Meschkowa, N. P. (1936). *Hoppe-Seyl. Z.* **240**, 199.
Mitchell, H. K. & Isbell, E. R. (1942). *Univ. Texas Publ.* 4237, 37.
Mitsuda, T. (1923). *Biochem. J.* **17**, 630.
Neuberger, A. & Webster, T. A. (1946). *Biochem. J.* **40**, 576.
Nielsen, N., Hartelius, V. & Johansen, G. (1944). *C.R. Lab. Carlsberg*, **24**, 40.
Potter, V. R. & Franke, K. W. E. (1934). *J. Nutrit.* **9**, 1.
Rose, W. C., Haines, W. C., Johnson, J. E. & Warner, D. T. (1944). *J. biol. Chem.* **148**, 453.
Schenck, J. R. & du Vigneaud, V. (1944). *J. biol. Chem.* **153**, 501.
Schenck, J. R., Simmonds, S., Cohn, M., Stevens, C. M. & du Vigneaud, V. (1943). *J. biol. Chem.* **149**, 355.
Schoenheimer, R., Rittenberg, D. & Keston, A. S. (1939). *J. biol. Chem.* **127**, 385.
Wolff, W. A. & Wilson, D. W. (1932). *J. biol. Chem.* **95**, 495.
Zapp, J. A. & Wilson, D. W. (1938). *J. biol. Chem.* **126**, 9, 19.

Metabolic Processes in the Gastric Mucosa

By C. LUTWAK-MANN, *Biochemical Laboratory, Cambridge*

(*Received* 18 *July* 1946)

The metabolic properties of the digestive tract have received attention from time to time, notably those of certain parts of the intestines (Dickens & Weil-Malherbe, 1941); but there is little recent work on the respiration and glycolysis of the gastric tissues apart from Rosenthal & Lasnitzki's (1928) measurements on the mucous membrane. Extensive studies on the localization of peptic and hydrolytic enzymes were carried out by Linderstrøm-Lang and his associates (relevant papers quoted by Rask-Nielsen, 1944); recently the presence of carbonic anhydrase was demonstrated in the gastric mucous membrane by Davenport (1939) and the enzyme was extracted from this material and purified by Keilin & Mann (1940).

In the present study, rather than to pursue the investigation of enzymes concerned with digestive processes in the stomach, an attempt was made to gather information about the respiration and glycolysis of the mucosa and the other gastric components so as to establish the metabolic requirements of the healthy normal mucosa and to gain insight into conditions which may lead to pathological changes such as erosions and ulcerations.

Certain aspects of the metabolism of the gastric mucosa were examined in 1944 in the course of research concerned with the mechanism of systemic poisoning with mustard gas (Lutwak-Mann, 1946); these studies were undertaken on behalf of the Ministry of Supply and the results were summarized by Dixon & Needham (1946) but full details will be published later. A part of the present work was communicated to the Biochemical Society (Lutwak-Mann & Barrett, 1946).

EXPERIMENTAL

Material. Rats were chosen as experimental animals. The anatomy of the rat stomach, an account of which may be found in a paper by Berg (1942), differs somewhat from the human, but it is probable that findings based on the behaviour of the rat stomach will have general application.

Usually animals weighing 100–150 g., but also younger and older rats, were examined. The rats received a mixed stock diet except in some experiments on special diets mentioned later. They were killed by decapitation, bled, and the whole stomach removed and placed in saline. The methods of preparing the tissue for an experiment varied and as the results depend to some extent upon the technique employed, the details are given below.

Methods. Anaerobic acid production and O_2-consumption were measured in Barcroft manometers with side bulbs and gas outlets. Ringer-phosphate (Krebs, 1933) was used for aerobic experiments; phosphate was replaced by bicarbonate for the assay of acid under anaerobic conditions in an atmosphere of 95% N_2+5% CO_2. Unless otherwise stated, the volume of the fluid in the manometer cups was 2 ml. and the dry weight of the tissue between 8 and 10 mg. The concentration of glucose or other metabolites added to saline was 0·2%. Volatile substances, e.g. alcohols, were placed in equal amounts in both sides of the manometric vessels.

Lactic acid was determined by the method of Friedemann, Cotonio & Shaffer (1927), phosphate by the method of Fiske & Subbarow (1925), N was determined by means of the Kjeldahl modification of Parnas & Wagner (1921), the content of adenosinetriphosphate (ATP) by the method of Parnas & Lutwak-Mann (1935), polypeptide-N following the procedure of Burstein (1937), the content of glutathione and ascorbic acid according to Hopkins & Morgan (1936).

RESULTS

Anaerobic glycolysis

Gastric mucosa detached from the rest of the stomach wall

As the gastric mucous membrane does not lend itself to the application of the ordinary slice technique for manometric experiments, the following procedure was employed in the earlier stages of this work.

The stomach was opened along the greater curvature and very thoroughly rinsed in several changes of saline. It was

spread on the convex surface of a large watch-glass, the loose mucus was gently wiped off and the mucosa itself separated from the rest of the stomach wall with a small scalpel. Some laceration was unavoidable, but it was noted that the removal of the mucous membrane was much easier in stomachs filled to capacity with food digest than in the contracted stomachs of the starved rats. The material from one or, if necessary, from two or more stomachs was pooled and divided into roughly equal parts (equivalent to 10–15 mg. dry weight) and at once immersed in Ringer solution. To ensure the even distribution of the tissue, which tends to form clumps, a thin glass rod was used to unfold the pieces of mucous membrane in the manometer cups.

