks, M. & Friedgood, C. E. (1945). *Science*, **102**, 447.
- Nachmansohn, M. D. (1937). *Bull. Soc. Chim. biol., Paris*, **19**, 453.
- Ostern, P., Herbert, J. & Holmes, E. G. (1939). *Biochem. J.* **33**, 1858.
- Saxton, S. A. & Miller, M. L. (1944). *Arch. Path. Lab. Med.* **37**, 34.
- Shipley, R. A. (1944). *Amer. J. Physiol.* **141**, 662.
- Somogyi, M. (1937). *J. biol. Chem.* **117**, 771.
- Stadie, W. C. (1944). *Yale J. Biol. Med.* **16**, 539.
- Tuerkischer, E. & Wertheimer, E. (1946). *Nature, Lond.*, **158**, 201.
- Van Dyke, H. B. & Hastings, A. B. (1928). *Amer. J. Physiol.* **83**, 563.

Hydrochloric Acid Production by Isolated Gastric Mucosa*

By R. E. DAVIES, *Unit for Research in Cell Metabolism (Medical Research Council), Department of Biochemistry, The University, Sheffield*

WITH AN APPENDIX BY R. E. DAVIES AND F. J. W. ROUGHTON

(Received 26 June 1947)

The experiments described in this paper were undertaken to throw light on the mechanism of hydrochloric acid production in the stomach. Isolated mucosa was used because it is easier to control the experimental conditions in excised tissue than in the intact organism.

The oxyntic cells of rats secrete continuously (Friedman, 1943), hence the starting materials for the production of HCl must come eventually from the blood and must be neutral. Several mechanisms which produce hydrogen ions have been envisaged and can be divided roughly into two types. (1) Those

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

producing the acid together with approximately neutral compounds. (2) Those producing the acid together with basic compounds (Fig. 1). Many manometric experiments have been carried out in Warburg cups with sheets of sheep, toad and frog gastric mucosa to test the possibility that mechanisms of type 1 are involved. In no case was evidence obtained that HCl was being formed, and liberating CO₂ from the HCO₃⁻ of the medium, although the rate of respiration of the tissues was normal and was increased by histamine. Since several glands can function *in vitro* (Delrue, 1930; Deutsch & Raper, 1936) it seemed unlikely that the failure to detect acid was due to the inability of the

mucosa to secrete it. These negative results suggest that mechanisms of type 1 are not operative, as would be expected if the old hypothesis of Bence Jones (1845) that 'the formation of acid sets free alkali' were true, assuming that the acid and alkali are formed in equal amount as in the type 2 mechanisms.

Fig. 1. Some biochemical reactions producing hydrogen ions. Type 1. Those producing the acid and approximately neutral compounds. Type 2. Those producing the acid and basic compounds. Type 1 mechanisms which are also reversed within the cell become type 2 mechanisms.

Experiments were devised to combine collection and estimation of the secretions with manometric measurements of the respiratory activity of the tissue; they show that when gastric mucosa secretes acid, an equivalent amount of alkali is also produced in the cell, which normally is at once neutralized by CO_2 and secreted into the surrounding medium.

METHODS

Saline media. The physiological salt solution (bicarbonate saline) of Krebs & Henseleit (1932), gassed with 5% (v/v) CO_2 + 95% (v/v) O_2 , or 5% CO_2 + 95% N_2 , was used during measurements of the balance of the O_2 uptake, and the CO_2 output, or of the anaerobic glycolysis of sheep gastric mucosa, and the phosphate saline of Krebs (1933) during experiments in 100% O_2 . The glucose concentration was 0.2 or 0.4%. In experiments with amphibian tissue these two solutions were used, after the addition of 0.29 vol. of water, and contained 0.2% (w/v) glucose. Another solution was used in the preparation of the mucosa and during some experiments in 100% O_2 . It was a basal saline similar to amphibian bicarbonate saline, but contained 0.004M HCO_3^- .

Preparation of the gastric mucosa. Sheep, frog and toad gastric mucosae were used. The abomasum was removed from the sheep within 5 min. of electric stunning, and

carried rapidly to the laboratory in ice-cold 0.16M-NaCl solution. After thorough washing a mucosal flap was dissected away, opened out and freed from submucosa. Pieces of tissue with a dry weight of 20–40 mg. were cut off and used at once. They were about 0.3–0.6 mm. thick, and did not curl up tightly.

Fig. 2. Diagrammatic representation of the overall reactions in a type 2 mechanism in A 'open', B 'tied' and C 'inside-out' frog gastric mucosa in saline media containing bicarbonate ions. In A and C the CO_2 liberated from the medium by the H^+ is equivalent to that needed to neutralize the OH^- , so no supplies of CO_2 are needed. In C the decrease of the HCO_3^- content in the external fluid is equivalent to the increase in the internal fluid. In B the acid secretion is collected inside the mucosal bag and supplies of CO_2 are needed to neutralize the OH^- .

Frogs (*Rana temporaria temporaria* L.) and toads (*Bufo bufo* L.) were captured locally and housed in a concrete froggery at 15–17° after the web had been tattooed with Indian ink for identification. An excess of cockroaches or worms was provided for food. The amphibians were pithed as required, and the stomach dissected out after cutting the oesophagus and duodenum. It was emptied by gentle squeezing and washed by passing 5 ml. of basal saline through it from a pipette. In none of more than 400 cases has any residual acid been found in the stomach after this procedure. The following technique was evolved for isolating the intact gastric mucosa. The stomach, with the greater curvature upwards, was gently pressed with the left thumb and index finger to tighten the muscle coats. A small longitudinal incision was made with a razor, and continued to both ends with fine scissors without damaging the mucosa. Starting from the pyloric end, the intact tube of mucosa was separated from the muscles with fine forceps and pulled free. It was then blotted and rapidly weighed on a torsion balance; with care the weights were reproducible to 1 mg. During the dissection the tissue was frequently damped with basal saline.

Amphibian tissue was used in three different ways: 'open', 'tied' and 'inside-out'. For experiments with open tissue the mucosa was cut along the greater and lesser curvatures and used directly. Tied tissue was used when collection of the secretion was required; the pyloric end of the mucosa was closed with fine silk thread, and the lumen completely emptied by gentle stroking with curved forceps. The cardiac end was next tied, and a loop made in the silk with which to hang the preparation on the hook of the torsion balance. Excess silk was cut off and the tissue blotted and reweighed. After clipping off the spare tissue past the knots, the unit was reweighed and was ready for use. The time from pithing the amphibian to placing the preparation in the cup was usually 9–12 min.

The balance of O_2 uptake and CO_2 output of a tied inside-out tube of acid-secreting mucosa should be similar to that of open sheets of acid secreting tissue, if as much alkali as acid is formed (Fig. 2). In this case, however, it is possible to prove that secretion occurs, by estimating the changes in the HCO_3^- content of the internal and external media.

The lumen of an inside-out gastric mucosa is not big enough to hold sufficient medium to supply the tissue, so a source of nutrient was attached to it. Tiny glass U-tubes were made, and a 4 ml. balloon of very thin rubber tied to one arm. A clean intact mucosa was then turned inside-out by passing fine forceps through the cardiac sphincter, seizing the pyloric end and sliding the tissue along the instrument. After tying the pyloric end, the cardiac end was attached to the other arm of the U-tube. The nutrient solution was injected into the balloon with a hypodermic syringe, and the air inside the preparation completely sucked out. After tying off the puncture hole, to prevent leakage, the preparation was weighed and was then ready for use (Fig. 2).