In the course of anaerobic incubation the mucosa gradually disintegrated and could not at the end of longer experiments be collected quantitatively. Therefore the contents of the manometer cups were emptied into centrifuge tubes and the deposit obtained after spinning was dried for 2 hr. at 110°. The values obtained in this way served as basis for the calculation of the earlier manometric results. However, it was later observed that even after as little as 20–30 min. anaerobiosis the saline in the manometer cup became increasingly opalescent and viscous, pointing to diffusion of protein from the tissue. To assess roughly the amount of soluble protein in the Ringer solution at the end of each experiment, after the removal of the tissue by spinning, dilute acetic acid was added dropwise to the supernatant fluid till no further precipitate was formed. The weight of this precipitate was determined after centrifugation and drying at 110°. The loss of protein from the gastric mucous membrane into the surrounding fluid during 1–2 hr. anaerobiosis was variable and amounted to between 20 and 50% of the whole dry weight. It also became clear that the larger losses occurred mostly in glucose-free samples, while in the presence of glucose as a rule less than 20% of the dry weight was attributable to the proteins which diffused into saline. The determination of 'initial' wet weight of tissue, apart from being time-consuming when speed was essential, was not practicable, owing to varying amounts of saline and loose mucus which adhered to the tissue after the rinsing in saline to remove food particles.

The graphs representing the progress of anaerobic acid production by the detached mucosa (Fig. 1) show that 20–30 min. from the start, the glycolysis, even in the presence of glucose, began to fall off. This could not be counteracted effectively by the addition of cozymase or a mixture of cozymase plus nicotinic amide; although there was some initial improvement in the rate of acid production, the effect of the coenzymes soon wore off. The addition to the Ringer solution of 0·2% glucose had some beneficial influence upon the maintenance of the tissue structure as judged by the smaller amounts of protein found in the saline, but the rate of acid production was either only slightly raised or not affected at all.

Even poorer glycolysis was obtained when, after separation from the stomach wall, the mucosa was ground into a uniform suspension (Fig. 1); the attempt to boost the low acid production by means of coenzymes was not successful, probably owing to destruction of these substances by enzymes liberated from the homogenized tissue.

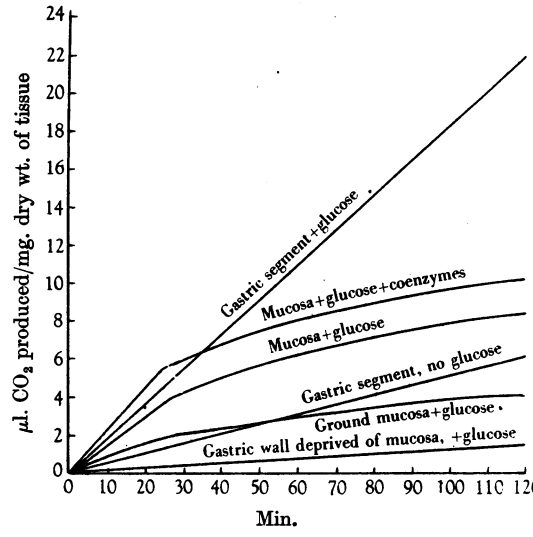

Fig. 1. Anaerobic glycolysis of gastric mucosa. Tissue incubated in Ringer-bicarbonate at 38°, in the form of segments of whole gastric wall, as mucosa detached from the rest of the stomach wall, in form of mucosa ground into uniform suspension, and stomach wall deprived mechanically of its mucous lining.

The anaerobic glycolysis of the separated gastric mucosa was not much improved when serum-bicarbonate was used instead of Ringer-bicarbonate. In view of the possible interference by serum with the determination of substances diffusing into the incubation fluid from the tissue, and the variable composition of serum, it was decided to employ Ringer solution rather than serum.

Segments of the whole stomach wall

The relatively rapid breakdown of the detached mucosa in anaerobic conditions, reflected in the progressive fall of glycolysis, made it desirable to find a more stable preparation. To this end, the gastric tissue was treated as follows.

The fore-stomach part was cut away and rejected, food particles were removed from the mucosa-lined portion of the stomach, and with very sharp scissors narrow (1·0–1·2 mm.) transverse annular segments of the entire wall were cut and washed in saline, till free from adhering food digest. During this, spontaneous eversion of these rings of tissue took place so that the outer circumference was that covered with the mucous lining. Usually sections were made in the region of the cardiac glands but in some experiments similar rings of gastric tissue were cut nearer the pyloric end of the stomach. With some practice it was possible to obtain segments the dry weight of which did not exceed 10 mg.

When gastric tissue prepared in this manner was used for manometric measurements of anaerobic glycolysis in Ringer-bicarbonate containing glucose, there was perfectly steady acid production (Fig. 1) for at least 2–4 hr. At the end of a 2 hr. period the saline surrounding the gastric segments remained almost water-clear; no debris of broken-up tissue was encountered and upon addition of acetic or trichloroacetic acid there was only slight precipitation of protein. Segments made from the same stomach showed good agreement but there were individual variations in the extent of anaerobic glycolysis when the gastric tissue of several batches of rats was examined.

In the absence of glucose, however, there was a marked difference in the behaviour of the tissue. The acid production was poor (Fig. 1) when anoxia had proceeded for 1–2 hr. and there was progressive disintegration of the tissue leading to a loosening of the surface epithelium which could be seen floating like a thin film still partly adhering to the deeper layers of the mucous membrane. At the same time the Ringer solution became opalescent and very faintly yellow. It was observed that gastric mucosa incubated in glucose retained its original reddish hue even after some hours of anaerobic incubation whereas the aglucotic material was almost colourless, confirming the loss of pigment to the solution.

When the glycolysis of sections of stomach wall from which the mucous lining had been mechanically removed was measured, very low values were obtained even in the presence of glucose (Fig. 1). In view of this it was felt justifiable to regard the values obtained with the gastric segments of the entire wall as representing chiefly the metabolism of the mucosa itself. It will be seen later that, deprived of the mucosa, the stomach wall was also incapable of utilizing O_2.

The optimum range of pH for the anaerobic glycolysis was rather narrow; best results were obtained between pH 7·4 and 7·8, but below pH 7·0 there was a considerable fall and hardly any glycolysis took place in unbuffered Ringer solution. The reaction proceeded in veronal buffer of pH 7·4–7·6, but less well than in bicarbonate or in phosphate buffer.

Lactic acid

The deproteinization with trichloroacetic acid of experimental samples which contained the separated mucosa invariably proved troublesome and no clear filtrates could be obtained. But there were no such difficulties with the gastric segments and these were used for the chemical determination of lactic acid. Table 1 shows the amount of lactic acid produced by gastric segments in the course of 2 hr. incubation in the presence of various substrates. With glucose or mannose the same quantities of lactic acid were formed, but in their absence the gastric mucosa was incapable of producing more than traces of lactic acid. Fructose, galactose, glycogen, also the Cori, Neuberg and Embden hexosephosphate esters, were poorly glycolyzed. The quantity of lactic acid as estimated chemically represented usually 70–85 % of the acid production measured manometrically. When segments were cut from the pyloric region of the stomach the discrepancy was somewhat bigger, the yield of lactic acid being as much as 40 % lower in some experiments than the amount of CO_2 produced. The possibility of another acid besides lactic acid, being produced anaerobically is not entirely excluded.

Table 1. *Anaerobic glycolysis in rat gastric mucosa in the presence of various substrates*

(Gastric segments from normal rats. Incubation 2 hr.; 38°; Ringer-bicarbonate; 95 % N_2 + 5 % CO_2, 0·2 % substrate.)

	Acid formation/10 mg. dry weight of tissue	
Substrate	Lactic acid (mg.)	CO_2 (μl.)
Glucose	0·68	220
Mannose	0·70	220
Fructose	0·26	80
Galactose	0·23	70
Glycogen	0·30	78
Cori ester	0·38	90
Embden ester	0·30	85
Neuberg ester	0·29	92
No addition	0·10	46

Influence of certain external factors on the anaerobic glycolysis of the gastric mucosa

Several experiments were carried out in which groups of 12 rats each were kept for some weeks on inadequate diets and on diets deficient in vitamins. Some groups received a full synthetic diet to which sulphanilamide or sulphasuxidine were added daily. In others the effect was studied of ether anaesthesia when relatively large amounts of ether were inhaled and presumably a proportion of ether entered the stomach. In addition, various age groups and differences between sexes were investigated.

No clear-cut difference was noticeable as regards the sexes, but the glycolysis of the gastric mucosa was undoubtedly higher when young animals were used. The results of the experiments with various diets are summarized in Table 2. When the rats were kept on a protein-free, high carbohydrate diet, they lost weight but the glycolysis was not greatly affected, even after several weeks. Rats fared very badly on a high-fat diet; the appearance of the gastric mucosa was pale with the folds almost smoothed out; the values for anaerobic glycolysis

7–10 days after the start of the fat diet were much less than the control values.

Rats maintained on a diet deficient in vitamin B_1 showed all the usual deficiency symptoms, but the gastric glycolysis remained largely unchanged until the late stage when the rats were moribund. After 2 months of a vitamin A-deficient diet the glycolysis of the gastric mucosa appeared normal.

The daily addition, for several weeks, of 1 % sulphanilamide caused some slight wavering in the weight curves but generally the rats tolerated the drug well and the glycolysis remained unchanged. The addition of 0·5 % sulphasuxidine produced diarrhoea after 2 weeks of feeding; in most rats the gastric glycolysis was normal but after continued treatment with the drug it fell in some animals. Brief ether anaesthesia, sufficient to induce sleep, but not lethal, had no effect.

Table 2. *Effect of various diets on the anaerobic glycolytic activity of rat gastric mucosa*

(Gastric segments from rats on various diets; 12 animals in each group.
Incubation 2 hr.; 38°; Ringer-bicarbonate; 0·2 % glucose, 95 % N_2 + 5 % CO_2.)

Diet	Duration (weeks)	Acid formation/10 mg. dry weight of tissue: Lactic acid (mg.)	Acid formation/10 mg. dry weight of tissue: CO_2 (μl.)
High carbohydrate	2	0·68	210
High carbohydrate	4	0·58	200
High fat	1	0·50	180
High fat	3	0·38	140
Full synthetic + 1 % sulphanilamide	1	0·72	210
Full synthetic + 1 % sulphanilamide	3	0·60	200
Full synthetic + 0·5 % sulphasuxidine	1	0·63	200
Full synthetic + 0·5 % sulphasuxidine	3	0·47	185
Vitamin B_1-deficient	1	0·74	240
Vitamin B_1-deficient	2	0·67	220
Vitamin B_1-deficient	3	0·40	160
Vitamin A-deficient	10	0·66	190

The effect of substances added in vitro

The anaerobic glycolysis of the gastric mucosa was inhibited by 0·002 M-iodoacetate and 0·01 M-NaF. In the presence of these inhibitors of glycolysis the tissue suffered gross visible damage in spite of the presence of glucose. But the degree of disintegration appeared less severe than in gastric segments incubated anaerobically in glucose-free Ringer solution.

Atropine sulphate in low concentrations, 0·002–0·01 M, did not alter the course of glycolysis; ten or twenty times this amount had a slight inhibitory effect. The injection of 0·5 mg. atropine/g. of body weight was well tolerated by the rats; their gastric glycolysis was examined at various intervals after the injections but the values obtained did not differ from controls. The addition *in vitro* of sulphanilamide (15 mg./2 ml. Ringer solution) had no effect. Dodecyl sulphate 0·08 % caused a marked change in the appearance of the gastric segments making them look shrunken and almost translucent; the surrounding saline was rather viscous and glycolysis was about half the normal value. The addition of 10–15 mg. of ethanol or methanol to 2 ml. glucose-containing Ringer-bicarbonate did not diminish the rate of glycolysis; amyl alcohol (1 ml. shaken with 9 ml. water; 0·2 ml. used per experiment) depressed the glycolysis perceptibly. When the acid production was measured in the presence of 15 mg. of ethyl or methyl acetate/2 ml. saline, with the usual amount of glucose, variable results were obtained; in some instances there was moderate inhibition, in others the acid production surpassed the controls by about 20 %.