Manometric procedure. The O_2 uptake, the resultant of O_2 uptake and CO_2 output, and the anaerobic glycolysis were measured with Warburg manometers in the usual way at 40–0° with mammalian, and 25–0° with amphibian tissue. Conical cups (20–30 ml. capacity), with a centre well, were used for most of the experiments. Histamine and other agents were added from the side arms, or from Keilin tubes (Keilin, 1929). The values of the Q_{O_2} , Q_{CO_2} and Q_{HCl} are in $\mu\text{l./mg. dry wt./hr.}$

Final treatment of tissues. At the end of the incubation, open tissue was washed in distilled water and dried overnight at 110°. Tied bags of mucosa, now distended by secretions, were uniformly blotted, the small blobs of mucus formed at the rosette ends removed, and the residue hung on the torsion balance by the silk loop and weighed. The contents were drained into a small sample tube, after cutting open the swollen bag of mucosa, which was reweighed when empty. The silk was removed and the tissue washed and dried as before. From the various weights, both the amount of secretion and the total content of the isolated mucosa can be estimated.

When the tissue had been used inside-out, care was taken to drain off as much as possible of the external fluid, whose volume was measured after transference to a 5 ml. graduated tube. The balloon, glass tube and mucosa were then blotted, weighed, and the internal fluid drained as completely as possible into a 5 ml. graduated tube. The volume of liquid transported by the mucosa could thus be calculated. The tissue was cut off, washed and dried as before.

Estimation of the amount of acid secreted. A sample (10–100 mg.) of the gastric contents was weighed into the side arm of a Warburg cup, and, after equilibration at 25° with 5% CO_2 + 95% O_2 , was tipped into 4 ml. of a solution containing 0.02 M $NaHCO_3$ and 0.1 M $NaCl$. This solution is isotonic with frog serum and secretion, so no gas exchanges due to differences in vapour pressure occur on mixing. From the output of CO_2 the acidity of the gastric contents can be calculated to an end point of pH 7.4. This pH was chosen because the secretion was ultimately formed from the medium, which had a pH of 7.4.

Bicarbonate estimation. After removing the tissue, a sample of the medium was placed in the main compartment of a Warburg cup and equilibrated at 25° with 5% CO_2 + 95% O_2 . 0.1 ml. N-HCl per ml. of medium was then added from the side arm. The output of CO_2 was compared with that from a sample of the original medium, to determine the change in bicarbonate concentration. Since CO_2 is less soluble in acid than in neutral solutions (Dixon, 1943), this technique does not give directly the absolute quantity of bicarbonate in either solution, but gives a sufficiently accurate value of the small difference between the two solutions.

Lactic acid was determined by the method of Friedemann & Graesser (1933).

RESULTS

A. The metabolism of sheets of gastric mucosa

Sheep gastric mucosa. It is necessary to know whether the tissue is sufficiently thin to allow adequate amounts of O_2 to diffuse into it to maintain its metabolic activity. The thickness of samples of sheep abomasal mucosa, determined from the weight of a known area, was from 0.3 to 0.6 mm., average 0.5 mm., assuming a density of 1. The ratio dry weight/wet weight was 0.2. The formula of Warburg (1923), which is of doubtful validity when applied to a tissue such as gastric mucosa, which contains cells of differing metabolic activities, gives the upper limiting value of the $|Q_{O_2}|$ as 16 at 38° at which the O_2 pressure at the centre of the tissue is just zero. The Q_{O_2} was measured in 56 out of 127 experiments, and was between –2.9 and –7.0, average –4.7, standard deviation 0.81. Sheep mucosa thus appeared to be a satisfactory tissue for the study of HCl secretion.

When the tissue was incubated aerobically in Warburg cups, the Q_{O_2} and Q_{CO_2} had the usual magnitudes in bicarbonate or phosphate salines or serum, and there was a slight excess CO_2 evolution which corresponded with the lactate formed in the medium. No excess CO_2 evolution due to HCl reacting with HCO_3^- in the media was detected, even after the addition of histamine to concentrations from 5×10^{-8} to 5×10^{-4} M, though in the middle of this range the Q_{O_2} was usually increased by 5–20%. Variations in the tissue/medium ratio had no effect, nor did the addition of extracts of gastric mucosa, so failure to detect acid secretion did not appear to be caused by a loss of diffusible coenzymes.

Various compounds, which might be expected to take part in mechanisms of type 1, were added to discover their effect on the Q_{O_2} . Acetyl choline and eserine, at concentrations from 5×10^{-7} to $5 \times 10^{-4}M$, did not alter the Q_{CO_2} , whilst 0.005 or 0.0025M- NH_4^+ had no effect and was not utilized, either alone or together with 0.005 or 0.0025M-ornithine, in the presence or absence of histamine or acetyl choline and eserine. 0.0025M-nicotinamide methochloride, either alone or together with 0.0025M-glycoeyamine, was without effect on the Q_{O_2} or Q_{CO_2} of sheep abomasal mucosa.

Frog gastric mucosa. Sheets of frog gastric mucosa also failed to show evidence of acid production, but, after the addition of histamine to a concentration of $5 \times 10^{-5}M$, the rate of O_2 uptake was usually increased, often by 10–20%, but sometimes by more than 100% (Fig. 3). No change was observed when

Fig. 3. Typical response curves of seven frog gastric mucosae. Open sheets and tied tubes of mucosa incubated in nutrient phosphate saline gassed with O_2 at 25.0° . Histamine added in six cases to a concentration of $5 \times 10^{-5}M$ at the arrows marked *H*.

Fig. 4. The balance of O_2 uptake and CO_2 output in five frog gastric mucosae. Preparations incubated at 25.0° in nutrient bicarbonate saline gassed with 5% $CO_2 + 95\% O_2$. Histamine added in three cases to a concentration of $5 \times 10^{-5}M$ at the arrows marked *H*. The vertical lines represent the readings and their probable error.

the balance of O_2 uptake and CO_2 output was measured (Fig. 4). This means that if HCl was produced an equivalent amount of alkali was also formed.

When a complete gastric mucosa was cut across to give samples of tissue from the cardiac, corpus and pyloric regions, which were then incubated, in all of three cases the pyloric mucosa had a low Q_{O_2} and failed to respond to histamine, whilst the other two were more active and responded in accordance with their content of oxyntic cells (Table 1). In all of four

Table 1. Effect of $5 \times 10^{-5}M$ -histamine on O_2 uptake of various regions of frog gastric mucosa

(A. Mucosa cut across into three sheets of approx. equal area, consisting largely of cardia, corpus and pyloric antrum of dry weight 20.1, 16.1 and 9.4 mg. respectively. B. Mucosa cut lengthways into three strips of approx. equal area and dry weight. Incubated at 25.0° in 4 ml. phosphate saline containing 0.2% glucose. Histamine added from side arm after 2 hr. Duration of response at least 5 hr.)

Source of mucosa	Av. Q_{O_2} before histamine ($\mu l./mg.$ dry wt./hr.)	Max. Q_{O_2} after histamine ($\mu l./mg.$ dry wt./hr.)
A		
Cardia	-1.4	-2.7
Corpus	-1.5	-3.5
Pyloric antrum	-1.2	-1.3
B		
Greater curvature	-1.0	-2.6
Posterior region	-0.9	-2.5
Anterior region	-0.9	-2.4

cases strips from a single mucosa, obtained by cutting the tissue from pylorus to oesophagus, had similar Q_{O_2} and Q_{CO_2} values to within about 10%. This fact was utilized to compare the O_2 uptake of a strip in phosphate saline with the uptake, calculated from the gaseous exchanges of two similar strips from the same mucosa in bicarbonate saline, using the indirect method of Warburg (Dixon, 1943). The use of this indirect method of measuring the Q_{O_2} presupposes that the ratio of tissue to medium has no effect on the O_2 uptake. This was checked by using three strips, cut lengthways from both of two mucosae, in phosphate saline. The tissue/medium ratio was varied by a factor of 7.5, and in both series the basal and histamine-stimulated rates agreed within the normal variation.

After a trial of many temperatures between 5° and 40° , 25° was chosen for experiments with frog gastric mucosa, because it is high enough to allow active metabolism, yet safely below 30° , above which temperature the tissue suffers heat damage.