The effect of aglucotic anaerobiosis upon the structural and enzymic make-up of the gastric mucosa

Gastric mucosa exposed to lack of O_2 coupled with the absence of a glycolyzable substrate such as glucose or mannose showed, as previously described, macroscopic evidence of serious damage, but it was desirable to locate more precisely the lesions thus caused. To this end a histological examination was undertaken by Dr A. M. Barrett the results of which are summarized in the Appendix. To explore the possibility of an accompanying biochemical trauma the following type of experiment was carried out.

Gastric segments from normal young animals were prepared and incubated in the usual manner in manometer cups in Ringer-bicarbonate, with and without glucose, for varying lengths of time during which the glycolysis was measured. Then they were quickly transferred to fresh, glucose-containing Ringer-bicarbonate and the further progress of glycolysis was again recorded manometrically as well as by measuring lactic acid. In such experiments the glucose-containing samples gave invariably almost water-clear saline, even after a total incubation period of 4 hr.; but in the absence of glucose tissue debris was noticeable 1–2 hr. after the beginning of the incubation in the sugar-free saline, which itself became increasingly opalescent. Contrary to earlier expectations, this behaviour, far from being alleviated or reversed, was intensified by the transfer of the aglucotic tissue to fresh glucose saline. In fact, the degree of destruction wrought by aglucotic anoxia was less during an unbroken period of 4 hr. without glucose, than when such tissue was placed in glucose at some point of the

anaerobic incubation. This was not merely judged from the gross appearance of the experimental samples but was confirmed by the estimation of N in the saline.

The formation of lactic acid continued at a steady rate throughout the entire experiment in glucose or mannose, a little more lactic acid being found after transfer of the tissue to fresh Ringer solution. But the aglucotic tissue, which itself formed only traces of lactic acid, when removed to fresh saline containing glucose, was no longer capable of transforming it into lactic acid. Complete loss of glycolytic activity in the segments of the gastric wall occurred usually after 1–2 hr. in N_2 without glucose; but the material from young rats appeared more resistant and after 1 hr. such gastric tissue still retained most of its glycolytic power. The same results were obtained if phosphate buffer was used instead of Ringer-bicarbonate, the mixture of CO_2+N_2 being replaced by N_2.

Table 3. *Diffusion of lactic acid, phosphate, ammonia and nitrogen from gastric rings into saline in the course of anaerobic incubation*

(Gastric segments incubated A, with glucose, and B, without glucose for 2 hr. at 38°.

Both A and B segments transferred to fresh Ringer-bicarbonate with 0·2% glucose, for a second incubation of 2 hr. at 38°. Results as mg./100 g. dry weight of tissue.)

	First incubation 2 hr.		Second incubation 2 hr.		Total experimental period 4 hr.	
	A (mg.)	B (mg.)	A (mg.)	B (mg.)	A (mg.)	B (mg.)
Lactic acid	6·0	0·8	8·0	1·1	14·0	1·9
Inorganic-P	0·16	0·25	0·09	0·15	0·25	0·40
Ammonia-N	0·035	0·031	0·005	0·017	0·040	0·048
Nitrogen	1·6	2·2	2·0	3·7	3·6	5·9

The salines at the end of incubation were examined for their content of P, ammonia-N and N (see Table 3). The gastric tissue deprived of glucose for 2 hr. had lost its ability to produce lactic acid from glucose. The amount of N which leaked into the saline was magnified by the transfer from glucose-free to glucose-containing saline. The total amounts of inorganic-P and ammonia-N which diffused into the fluid were higher in the experiments in which the glycolytic activity of the gastric mucosa had been destroyed as a result of aglucotic anoxia.

The content of pyrophosphate-P (hydrolyzable in 7 min. by N-HCl at 100°) in the gastric tissue during its anaerobic incubation was also examined. In the presence of glucose or mannose, the value of this labile P fraction fell after 30 min. incubation to less than half the amount found in fresh tissue (see p. 26). It continued to diminish progressively, reaching a low but apparently constant level. In the absence of glycolyzable sugars only a mere trace of pyrophosphate was detectable after the 30 min. interval, when material damage was already perceptible; at later stages of aglucotic exposure no pyrophosphate was found. The addition of glucose or mannose in the course of sugar-free incubation, or transfer to fresh glucose-containing saline, failed to bring about the reappearance of this P fraction.

It was important to establish the amount of glucose (or mannose) required by the rat gastric mucosa under anaerobic conditions for preservation of its structure and ability to form lactic acid from carbohydrate. A search was also made for substances which could replace glucose or mannose. To offset the suspected loss into the saline of labile coenzymes, various substances known to be essential for glycolysis were added alone or in combinations. Fig. 2 shows that as little as 1 mg. glucose (roughly M/400 under the conditions of the experiment) was already protective, but far better results were achieved using five to ten times more; excess beyond this led to little further protection of glycolysis. Fructose, galactose, glycogen, or the hexosephosphoric esters of Cori, Neuberg and Embden, were unable to secure the maintenance of glycolytic activity or to check the disintegration of the tissue. The only substitute for glucose was mannose, which preserved both the structure and the glycolytic system under anaerobic conditions. Some beneficial effect upon the acid production was observed when a mixture of cozymase, ATP and ADP (0·1 mg. of each/2 ml. fluid) was added to saline in which the aglucotic sample was incubated. Nicotinic amide and guanosine were without effect. Among other substances, examined in similar experiments, lactate and pyruvate as well as *dl*-methionine exerted a small positive effect, but the addition of glucosamine-HCl, *N*-acetyl-glucosamine or K hyaluronate, did nothing to protect the structural integrity of the mucous membrane or its glycolytic activity.

In view of the inability of the rat gastric mucosa to convert fructose to lactic acid, the possibility was examined of an interference with the action of glucose. Various concentrations of fructose were added to glucose but did not interfere with its protective effect (Fig. 2).

The Ringer-bicarbonate, in which gastric segments were incubated anaerobically without glucose for 3–4 hr. till it became opalescent and tissue debris accumulated, was collected and replenished with glucose and then used as medium for a manometric experiment with fresh gastric segments. The fact that glycolysis was unimpeded proved that there had been no leakage of substances from the asphyxiated aglucotic tissue, capable of interfering with the glycolysis.

The ability of the mucosa to respire was examined after various intervals of anaerobic aglucotic incubation. After 60–80 min. the tissue was still fully capable of taking up O_2, but longer anaerobic exposure caused significant decrease in the O_2 consumption.

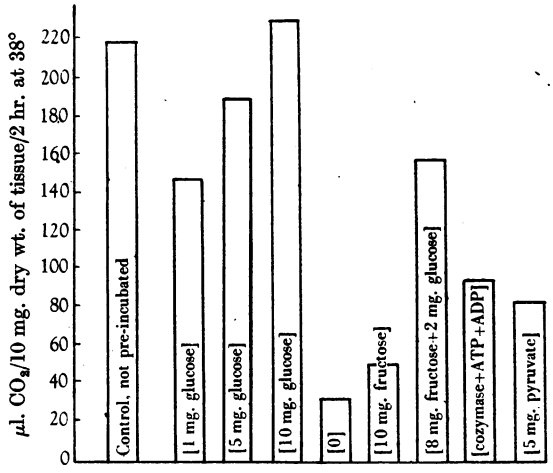

Fig. 2. Glycolytic activity in the gastric mucosa after a preceding period of anoxia. After 2 hr. anaerobic pre-incubation with various substances [indicated in square brackets] the gastric segments were transferred to fresh Ringer-bicarbonate containing 0·2% glucose and the anaerobic acid production measured for 2 hr. at 38°. (Average data from 4 experiments each.)

The possibility was considered that while enzymic systems dependent upon labile coenzymes might suffer irreparable damage from exposure to anaerobic conditions without utilizable substrate, others might fare much better, either because they were situated in less vulnerable layers of the gastric mucosa, or owing to greater inherent resistance. It was decided to explore the fate of carbonic anhydrase which is present in the parietal cells of the gastric mucous membrane (Davenport, 1939). The experiments were arranged in the following manner.

The stomachs of young rats were opened along the greater curvature, washed very thoroughly in saline, weighed and placed in manometer vessels in 5 ml. Ringer-bicarbonate or Ringer-phosphate, with or without glucose. They were kept for varying periods in strictly anaerobic conditions. At the end of the incubation the saline was quantitatively collected, the stomachs were rinsed with 5 ml. water which was added to the saline. The mucosa was then collected, weighed, ground with 3 ml. water and centrifuged; the residue was rejected, and the supernatant extract, as well as the previously collected saline+washings, were used for the assay of carbonic anhydrase by means of the colorimetric method of Philpot & Philpot (1936) as described by Keilin & Mann (1940). The main results were also confirmed with the manometric boat apparatus of Brinkmann, Margaria & Roughton (1933) following the procedure of Meldrum & Roughton (1933).

In the experiments recorded in Table 4 the wet weights of the whole stomachs did not differ appreciably (between 0·75 and 0·9 g. wet weight); the amounts of separated mucosa were equal (0·18 g. wet weight); and the same volumes of saline and water were used for each sample. It can be seen that, whereas after 2 hr. in anaerobic conditions carbonic anhydrase was detected in the incubation fluid both in the presence and absence of glucose, distinctly more enzyme was found in the glucose-free saline. When the incubation period was extended to 4 hr. there was several times more enzyme in the absence than in the presence of glucose. The enzyme in the aqueous extract from the mucosa itself was little affected by 2 hr. anaerobiosis, but after longer exposure there appeared to be some loss of activity in the mucosa deprived of glucose. In analogous experiments carried out aerobically, after 2 hr. incubation in O_2 with and without glucose, little more than traces of carbonic anhydrase activity were found in the saline. Somewhat more was detectable after longer incubation, but the dependence upon glucose was slight. The figures for the N content of the saline samples show that under anaerobic conditions much more N was lost into the fluid surrounding the tissue than in O_2, and how this loss of N was magnified by the lack of glucose (Table 4).

Table 4. *Behaviour of carbonic anhydrase in the gastric mucosa during anaerobic and aerobic incubation*

(Rat stomachs incubated in 5 ml. saline, with and without glucose, for 2 hr. and 4 hr. at 38°. After incubation, rinsed with 5 ml. water. Saline+rinsing water from each experiment combined, and used for enzyme assay as 'saline' with and without glucose. Mucosa ground in 3 ml. water, aqueous extract used for assay as 'mucosa' with and without glucose. The colorimetric test of Philpot & Philpot (1936) was used to determine the activity of carbonic anhydrase in these fluids.)

Material examined	Enzyme activity as vol. of fluid required to complete colorimetric test in 30 sec. Stomachs incubated 2 hr. (ml.)	Enzyme activity as vol. of fluid required to complete colorimetric test in 30 sec. Stomachs incubated 4 hr. (ml.)	N content in 'saline' after 4 hr. incubation (mg.)
1. Anaerobic incubation			
'Saline' with glucose	2·0	1·6	2·7
'Saline' without glucose	0·8	0·4	4·4
'Mucosa' with glucose	0·2	0·15	—
'Mucosa' without glucose	0·2	0·30	—
2. Aerobic incubation			
'Saline' with glucose	3·0	2·0	1·6
'Saline' without glucose	2·5	1·4	1·9
'Mucosa' with glucosa	0·15	0·15	—
'Mucosa' without glucose	0·10	0·15	—

Oxygen uptake

Aerobically, the behaviour of the gastric tissue differed in several aspects from that exhibited in the absence of O_2. The gastric mucosa separated mechanically from the rest of the stomach wall was clearly capable of preserving its coherence without undue loss of protein into the surrounding saline, even in the course of fairly long aerobic incubation. Secondly, the ability to maintain satisfactorily the structure was largely independent of the addition of glucose, except during very long incubation periods when a slight beneficial effect was noticeable.