B. The production of HCl by tied intact tubes of frog gastric mucosa

The equivalence of the acid and alkali produced. When an intact tube of frog gastric mucosa was tied with silk at both ends and placed in a Warburg cup in bicarbonate saline gassed with 5% $CO_2 + 95\% O_2$,

the change in the gas pressure was similar to that with open sheets of tissue. The small negative pressure change was the resultant of O_2 uptake and CO_2 output, and was usually of the order of 1–2 mm. Brodie solution/0.5 hr. The addition of histamine to a concentration of $5 \times 10^{-5} M$ was usually followed by a large increased uptake of gas (up to 55 mm. Brodie solution/0.5 hr.) (Fig. 5), and the tube of mucosa became distended with secretion containing mucus and HCl. Histamine-resistant mucosae secreted mucus only (Fig. 4, Table 4), and did not have an increased uptake of gas.

Fig. 5. Typical histamine response curves of eight tied tubes of frog gastric mucosa. Preparations incubated at 25.0° in nutrient bicarbonate saline gassed with 5% CO_2 + 95% O_2 . Histamine added in all cases to a concentration of $5 \times 10^{-5} M$ at the arrows marked H.

The following experiments show that this increased gas uptake was due to CO_2 absorption. The experiments on sheets of mucosa showed that the balance of the gas exchanges was not significantly altered by the addition of histamine, and that the loss of HCO_3^- in the medium was accounted for by the amount of lactic acid produced (Table 2). In experiments on acid-secreting closed tubes of mucosa, bicarbonate estimations showed a large increase of this ion in the medium. This increase does not represent the total amount of HCO_3^- formed, because some was decomposed by the lactic acid formed by aerobic glycolysis. The amount of lactic acid in the medium was determined, and a corrected value of the increase of HCO_3^- thus obtained. This value gave good agreement with the extra uptake of gas concomitant with acid secretion, measured in mm. Brodie solution and multiplied by the manometer constant k_{CO_2} for the experimental conditions. This proves that the gas absorbed was CO_2 .

The quantity of gas absorbed was also equivalent to the amount of HCl found inside the bag of mucosa (Table 2), and this proves that the mucosa produces

Table 2. Relation between change in gaseous balance, HCl, HCO_3^- and lactate production in typical frog gastric mucosae

(Open or tied mucosae were placed in 4 ml. nutrient bicarbonate saline and the gaseous balance in 5% CO_2 + 95% O_2 followed at 25.0° . After the experiments the weight of secretions and the amount of acid in the mucosal bag and of HCO_3^- and lactate in the medium were determined.)

	Dry wt. of tissue (mg.)	Wet wt. of secretion (mg.)	HCl produced (μ l.)	CO_2 uptake due to secretion (from change in gaseous balance) (μ l.)	ΔHCO_3^- in medium (μ l.)	Lactate formed (μ l.)	ΔHCO_3^- + lactate (μ l.)	HCl - (ΔHCO_3^- + lactate) (μ l.)	Duration of experiment (hr.)	Time of addition of histamine (hr.)
A. Histamine to $5 \times 10^{-5} M$ added during experiment										
Tied	12.6	86 $\pm$ 1	+ 183 $\pm$ 2	+ 180 $\pm$ 2	+ 154 $\pm$ 6	+ 38 $\pm$ 4	+ 192 $\pm$ 10	- 9 $\pm$ 12	5 $\frac{1}{2}$	1 $\frac{1}{2}$
Tied	13.8	32 $\pm$ 1	+ 41 $\pm$ 2	+ 46 $\pm$ 2	+ 13 $\pm$ 6	+ 23 $\pm$ 4	+ 36 $\pm$ 10	+ 5 $\pm$ 12	4	1
Open	15.5	—	—	0 $\pm$ 1	- 24 $\pm$ 6	+ 25 $\pm$ 4	+ 1 $\pm$ 10	—	6	1 $\frac{1}{2}$
B. No histamine added										
Tied	20.0	11	0 $\pm$ 1	0 $\pm$ 1	- 33 $\pm$ 6	+ 28 $\pm$ 4	- 5 $\pm$ 10	+ 5 $\pm$ 11	6 $\frac{1}{2}$	—
Open	23.9	—	—	0 $\pm$ 1	- 40 $\pm$ 6	+ 33 $\pm$ 4	- 7 $\pm$ 10	—	5	—

alkali, which is neutralized by CO_2 at the same rate as it secretes acid. The changes in the rate of gaseous uptake can therefore be used to follow the changes in the rate of HCl secretion during the experiment. It should be pointed out that, if the ratio volume of fluid (V_F)/volume of gas (V_G) is made large, then the change in the metabolic rate of the tissue after stimulation with histamine can cause an appreciable change in the now large resultant balance of the gas exchanges, which does not occur if the ratio V_F/V_G is small, as in the experiments described (cf. Warburg, 1926).

The relationship between the Q_{O_2} and the Q_{HCl} . In order to correlate HCl production with O_2 uptake, experiments were carried out in phosphate saline gassed with O_2 , with alkali in the centre well of the Warburg cup. The increase in the Q_{O_2} following the addition of histamine (Fig. 3) was associated with acid production, and was less for small than for high rates of secretion (Table 4). In solutions such as phosphate saline and blood, which contain buffers other than the $\text{H}_2\text{CO}_3\text{:HCO}_3^-$ system, retention occurs so that, although the mucosa still produces as much alkali as acid, the HCO_3^- formed reacts with these buffers when it is secreted into the medium. Experiments using frog serum showed that the metabolic activities of frog gastric mucosa were indistinguishable in this medium from those in nutrient saline solutions.

From a knowledge of the magnitudes of the Q_{O_2} and Q_{HCl} during secretion it is possible to assess some theories of the elaboration of HCl by gastric mucosa. A difficulty is that when the variations in O_2 uptake in a Warburg cup are measured, only the average Q_{HCl} can be estimated from the total amount of HCl produced by the mucosa, and this is less than the maximum value. If the variations in the gaseous balance are followed, the Q_{O_2} of the tissue is not known, and the regional differences in the tissue preclude the use of the material clipped off outside the knots to find the Q_{O_2} .

The maximum $|Q_{O_2}|$ of 92 stimulated mucosae was from 1.1 to 4.3, average 2.6, standard deviation 0.56. The maximum $|Q_{O_2}|$ of 28 sheets of stimulated mucosal tissue was from 1.0 to 3.8, average 2.5, standard deviation 0.57. This shows that tied mucosae were effectively oxygenated by diffusion from one side of the tissue only. The largest frog so far used weighed 64 g. and its mucosa was 0.29 mm. thick. With $|Q_{O_2}|$ values < 5 this thickness is well within the safety limits given by Warburg's (1923) formula, even if the tube of mucosa is tied. The maximum Q_{HCl} observed in 130 experiments (98 with males) was 8.1 and in 20 cases (16 males) the maximum Q_{HCl} rose to more than 4. No sex difference is thus apparent. Secretion could last for only 1 hr. or for at least as long as 8 hr. but usually stopped 4–6 hr. after the addition of histamine (Fig. 5).

All the mucosae with high average rates of secretion were in bicarbonate saline, and it is therefore important to know the Q_{O_2} of secreting tied mucosae in this medium. The ordinary way of calculating the results, using the second method of Dickens & Šimer (Dixon, 1943), gives an apparent negative glycolysis with secreting tied mucosae. Since with open tissue the amount of glycolysis and the lactic acid found in the medium are in agreement, the true glycolysis in tied tissue can be found by estimating the lactic acid formed, and thus the Q_{alkali} of the mucosa can be calculated. As previous experiments have shown, this is equal to the Q_{HCl} . The disadvantage of this method is that only the overall rates of O_2 uptake, respiratory CO_2 output and glycolysis can be measured, though an estimate of the variations in the Q_{HCl} can be obtained from the changes in the gaseous uptake after histamine stimulation. The mean average Q_{O_2} in 20 experiments in bicarbonate saline was -2.6 and in four cases the average Q_{HCl} over the whole period of the experiment was greater than the average $|Q_{O_2}|$. From the normal shapes of the response curves (Figs. 3, 5) it seems probable that in these experiments the maximum Q_{HCl} was much higher than the maximum $|Q_{O_2}|$. In any case, only that part of the tissue between the silk loops produced the acid found inside the mucosal bag, whilst the whole unit respired. No account has been taken of this in the calculations, so the evidence is overwhelming that the Q_{HCl} can be higher than the $|Q_{O_2}|$ in isolated frog gastric mucosa provided with external supplies of CO_2 . Several toads have been used, and mucosae from this amphibian behaved in every way similarly to frog mucosae.

The response to histamine. Histamine was more effective at $5 \times 10^{-6}\text{M}$ than in more dilute solutions such as 10^{-6} or 10^{-7}M . This concentration of $5 \times 10^{-6}\text{M}$ is relatively high, but, since the gastric mucosa removes histamine and is relatively thick compared with the distance from the blood capillaries to the oxyntic cells, diffusion will be slow and the actual concentration of histamine in the central layers of the tissue, which contain the oxyntic cells, will be much smaller than $5 \times 10^{-6}\text{M}$, so the necessity for this high concentration in the medium for optimal results may not be unexpected.

Some mucosae failed to secrete HCl even after the addition of histamine to a concentration of $5 \times 10^{-3}\text{M}$. The fraction of histamine-resistant mucosae was lower in autumn 1945 and spring 1946 than during the winter; spontaneous secretion appears to be largely a summer phenomenon and did not occur during the investigations described in this paper.

Experiments with 'inside-out' mucosa. Tubes of mucosa turned inside-out, and attached to a supply of nutrient saline in small balloons (Fig. 2), were

placed in nutrient saline in a Warburg cup, and the gaseous balance followed in four cases whilst the O_2 uptake was observed in another four experiments. The manometric readings obtained with the inside-out tissue were the same as those obtained with open sheets, both before and after the addition of histamine. This is to be expected only if no secretion occurred, or if the acid secreted into the external fluid liberated a quantity of CO_2 equivalent to that diffusing through the mucosa to neutralize the alkali formed concomitantly with the acid (Fig. 2). Proof that histamine is effective from the acid-secreting side of the mucosa, and that secretion did take place, was obtained by estimating the change in liquid volume and $[HCO_3^-]$ in the internal and external fluids. These estimations show that the acid and alkali are equivalent, with a possible error of about 10% (Table 3).

Table 3. *Secretion by mucosa turned inside-out*

(Inside-out frog gastric mucosae tied at pyloric end and attached by bent glass tubes to thin balloons containing 2 ml. nutrient bicarbonate saline. The units then placed in 2 ml. nutrient bicarbonate saline and the gaseous balance in 5% CO_2 + 95% O_2 followed at 25.0° before and after addition of histamine to a concentration of $5 \times 10^{-6}M$ at 2 hr. The experiments stopped at 4 hr. and estimations of HCO_3^- and lactate made on internal and external solutions. Typical results.)

	Exp. 1	Exp. 2
Dry wt. of tissue (mg.)	20.6	34.3
Fluid transport (ml.)	0.250 ± 0.010	0.200 ± 0.010
ΔHCO_3^- internal (μ l.)	$+61 \pm 2$	$+36 \pm 2$
ΔHCO_3^- external (μ l.)	-73 ± 2	-58 ± 2
Lactate internal (μ l.)	$+8 \pm 4$	$+15 \pm 4$
Lactate external (μ l.)	$+2 \pm 4$	$+5 \pm 4$
ΔHCO_3^- internal due to secretory activity (μ l.)	$+69 \pm 6$	$+51 \pm 6$
ΔHCO_3^- external due to secretory activity (μ l.)	-71 ± 6	-63 ± 6
Change in gaseous balance after histamine (mm. Brodie solution)	0 ± 0.5	0 ± 0.5

The anaerobic metabolism of frog gastric mucosa. Unstimulated or histamine-resistant tied mucosae secreted appreciable amounts of neutral mucus (Table 4). In the absence of O_2 , none of four mucosae produced any secretion. The preparations were as flat and empty at the end of the experiment as at the beginning, and the weight was unaltered. The anaerobic glycolysis, average $+1.9 \mu$ l./mg. dry wt./hr., was quite unaffected by the addition of histamine. Whilst aerobically the tissue could respire and secrete HCl, for some hours at least, in the absence of external supplies of glucose, anaerobically the glycolysis fell off rapidly under these conditions (cf. Lutwak-Mann, 1947).

Table 4. *Amount and concentration of acid in secretion, aerobically and anaerobically*

Gas		Wet wt. of tissue (mg.)	Dry wt. of tissue (mg.)	Wet wt. of secretion (mg.)	Wet wt. of contents (mg.)	Consistency of contents	HCl produced (μ l.)	H ⁺ in contents (M)	Average Q_{HCl}	$\Delta Q_{O_2} $ due to histamine	Duration of experiment (hr.)	Time of addition of histamine (hr.)
A. Histamine to $5 \times 10^{-6}M$ added during experiment												
5% CO_2 + 95% O_2		109	18.3	52	60	Clear jelly	72	0.055	0.71	—	7	1½
5% CO_2 + 95% O_2		120	18.2	156	192	Clear liquid	198	0.046	2.0	—	6½	1
5% CO_2 + 95% O_2		148	24.3	198	234	Clear liquid	424	0.081	3.5	—	6	1
5% CO_2 + 95% N_2		101	19.7	1	0	—	0	—	0.00	—	6	2
5% CO_2 + 95% N_2		81	17.5	0	0	—	0	—	0.00	—	4	1
O_2		93	13.3	49	59	Whitish jelly	74	0.056	1.2	0.3	5½	1
O_2		119	16.5	81	111	Whitish jelly	151	0.061	1.8	0.6	6	1
B. No histamine added												
O_2		152	36.6	47	55	Clear jelly	0	0.000	0.0	—	6½	—

(Typical results for tied tubes of mucosa placed in 4 ml. of nutrient phosphate saline gassed with O_2 , or nutrient bicarbonate saline gassed with 5% CO_2 + 95% O_2 , or 5% CO_2 + 95% N_2 at 25.0°. Complete anaerobiosis secured with yellow phosphorus in centre well. The tube weighed before and after incubation and after emptying. The differences between the increase in weight of the tube, the 'secretion' and the actual 'contents' are probably accounted for by extrusion of mucus into the lumen of the mucosa.)

DISCUSSION

The equivalence of the acid and alkali produced by oxyntic cells. In 1859 Brücke assumed that if a free acid was liberated in the gastric tubules, an alkaline fluid of corresponding strength must at the same time pass into the blood and lymph systems (Brücke, 1859). Peters & Van Slyke (1931) have reviewed the many investigations since then into the acid-base balance of the blood, which have demonstrated an increase in its HCO_3^- content apparently associated with gastric HCl secretion. Hanke, Johannesen & Hanke (1931) found that the amount of alkali liberated by the stomach was roughly proportional to the acid secreted, and claimed that the amounts were therefore approximately equivalent. This claim was repeated by Hanke (1937) without support from published evidence. Delrue (1930) first obtained HCl secretion *in vitro* using two chambers, filled to the same level, separated by a sheet of frog gastric mucosa. Although he showed that the pH of the solution at the sub-mucosal side of the tissue rose during secretion, he apparently did not realize the significance of his results. Gray, Adkison & Zelle (1940) repeated these experiments and obtained results 'suggesting that alkali was being liberated from the muscularis surface'.

The experiments described in this paper show that no secretion of HCl could be detected manometrically when sheets of gastric mucosa were incubated in Warburg cups. When the secretion was kept enclosed within the intact tied gastric mucosa HCl production was readily observed. This result in itself shows, with a possible error of about 1%, that 1 mol. of alkali is produced with each mol. of acid, because the HCl produced by a mucosa of dry weight 40 mg. and a Q_{HCl} 5 $\mu\text{l./mg. dry wt./hr.}$ liberates 200 $\mu\text{l./hr. CO}_2$, and a change of 1 $\mu\text{l./hr.}$ in the overall gaseous balance can be detected with both inside-out and open tissue. As this change does not occur, $200 \pm 1 \mu\text{l./hr. CO}_2$ must be absorbed to neutralize the alkali. The experimentally observed equivalence of this acid and alkali, in experiments with the tissue tied and inside-out, supports the conclusion that they are exactly equivalent. This equivalence explains the numerous failures to observe the presence of acid in fresh excised gastric mucosae (Hoerr & Bensley, 1936; Linderstrøm-Lang, Holter & Ohlsen, 1935).