When, instead of the detached mucosa, circular sections or simply thin strips of the whole wall were incubated in saline in O_2, there were even fewer signs of tissue damage; the diffusion of N into saline was much less than in anaerobic experiments, and the effect of glucose negligible. Some of the earlier experiments were carried out in air, as it was felt that the gastric mucous membrane might be sensitive to pure O_2. However, it would appear from the histological findings that in air there may have been at least partial anaerobiosis which expressed itself as loss of surface epithelium (see Appendix), a phenomenon found to be characteristic of gastric mucosa suffering from lack of O_2. As there was no evidence of any special sensitivity of the mucosa to pure O_2, all later experiments were done in O_2, but the duration of the routine experiments was limited to 2 hr. to minimize the risk of microbial contamination. The O_2 uptake in air was found to be several times less than in pure O_2, both with segments of the whole gastric wall and with the detached mucosa which consumed only about $\frac{1}{3}$ to $\frac{1}{2}$ of the amount utilized in O_2.

Except in very young rats, the thickness of the stomach wall was greater than 1 mm.; none the less, when very thin (0·8–1·0 mm.) strips were prepared, their dry weight not exceeding 6–8 mg., satisfactory and reproducible manometric results were obtainable (Fig. 3), while at the same time the structure of the tissue as a whole was reasonably well preserved. Moreover, the respiration of the stomach wall from which the mucous lining had been removed represented not more than 10% of the values reached by the segments with their mucosa intact (Fig. 3). The detached mucosa (10–15 mg. dry weight) consumed less O_2 than segments of the entire wall and the reaction was not linear beyond 1 hr. (Fig. 3).

Substrate

Practically the same results were obtained whether or not glucose was added, both with detached mucosa and whole stomach wall. Occasionally, an enhancing influence of glucose upon the O_2 uptake was observed, particularly in starved rats, and in old animals in which the gastric mucosa showed generally lower values than in young rats (Fig. 3), but the respiration of the detached mucosa was sometimes actually depressed by the addition of glucose. The addition of fructose to both types of tissue preparation slightly lowered the O_2 consumption. The addition to Ringer-phosphate of succinate, glucosamine-HCl, *N*-acetylglucosamine, had no effect upon the respiration of the segments of gastric mucosa.

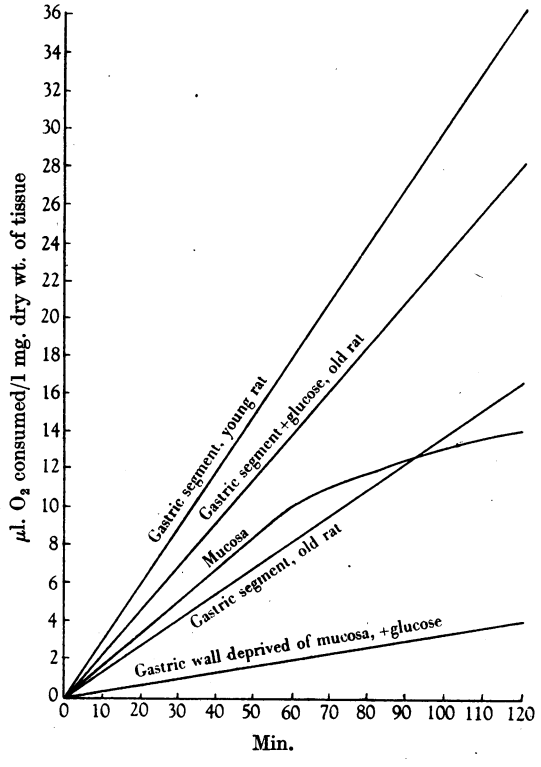

Fig. 3. O_2 uptake of gastric mucosa. Tissue incubated in Ringer-phosphate at 38°. Segments of entire stomach wall, mucosa separated from the wall, and stomach wall deprived of mucous membrane.

Unlike the anaerobic glycolysis, the O_2-uptake was not excessively sensitive to changes of pH, values obtained between pH 6·5 and 7·8 differed little. The effect of various diets, similar to those already described, upon the utilization of O_2 by the gastric mucosa was also examined. Generally, it was found that where the animals suffered badly from inadequate food composition, the O_2 consumption was less than in control rats; otherwise, there were no significant changes.

Aerobic glycolysis

Some lactic acid was produced by the gastric mucosa from added glucose during incubation in O_2. The amounts found at the end of a 1 or 2 hr. period represented about half or even less of the values formed in corresponding experiments in the absence of O_2. The aerobic glycolysis was slightly higher in Ringer-phosphate than in Ringer-bicarbonate. Without added glucose, the respiring mucosa did not produce any lactic acid.

The effect of inhibitors and other substances added in vitro

There were some differences in the response to inhibitors, as between the separated mucosa and the gastric segments. In presence of KCN (0·001 M) the respiration of the gastric mucosa was at a standstill (Fig. 4); weak NaF (0·0025–0·01 M) had little