The utilization of external supplies of CO_2 by oxyntic cells. As the maximum observed Q_{HCl} is about twice the maximum observed $|Q_{\text{O}_2}|$, an actively secreting gastric mucosa may produce acid and alkali at such a rate that a supply of CO_2 in excess of its own respiratory output is needed to neutralize the alkali. There are several reports which support this statement. The neglected work of Edkins & Murray (1924) can now be interpreted as showing

that added CO_2 disappears entirely from the lumen of a secreting stomach, and reaches the tissue CO_2 pressure in a non-secreting stomach. McIver, Redfield & Benedict (1926) claimed, by showing that the movement of CO_2 into and out of the stomach is governed by the physical laws of diffusion, that they had refuted this work. As no HCl was secreted during their experiments their claim is unjustified. Kurtz & Clark (1946) recently demonstrated an inverse relationship between the amount of HCl secreted and the amount of CO_2 diffusing into the gastric cavity, whilst Teorell (1933) found negative respiratory quotients using secreting cats' stomachs. These results are all explicable if the actively secreting mucosa utilizes external supplies of CO_2 .

The rates of respiration and of acid secretion in mammalian gastric mucosa. The result obtained with frog mucosa that the Q_{HCl} can be greater than the $|Q_{\text{O}_2}|$ applies also in mammalian mucosa, as can be shown by calculations based on previously published work. Concomitant measurements of the rates of respiration and secretion of a mammalian stomach have been recorded in at least three papers. Using operative procedures which probably damaged the tissue, as judged by the low activities, Teorell (1933) found that 12 g. cat mucosa used 0.45 ml. $\text{O}_2/\text{min.}$ and produced up to 0.20 ml. 0.1N-gastric juice/min. The writer has found that the dry weight of cat mucosa is about 20% of the wet weight. Therefore the Q_{O_2} was -11 and the Q_{HCl} was +11. Similar values can be obtained from the data of Ni & Lim (1928) and Hanke (1937) who worked on dogs. Since all the acid was produced by the oxyntic cells, but only a fraction of the O_2 was used by them, the Q_{HCl} of these cells must have been much higher than their $|Q_{\text{O}_2}|$. However, the rates of acid secretion by these *vivi-perfused* stomachs were much lower than those for gastric tissue prepared after less operative interference.

In unpublished experiments Mackay & Davies have obtained 51 mg. HCl/15 min. for 2 hr. from a chloralosed cat with an acid-secreting mucosa of dry weight 2.7 g. In this case the Q_{HCl} was 46. Rehm & Enelow (1945) obtained 16 mg. HCl/10 min. from a 21 sq.cm. sheet of dog gastric mucosa. It can be calculated from the figures of Ni & Lim (1928) that their dog's mucosa was 1.3 mm. thick, and it is unlikely that the mucosa used by Rehm & Enelow (1945) was thicker than 1.5 mm. Using this value and a dry weight/wet weight ratio of 0.2 the Q_{HCl} was 93. These values of the Q_{HCl} are far higher than the $|Q_{\text{O}_2}|$ of cat and dog mucosa.

Many figures are available for the peak value of secretory activity in man. Glenn (1946) recorded the results of 450 histamine-stimulated fractional gastric analyses. The mode of peak volume (ml./15 min.) $\times$ free acidity (clinical units) was 4000 and the maximum value 16,000. Mrs Bradford and the author

have found the dry weight in g. of 11 human acid-secreting mucosae to be from 8.6 to 15.5, average 12.0, standard deviation 2.0. The mode of the maximum Q_{HCl} was therefore 30. If it is assumed that the subject with the highest rate of secretion had an acid-secreting mucosa of even 16 g., the Q_{HCl} must have been 90. Rosenthal & Lasnitzki (1928) found that the Q_{O_2} of 11 samples of isolated, unstimulated, human gastric mucosa lay between -5 and -20. The average was -9.2.

Since the R.Q. of any gastric mucosa is unlikely to be much more than 1.0 it is clear that the Q_{HCl} of the oxyntic cells and even of the entire acid-secreting mucosa can rise much higher than both Q_{O_2} and Q_{CO_2} in the frog, cat, dog and man.

Bearing of the relationship between Q_{O_2} and Q_{HCl} on some recent theories of HCl elaboration. The findings discussed in the preceding paragraph bear on some recent theories of gastric acid elaboration. Davenport (1943), Bull & Gray (1945) and Conway, Fitzgerald & Walls (1945) have variously suggested that the H^+ ions are produced at the same rate as, or arise from, acids such as pyruvic or carbonic formed during oxidative metabolism within the oxyntic cells. Since 2 mol. of pyruvic acid are formed from 1 mol. of glucose, and 6 mol. of O_2 are required to oxidize the glucose to 6 mol. of CO_2 and 6 mol. of H_2O , in the first case the Q_{HCl} could never be greater than one third of the Q_{O_2} and in the last case the Q_{HCl} could never be greater than the Q_{O_2} . The possibility that the oxyntic cells remove pyruvic acid from the blood, secrete the H^+ ions and metabolize the anions is unlikely, because the cells do not require supplies of this acid in order to produce HCl.

The observed relation between the Q_{HCl} and the Q_{O_2} renders untenable any theory that the H^+ ions are produced by the oxidative degradation of any substrate known to take part in intermediary metabolism. The results of these experiments support the view that the reaction fundamentally concerned in the production of HCl in gastric mucosa is

The H^+ ions are secreted, and the OH^- ions finally neutralized by CO_2 and passed into the blood or nutrient medium. This is comparable with the membrane hydrolysis theory of Hollander (1943), and explains the CO_2 uptake which occurs during acid secretion.

The function of carbonic anhydrase in oxyntic cells. Davenport (1939) found that oxyntic cells contain a high concentration of carbonic anhydrase, and suggested that this enzyme has an important role in the mechanism of the formation of acid. However, Feldberg, Keilin & Mann (1940), using sulphanilamide, and Davenport (1946b), using thiophen-2-

sulphonamide, both powerful inhibitors of carbonic anhydrase, failed to inhibit acid secretion. On the basis of these results, and some rough calculations, Davenport (1946b) believed that the necessity for the enzyme in the gastric mucosa had not been demonstrated. To do this it must be shown that, under conditions in the cell, the uncatalyzed uptake of CO_2 is slower than the observed rate, and that the amount of enzyme present is sufficient to produce the necessary acceleration of this reaction.

The calculations given in the Appendix show that the uncatalyzed rate of CO_2 uptake, to neutralize alkali within actively acid-secreting oxyntic cells, is too slow by a factor which depends on the extent to which the pH of the cell is alkaline to the equilibrium value. For example, in mammalian cells at a pH difference of 0.01, a magnification of 400-fold is required, whereas at a pH difference of 0.1 this is reduced to 40-fold. On the assumptions mentioned in the Appendix, the amount of carbonic anhydrase present in the oxyntic cells of cats is sufficient to produce a magnification of the order of 15,000-fold. This means that even if 99.7% of the enzyme were inhibited, the residual quantity could still produce a 40-fold magnification of the uncatalyzed CO_2 uptake. This may account for the results of Feldberg *et al.* (1940) and Davenport (1946b). A concentration of sulphonamide sufficient to produce 99.7% inhibition of the carbonic anhydrase of the gastric cells would necessarily have inhibited the enzyme of the red cells to a similar degree. As the red cells contain only about one fifth as much carbonic anhydrase per unit volume as the gastric cells, the carbon dioxide transport by the blood should have been so seriously affected as to lead to speedy death of the animal (cf. the calculations of Roughton, 1943). It would therefore seem that the gastric enzyme could not have been sufficiently inhibited in any published experiment.

SUMMARY

1. Intact tied tubes of frog and toad gastric mucosa secreted acid and mucus *in vitro* aerobically, and also produced an equivalent amount of alkali ($\pm < 1\%$), which was neutralized by CO_2 and passed into the nutrient solution as HCO_3^- ions.

2. No evidence of HCl secretion was obtained when sheets of sheep, frog or toad gastric mucosa were incubated in Warburg cups, although their respiration was enhanced by histamine. This is in agreement with the conception that HCl secretion into the lumen of the stomach is linked with an equivalent bicarbonate secretion into the nutrient solution.

3. The Q_{HCl} of active mucosae was often higher than the $|Q_{\text{O}_2}|$ or the respiratory Q_{CO_2} , and in these

cases external supplies of CO_2 were needed for the neutralization of alkali.

4. Calculations presented in the Appendix show that the uncatalyzed rate of CO_2 uptake, to neutralize alkali within very active oxyntic cells, is too slow by a factor of the order of 100, and make it probable that the necessary extra uptake can be readily produced by the carbonic anhydrase within oxyntic cells.

5. Anaerobically the tissue secreted neither acid nor mucus.

6. Evidence is presented which disproves the theories of HCl elaboration due to Bull & Gray (1945) and Conway *et al.* (1945).

I am greatly indebted to Mrs N. M. Bradford (née Longmuir) for assistance during part of these investigations, and to Prof. H. A. Krebs, F.R.S., for his encouragement, interest and advice in this study.

Appendix

By R. E. DAVIES AND F. J. W. ROUGHTON, *Unit for Research in Cell Metabolism (Medical Research Council), Department of Biochemistry, The University, Sheffield, and Department of Colloid Science, The University, Cambridge*

The uncatalyzed rate of uptake of CO_2 in oxyntic cells. The value of the fraction, total volume of oxyntic cells/volume of acid secreting mucosa, is needed for the calculations. From the figures of Linderstrøm-Lang *et al.* (1935) it can be estimated that the fraction in pig mucosa, including the muscularis mucosa, is about 10%. Estimations on photographs of sections of human gastric mucosa varied from 8 to 11%, with an average just below 10%. Davenport (1939) found in cats that the average concentration of carbonic anhydrase was 39 Davenport units (E_D)/mg. wet weight in oxyntic cells and 2.7 E_D /mg. wet weight in acid-secreting mucosa. The surface cells contained small amounts, so the required fraction is not greater than 7%.

The following calculations are made for a mammalian mucosa with a Q_{HCl} of 50 μl /mg. dry wt./hr. at 38° containing 10% oxyntic cells, both well within the extremes. The output of CO_2 is given by the equation

$$\frac{d}{dt} [\text{CO}_2] = -k_u(1 + l[B]) [\text{CO}_2] + k_0(1 + l[B]) [\text{H}_2\text{CO}_3] \quad (\text{Roughton, 1943})$$

in solutions with $\text{pH} < 8$. $[B]$ is the concentration of the proton acceptor in the buffer and l is its catalytic coefficient. Since $[\text{HPO}_4^-]$ is probably about 0.002M inside the oxyntic cell and its l value is 8 at 0°, this term, and a correction for the protein buffers, can be neglected and the equation used in the form

$$\frac{d}{dt} [\text{CO}_2] = -k_u[\text{CO}_2] + k_0 \frac{[a_H][\text{HCO}_3^-]f_{\text{HCO}_3}}{K_1}, \quad (1)$$

where $K_1 = \frac{[a_H][\text{HCO}_3^-]f_{\text{HCO}_3}}{[\text{H}_2\text{CO}_3]}$;

a_H = hydrogen ion activity, and

f_{HCO_3} = activity coefficient of HCO_3^- ion.

$[\text{CO}_2]$ is unlikely to be much more than 0.0011M, and Irving (1932) gives the 'total CO_2 ' in dog fundic mucosa as about 30 ml./100 g. so $[\text{HCO}_3^-] = 0.0134\text{M}$.

Taking the ionic strength of blood as 0.13 (Hastings & Sendroy, 1925) and assuming similar conditions within oxyntic cells then

$$\log f_{\text{HCO}_3} = -0.5 \sqrt{0.13} \quad (\text{cf. Roughton, 1935}) \\ = -1.820$$

and $f_{\text{HCO}_3} = 0.66$.

Roughton (1943) gives $k_0 = 89 \text{ mol./l./sec.}$, $k_u = 0.131 \text{ mol./l./sec.}$, and $\text{p}K_1 = 3.47$ at 38° at infinite dilution.

Substituting these values in equation (1), at equilibrium

$$\frac{d}{dt} [\text{CO}_2] = 0 = - (0.131 \times 0.0011) + \left(\frac{89 \times a_H \times 0.0134 \times 0.66}{10^{-3.47}} \right),$$

therefore, $\text{p}a_H = 7.20_7$.

If the $\text{p}a_H$ of the cell is maintained below this value, CO_2 is evolved, whilst above it CO_2 is absorbed.

At 0.01 $\text{p}a_H$ units on the alkaline side of equilibrium, application of equation (1) shows that

$$\frac{d}{dt} [\text{CO}_2] = -0.03 \times 10^{-4} \text{ mol./l./sec.}$$

At 0.05 $\text{p}a_H$ units above equilibrium,

$$\frac{d}{dt} [\text{CO}_2] = -0.16 \times 10^{-4} \text{ mol./l./sec.}$$

At 0.10 $\text{p}a_H$ units above equilibrium,

$$\frac{d}{dt} [\text{CO}_2] = -0.30 \times 10^{-4} \text{ mol./l./sec.}$$

The oxyntic cells of the mucosa considered have a Q_{HCl} and a $Q_{\text{HCO}_3^-}$ of 500. Taking the dry weight as 0.2 wet weight, the usual value for mammalian mucosa, the rate of CO_2 uptake needed to produce this bicarbonate $\approx 12 \times 10^{-4} \text{ mol./l./sec.}$ The magnification of the CO_2 uptake required is therefore 400-fold at 0.01 $\text{p}a_H$, 75-fold at 0.05 $\text{p}a_H$, and 40-fold at 0.10 $\text{p}a_H$ units on the alkaline side of equilibrium.

When the back reaction is neglected the required rate is 8.5 times the maximum uncatalyzed rate of CO_2 uptake. (In his calculations Davenport (1946b) neglected the back reaction and also used the low Q_{HCl} of about 11.)

In these calculations it is assumed that the whole volume of the cell is available to absorb CO_2 and that $[\text{HCO}_3^-]$ and $[\text{CO}_2]$ remain constant. When the cell is dependent upon a supply of CO_2 from arterial blood, diffusion limitations will reduce the steady state level of $[\text{CO}_2]$ below 0.0011M. Thus these figures for the required magnification are minimal values and show the necessity for carbonic anhydrase in actively secreting oxyntic cells.

In frogs, while the Q_{HCl} values are lower by about 6-fold, the velocity constants for the hydration of CO_2 are lower by about 3.5-fold at 25.0° , so the enzyme is also required in frog gastric mucosa.

The effect of the carbonic anhydrase in oxyntic cells on the CO_2 uptake. The enzyme units used by Davenport (1939) were determined by the method of Meldrum & Roughton (1933) except that the temperature was 0° instead of 15° . One Davenport unit (E_D) is the amount of carbonic anhydrase which, when dissolved in 2 ml. of 0.1M- Na_2HPO_4 in 0.1M- KH_2PO_4 and mixed with 2 ml. of 0.2M- NaHCO_3 in 0.02M- NaOH , halves the time for the evolution of the second quarter of the total CO_2 . If R = the reciprocal of the time with the catalyst, and R_0 = the reciprocal of the time without the catalyst, then there is one E_D unit in 4 ml. when

$$\frac{R - R_0}{R_0} = 1.$$

To calculate the effect of enzymic activities, as expressed in Davenport units, on the rate of CO_2 uptake under the conditions prevailing in the gastric mucosa, it is first necessary to relate the Davenport unit to the more fundamental constants of carbonic anhydrase activity used by Roughton & Booth (1946).