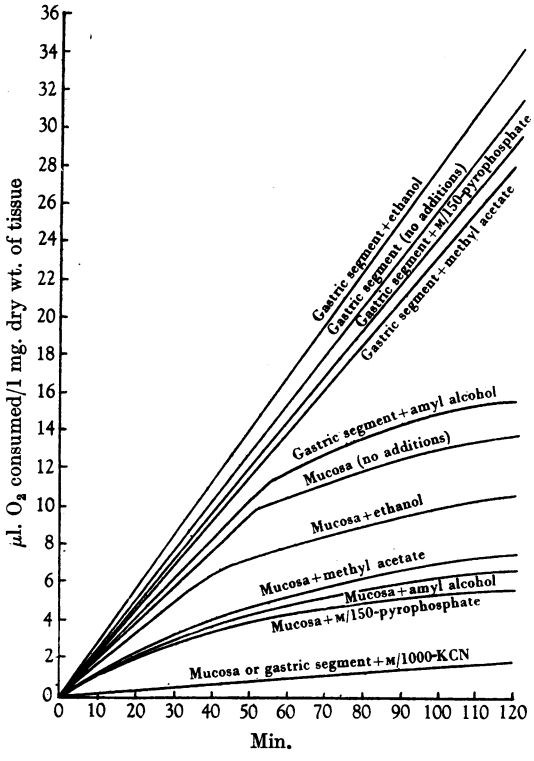

Fig. 4. Effect of various substances on O_2-uptake of gastric mucosa. Tissue incubated in Ringer-phosphate at 38°. Segments of whole stomach wall, or mucosa alone. Concentration of alcohols or esters, 15 mg./2 ml. saline.

affect on the respiration of the gastric strips but decreased that of the separated mucosa; NaF (0·0125 M) considerably inhibited O_2 uptake in both kinds of tissue preparation. Pyrophosphate (0·005–0·01 M) did not alter the respiration of gastric segments but caused considerable inhibition of separated respiring mucosa (Fig. 4). Substances which inhibited the O_2 uptake of the entire wall and the detached mucosa, were: 0·08% dodecyl sulphate; 0·005 M-Na_2S; 0·005 M-dimethyldithiocarbamate; malonate, 0·005–0·01 M, was without effect, but in higher concentration (0·02 M) inhibited the respiration. Atropine in the amounts mentioned above had no effect. Ethyl or methyl acetate slightly inhibited gastric strips but decreased the O_2 uptake of the detached mucosa. Ethanol and methanol had either no effect on the gastric segments or slightly enhanced their O_2 consumption, but lowered significantly the O_2 uptake of the mucous membrane alone; amyl alcohol strongly inhibited the respiration regardless of the kind of tissue preparation (Fig. 4).

ASSAY OF THE CONTENT OF CERTAIN SUBSTANCES IN THE RAT GASTRIC MUCOSA

The content in the rat gastric mucosa of the following substances was estimated: adenosinetriphosphate (ATP), acid-soluble P fractions, polypeptide-N, glutathione, ascorbic acid, glycogen. In all experiments the rumen portion was rejected and the whole mucosa-covered part of the stomach was used without separating the mucous membrane from the rest of the wall. The material from 6 to 8 rats was used for each determination.

For both freshly fed and starved rats, 1·6 mg. ATP amino-N/100 g. wet weight of stomach was found. The values for acid-extractable P content/100 g. wet weight were: 50–60 mg. total acid-soluble P, of which 20–25 mg. was directly determinable, 6–8 mg. belonged to pyrophosphate-P, 5 mg. to esters hydrolyzable in 1 hr. and the rest was made up of difficulty acid-hydrolyzable P esters.

The total non-protein-N content of the rat gastric mucosa was 116 mg. and the polypeptide-N was 20 mg./100 g. wet weight of tissue.

The content of ascorbic acid and glutathione (GSH) was estimated in normal animals and also in rats maintained for 3 weeks on diets containing 1% sulphanilamide or 0·5% sulphasuxidine. The normal values were 0·65 mg. GSH and 0·13 mg. ascorbic acid/g. wet weight. In rats on diets containing sulphonamide drugs, the values for GSH were less than in controls, 0·42 and 0·51 mg., in the sulphanilamide- and sulphasuxidine-fed animals, respectively; but the amounts of ascorbic acid appeared unaltered.

In a few experiments the Pflüger procedure for the determination of glycogen was applied to rat gastric mucosa, but no precipitate was obtained after the addition of 1–2 vol. of ethanol to the

KOH hydrolyzate. When 10 vol. of ethanol were added the precipitate which formed was not glycogen.

DISCUSSION

The biochemical study of the metabolic properties of the digestive tract necessitated the adoption of an approach varying from that generally accepted as suited to the investigation of such organs, as for instance the liver. In its make-up the digestive tract lacks the uniformity of structure characteristic of liver tissue (in which one type of cell largely predominates) and is built from different interpenetrating morphological elements; attempts to separate them artificially for experimental purposes tend to cause a fall in enzymic processes of the tissue such as the respiration and glycolysis. The mucous membrane under physiological conditions comes into contact with metabolites and other substances not only through the blood-stream like all other tissues, but also from the surface lining of the lumen. In this respect, the gastric differs from the intestinal mucosa in that it comes into contact with largely undigested matter. It would not be surprising if the mucous membrane were to react differently according to whether substances reached its epithelium-lined surface facing the stomach cavity or entered the cells *via* the blood capillaries. Normally secretion of gastric mucin by the epithelial cells protects the exposed surface against noxious agents but when this function of mucin production is interrupted owing to material damage to the epithelium, the deeper layers of the stomach wall may easily suffer.

The great frailty of the gastric mucosa after separation from the rest of the stomach wall in absence of O_2, even when supplied with glucose, makes it unsuited for anaerobic experiments. The use of segments of the entire gastric wall meant that some precision in manometric technique had to be sacrificed, as their thickness mostly exceeded that considered optimal for manometric assays with tissues such as liver, kidney and brain. On the other hand, even when O_2 was excluded, they maintained for a time at least a reasonable degree of tissue integrity, reflected in the steady rate of the metabolic processes investigated. With the detached mucosa it was impossible to determine satisfactorily whether the addition of glucose increased the anaerobic acid production; but with the sections of the entire wall the results were clear-cut and gave unequivocal proof of increase by glucose (cf. Fig. 1). Fortunately, the stomach wall mechanically deprived of its mucous lining was almost without glycolytic and respiratory activity.