According to these authors

$$R_{eo} = \frac{k_{eo}xE}{x + K_{mo}}, \quad (2)$$

where R_{eo} = the extra rate of CO_2 output in the presence of the enzyme,

k_{eo} = velocity constant for dissociation of the enzyme-complex $E \cdot \text{H}_2\text{CO}_3$,

$x = [\text{H}_2\text{CO}_3]$,

E = enzyme concentration, and

K_{mo} = Michaelis constant for CO_2 output.

Equation (2) is not convenient to work with, in view of the difficulty of calculating x exactly, but it may be transformed into a much more manageable form as follows.

Roughton & Booth (1946) have shown that

$$k_{eo} = k_{eu} \text{ and } K_{mo} = K_{mu}/K,$$

where k_{eu} = velocity constant for dissociation of the enzyme-complex $E \cdot \text{CO}_2$,

K_{mu} = Michaelis constant for CO_2 uptake = 0.009M at 0° ,

K = equilibrium constant of the reaction

$$\text{i.e. } K = \frac{[\text{CO}_2]}{[\text{H}_2\text{CO}_3]} = c. 900 \text{ at } 0^\circ.$$

We then have

$$R_{eo} = \frac{k_{eo}xE}{x + K_{mo}} = \frac{k_{eo}KxE}{Kx + K_{mo}} = \frac{k_{eu}EC'}{C' + K_{mu}},$$

where $C' = Kx$ = the hypothetical concentration of dissolved CO_2 which would be in equilibrium with the $[\text{H}_2\text{CO}_3]$ at the stage used in Davenport's determination for the calculation of the enzyme units present.

This hypothetical or virtual $[\text{CO}_2]$ can be readily obtained if the CO_2 -dissociation curve of the Meldrum-Roughton (M-R) mixture used by Davenport is known. The curve in question gives the relation between $[\text{HCO}_3^-]$ and $[\text{CO}_2]$ at complete equilibrium. It has been specially determined by us over the temperature range $0-38^\circ$ (see Fig. 6 for

Fig. 6. CO_2 dissociation curve of the Meldrum-Roughton phosphate-bicarbonate mixture. The arrows at A, B and C indicate respectively the conditions at the start, at 3/8 completion and at the end of the reaction, when 4 ml. of M-R solution are shaken in a 65 ml. boat.

results at 0°), by equilibrating 4 ml. of M-R mixture with CO_2 pressures varying from 0 to 200 mm. Hg in a modified Van Slyke constant-volume gasometric apparatus, and then determining the CO_2 contents of the gas and liquid phases. The usual 50 ml. Van Slyke gas chamber was replaced by a special 65 ml. chamber, which was the size of the boats used by Davenport. A more detailed account of the technique and experiments will be published elsewhere.

By this procedure we found that, at 0° , under the conditions prevailing in the boat experiments,

$$[\text{HCO}_3^-] = 0.0878\text{M just after mixture,}$$

$$= 0.0611\text{M at the end of reaction,}$$

$$= 0.0778\text{M when the reaction is 3/8 completed,}$$

i.e. at the stage used for calculating the enzyme units present.

At the 3/8 stage, virtual $[\text{CO}_2]$ is the abscissa on Fig. 6, corresponding to 0.0778M- HCO_3^- , and therefore = 0.0094M. $[\text{HPO}_4^-] = 0.07\text{M}$ and $p_{\text{H}} = 7.24$, by calculation.

With Davenport's conditions and 1 E_D enzyme unit in 4 ml., we then have

$$\frac{\left\{ \frac{k_{eu}E \times 0.0094}{(0.0094 + 0.009)} + k_u \times 0.0094(1 + l[\text{HPO}_4^-]) \right\} - k_u \times 0.0094(1 + l[\text{HPO}_4^-])}{k_u \times 0.0094(1 + l[\text{HPO}_4^-])} = 1 \text{ at } 0^\circ.$$

$$\text{Therefore } \frac{k_{eu}E}{k_u \times 0.0184 \times 1.56} = 1.$$

Since $k_u = 0.0021$, $k_{eu}E = 6 \times 10^{-5}$ mol. CO_2 /l./sec. in the case of 1 Davenport unit.

In cat oxyntic cells there are 39 E_D /mg. wet weight, so 4 ml. of the cells contain $1.56 \times 10^5 E_D$ units, and therefore in the latter case

$$k_{eu} = 6 \times 10^{-5} \times 1.56 \times 10^5 \\ = 9.4 \text{ at } 0^\circ.$$

Within the oxyntic cell, taking $[\text{CO}_2]$ as 0.0011M, and neglecting the catalytic effects of the buffers for reasons given earlier, the magnification of the non-enzymic rate of CO_2 uptake at 0°

$$= \frac{k_{eu}E \times 0.0011}{0.0011 + K_{mu}} \div k_u \times 0.0011 \\ = \frac{9.4}{0.0021(0.0011 + 0.009)} = 440,000\text{-fold.}$$

This figure is greater than the magnification of 1.56×10^5 for the M-R solution at 0° because (i) the substrate concentration in the latter case (i.e. 0.0094M-virtual CO_2) is about the same as the value of the Michaelis constant, whereas in the former case the substrate concentration (i.e. 0.0011M- CO_2) is only about one-eighth as great; (ii) the oxyntic cells are not known to contain inorganic catalysts corresponding to the $[\text{HPO}_4^-]$ in the M-R solution.

According to Kiese (1941), and to the rough preliminary data of Meldrum & Roughton (1933), the Michaelis constant increases about 3-fold per 10° rise, and should thus be about 50 to 60 times greater at 38° than at 0° . The substrate concentration, both in the M-R solution and in the case of 0.0011M- CO_2 solution, is therefore very small compared with the value of the Michaelis constant at 38° ; this means, if the effect of $[\text{HPO}_4^-]$ is neglected, that the magnification must be sensibly the same in the two cases and equal to $k'_{eu}E/k'_uK'_{mu}$, where symbols with primes denote the corresponding constants at 38° .

From these arguments it would follow that

$$\frac{\text{magnification in } 0.0011\text{M-}\text{CO}_2 \text{ at } 0^\circ}{\text{magnification in } 0.0011\text{M-}\text{CO}_2 \text{ at } 38^\circ} \\ = 1.8 \times \frac{\text{magnification in M-R solution at } 0^\circ}{\text{magnification in M-R solution at } 38^\circ}.$$

According to the recently amended data of Meldrum & Roughton (Roughton, 1948) on the enzymic and non-enzymic rates of CO_2 output from M-R solutions at temperatures ranging from 0.2° to 34.95° ,

$$\frac{\text{magnification in M-R solution at } 0^\circ}{\text{magnification in M-R solution at } 38^\circ} = c. 17.$$

Assuming that the value of l for HPO_4^- does not alter greatly from 0° to 38° (see below), the magnification in 0.0011M- CO_2 at 38° should be

$1/1.8 \times 17 = 1/30$ times the magnification at 0° , so that for the conditions prevailing in the gastric mucosa, the magnification should be $440,000/30$, i.e. about 15,000-fold. This figure is only about one fifth of the figure of 70,000-fold at 38° previously given by Davies, Longmuir & Crane (1947). The discrepancy is in part due to the fact that the figure, in the literature at the time, for the effect of temperature on the rate of the enzymic reaction was too high (Roughton, 1943), and in part to a theoretical error in the mode of calculation adopted. It is hoped that the latter is now reliable.

The concentration of carbonic anhydrase in frog gastric mucosa is about $0.1\text{--}0.2 E_D$ /mg. wet weight (Davenport, 1946a). Assuming that the concentrations of HCO_3^- and CO_2 are similar to those in the mammalian oxyntic cells, but that the temperature is 25° and the enzyme content $0.15 E_D$ /mg. wet weight, this concentration of enzyme could produce a magnification of about 1,200-fold.

Since Roughton (1943) states that the phosphate in the Meldrum-Roughton solution and the chloride in the red cell both inhibit carbonic anhydrase to about the same extent at the pH of red cells, no correction need be made for this effect in oxyntic cells.

The uncertainties in these calculations are (1) the extrapolation of the temperature coefficient of the magnification up to 38° , (2) the assumption of a linear relation between enzyme concentration and activity at high concentrations, (3) the possibility that the general environment of the enzyme in the cell may modify its activity, (4) the assumption that diffusion has no limiting effect on the reaction, and (5) the assumption that l is constant from 0° to 38° .

As regards (1) and (5), the uncertainty is probably now not large. In the case of these gastric cells we have no information in regard to (2) and (3), but unpublished calculations by Roughton, based on the rough data of Dirken & Mook (1930) on the rate of CO_2 reactions in red cell suspensions and strong haemoglobin solutions, suggest that the uncertainty may well be only of the order of 2- or 3-fold. The effect of diffusion is very difficult to assess until more exact knowledge is available as to the distances which the CO_2 has to diffuse from the capillaries to the highly concentrated enzyme in the cells.

Apart from the uncertainty as to what limiting effect is caused by diffusion, these calculations make it probable that the necessary extra uptake can be readily produced by the carbonic anhydrase within the oxyntic cells.

REFERENCES

- Brücke, E. (1859). *S.B. Akad. Wiss. Wien*, **37**, 131.
 Bull, H. B. & Gray, J. S. (1945). *Gastroent.* **4**, 175.
 Conway, E. J., Fitzgerald, O. & Walls, D. (1945). *Nature, Lond.*, **156**, 477.
 Davenport, H. W. (1939). *J. Physiol.* **97**, 32.
 Davenport, H. W. (1943). *Gastroent.* **1**, 383.
 Davenport, H. W. (1946a). Private communication.
 Davenport, H. W. (1946b). *Physiol. Rev.* **26**, 560.
 Davies, R. E. (1946). *Biochem. J.* **40**, xxxv.
 Davies, R. E., Longmuir, N. M. & Crane, E. E. (1947). *Nature, Lond.*, **159**, 468.
 Delrue, G. (1930). *Arch. int. Physiol.* **33**, 196.
 Deutsch, W. & Raper, H. S. (1936). *J. Physiol.* **87**, 275.
 Dirken, M. N. J. & Mook, H. W. (1930). *J. Physiol.* **73**, 273.
 Dixon, M. (1943). *Manometric Methods*, 2nd ed. Cambridge: University Press.

- Edkins, N. & Murray, M. M. (1924). *J. Physiol.* **59**, 271.
- Feldberg, W., Keilin, D. & Mann, T. (1940). *Nature, Lond.*, **146**, 651.
- Friedemann, T. E. & Graesser, J. B. (1933). *J. biol. Chem.* **100**, 291.
- Friedman, M. H. F. (1943). *Proc. Soc. exp. Biol., N.Y.*, **54**, 42.
- Glenn, P. M. (1946). *Gastroent.* **6**, 409.
- Gray, J. S., Adkison, J. L. & Zelle, K. (1940). *Amer. J. Physiol.* **130**, 327.
- Hanke, M. E. (1937). *Science*, **85**, 54.
- Hanke, M. E., Johannesen, R. E. & Hanke, M. M. (1931). *Proc. Soc. exp. Biol., N.Y.*, **28**, 698.
- Hastings, A. B. & Sendroy, J. (1925). *J. biol. Chem.* **66**, 445.
- Hoerr, N. L. & Bensley, R. R. (1936). *Anat. Rec.* **65**, 417.
- Hollander, F. (1943). *Gastroent.* **1**, 401.
- Irving, L. (1932). *J. cell comp. Physiol.* **2**, 149.
- Jones, H. Bence (1845). *Philos. Trans.* p. 335.
- Keilin, D. (1929). *Proc. Roy. Soc. B*, **104**, 206.
- Kiese, M. (1941). *Biochem. Z.* **307**, 400.
- Krebs, H. A. (1933). *Hoppe-Seyl. Z.* **217**, 191.
- Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
- Kurtz, L. D. & Clark, B. B. (1946). *Fed. Proc.* **5**, 188.
- Linderström-Lang, K., Holter, H. & Ohlsen, A. S. (1935). *C.R. Lab., Carlsberg*, **20**, 66.
- Lutwak-Mann, C. (1947). *Biochem. J.* **41**, 19.
- Melver, M. A., Redfield, A. C. & Benedict, E. B. (1926). *Amer. J. Physiol.* **76**, 92.
- Meldrum, N. U. & Roughton, F. J. W. (1933). *J. Physiol.* **80**, 113.
- Ni, T. G. & Lim, R. K. S. (1928). *Chin. J. Physiol.* **2**, 45.
- Peters, J. P. & Van Slyke, D. D. (1931). *Quantitative Clinical Chemistry*, **1**, Interpretations. London: Baillière, Tindall and Cox.
- Rehm, W. S. & Eneelow, A. J. (1945). *Amer. J. Physiol.* **144**, 701.
- Rosenthal, O. & Lasnitzki, A. (1928). *Biochem. Z.* **196**, 340.
- Roughton, F. J. W. (1935). *Physiol. Rev.* **15**, 241.
- Roughton, F. J. W. (1943). *Harvey Lect.* **39**, 96.
- Roughton, F. J. W. (1948). *J. Physiol.* **107**, 12 P.
- Roughton, F. J. W. & Booth, V. H. (1946). *Biochem. J.* **40**, 319.
- Teorell, T. (1933). *Skand. Arch. Physiol.* **66**, 225.
- Warburg, O. (1923). *Biochem. Z.* **142**, 317.
- Warburg, O. (1926). *Biochem. Z.* **172**, 432.

Production of Ulcers in Isolated Frog Gastric Mucosa*

By R. E. DAVIES AND NORAH M. LONGMUIR, *Unit for Research in Cell Metabolism (Medical Research Council), Department of Biochemistry, The University, Sheffield*

(Received 26 September 1947)

Previous experiments (see Davies, 1948) have shown that when isolated frog gastric mucosa secretes acid, an equivalent amount of alkali is also produced. Normally this is neutralized by CO_2 and the resulting HCO_3^- ions are passed into the nutrient medium. The amount of HCl produced in highly active mucosae in bicarbonate saline gassed with 5% CO_2 + 95% O_2 (vol. % of gases throughout) was up to 100% above the metabolic CO_2 production and therefore external supplies of CO_2 were needed to neutralize this alkali. During these experiments (done in autumn, winter and early spring) it was found that the more active 'tied bags' of mucosa in phosphate saline gassed with 100% O_2 produced viscous whitish secretions containing much cell debris (indicating tissue damage), although in bicarbonate saline gassed with 5% CO_2 + 95% O_2 the secretions were clear and mobile.

The experiments described in this paper were designed to discover whether ulceration would occur when the experimental conditions interfered with the neutralization of the alkali by CO_2 ; this would lead to an accumulation of unneutralized alkali.

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

It was found that whilst mucosae with high secretory activities remained undamaged in bicarbonate saline, active mucosae were regularly ulcerated and even perforated in phosphate salines. Variations in the concentration of the phosphate buffer of the saline medium did not affect the occurrence of this ulceration.

EXPERIMENTAL

Saline media. The bicarbonate saline used for measurements of the balance of the O_2 uptake and the CO_2 output was the physiological salt solution of Krebs & Henseleit (1932) diluted by 0.29 vol. of water and gassed with 5% CO_2 + 95% O_2 or with 5% CO_2 + 95% N_2 . During measurements of the O_2 uptake two alternative solutions were used. These were the phosphate saline of Krebs (1933) diluted with 0.29 vol. of water, and a saline solution similar to this phosphate saline, but containing only 0.0011 instead of 0.0084 M-phosphate. Both were gassed with 100% O_2 . All salines contained 0.2% glucose.

Material. Frogs (*Rana temporaria temporaria* L.) were captured locally, the web tattooed with Indian ink for identification, and housed in a concrete frogger at 15–17°. An excess of cockroaches or worms was provided for food.

The gastric mucosa was washed, isolated and closed with silk thread at both ends (Davies, 1948). The amount of secretion produced and the total content of the mucosa