This investigation has shown the utter dependence of the normal gastric mucosa, under anaerobic conditions upon the presence of a glycolyzable substrate, both for the preservation of important enzymic systems (e.g. glycolysis) and for the maintenance of tissue integrity. The first tissue element to succumb in absence of O_2 and glucose appears to be the epithelium lining the surface of the mucosa, but deeper elements were also somewhat affected. The concentration of glucose which was found to be protective lay well within the physiological range, though in the experiments *in vitro* somewhat higher concentrations were required to achieve full protection for the preservation of the tissue and the glycolytic activity. The only substance which could replace glucose was mannose; other sugars, which are not glycolyzed by the rat gastric mucosa, did not protect.

The phenomenon of anaerobic dependence of tissues upon a glycolyzable carbohydrate is undoubtedly a general one and not limited to the gastric mucous membrane. Segments of caecum were also observed to be disintegrated and to lose their glycolytic activity as a consequence of exposure to aglucotic anaerobic conditions. Dickens & Greville (1933) similarly found that in brain slices even very brief anaerobic periods of substrate deprivation resulted in loss of glycolytic power and Macfarlane & Weil-Malherbe (1941) established the disappearance under such conditions of the pyrophosphate-P in brain tissue.

On the basis of the older work as well as the experiments described here, there is every reason to believe that the failure of the labile adenosinepolyphosphates to be resynthesized when the glycolytic cycle is interrupted, is an important factor explaining the loss of glucolytic activity by the anoxic tissue. But it still remains for future study, biochemical and cytological, to determine precisely the point of juncture between the chemical reactions involving the carbohydrate metabolism and the structural damage which seems to affect cell proteins and probably other complex components of the tissue, as soon as the supply of essential glycolytic coenzymes is exhausted. The altered and rapidly deteriorating cell-permeability of the gastric mucosa under anaerobic conditions was best indicated by the diffusion into the surrounding saline of an enzymic protein, carbonic anhydrase, the presence of which could sometimes be detected in the incubation fluid before the glycolysis of the mucosa was wholly destroyed. Another instance of the close relationship between structural integrity and glycolysis was provided by experiments with iodoacetate and NaF, in the presence of which tissue damage was clearly visible.

It is too early to speculate upon the possibility of erosions or even ulcerations being a direct outcome of such changes as were observed in the gastric mucosa when deprived of substrate anaerobically. But it is conceivable that owing, e.g., to temporary

breakdowns in blood circulation, especially in poorly vascularized areas, parts of the mucous surface might suffer losses in the continuity of the epithelial lining whereby gastric mucin would no longer be produced and chemical agents could penetrate into deeper layers.

In contrast to its behaviour under anaerobic conditions, the gastric mucosa in O_2 was found to be quite independent of glucose. The preformed substrate utilized aerobically by the mucosa, has not yet been identified. The sensitivity of the O_2 uptake to KCN makes it probable that the cytochrome system is involved in the respiration of the mucous membrane.

When as the result of a faulty diet the condition of the rats was poor, the metabolism of the gastric tissue was usually also lowered. A more detailed examination of very much larger numbers of animals and other species might perhaps reveal an association of metabolic changes with specific nutritional disorders.

SUMMARY

1. The anaerobic glycolysis and O_2 uptake of the gastric mucosa of the rat were studied. The experimental material was used either in the form of mucous membrane detached from the rest of the stomach wall or as segments of the entire wall. The stomach wall itself, freed from its mucous lining, had little glycolytic or respiratory activity.

2. The anaerobic glycolysis was best studied using segments of the whole gastric wall, because anaerobically the separated mucosa disintegrated rapidly. Glucose and mannose were readily converted into lactic acid by the gastric segments, but fructose, galactose, glycogen and several hexose-phosphates, were poorly glycolyzed. The glycolysis was inhibited by low concentrations of iodoacetate or NaF.

3. In absence of O_2 the maintenance of both tissue integrity and glycolytic activity in the gastric mucosa depended upon the presence of a glycolyzable substrate such as glucose or mannose, of which concentrations as low as 0·0025-M sufficed to prevent the structural damage and preserved the glycolytic system. Various substances examined were ineffective as substitutes for these two sugars.

4. Enzyme systems not dependent upon labile coenzymes were less susceptible to destruction under anaerobic aglucotic conditions. It was found that the respiration was little affected by preceding anaerobiosis; the gastric carbonic anhydrase was also resistant.

5. Aerobically, without glucose, the gastric mucosa maintained its enzymic activity and structure; the addition of glucose had no significant effect upon the rate of O_2 consumption. Low concentrations of KCN completely inhibited the respiration of the gastric mucosa. The rate of the aerobic glycolysis was not more than about half of that due to the lactic acid formed from glucose anaerobically.

The author is indebted to the Medical Research Council for a whole-time personal as well as material expenses grant. She wishes to express her gratitude to Prof. A. C. Chibnall, F.R.S., for his encouragement and interest in this study. Miss R. Leader's untiring and skilful assistance in dealing with the animals is recorded with much appreciation.

REFERENCES

Berg, B. N. (1942). *Amer. J. Path.* **18**, 49.
Brinkmann, R., Margaria, R., & Roughton, F. J. W. (1933). *Philos. Trans.* A, **232**, 65.
Burstein, M. (1937). *J. Physiol. Path. gén.* **35**, 71.
Davenport, H. W. (1939). *J. Physiol.* **97**, 32.
Dickens, F. & Greville, G. D. (1933). *Biochem. J.* **27**, 1134.
Dickens, F. & Weil-Malherbe, H. (1941). *Biochem. J.* **35**, 7.
Dixon, M. & Needham, D. M. (1946). *Nature, Lond.*, **158**, 432.
Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Friedemann, T. E., Cotonio, M. & Shaffer, P. A. (1927). *J. biol. Chem.* **73**, 335.
Hopkins, F. G. & Morgan, J. (1936). *Biochem. J.* **30**, 1146.
Keilin, D. & Mann, T. (1940). *Biochem. J.* **34**, 1163.
Krebs, H. A. (1933). *Hoppe-Seyl. Z.* **217**, 193.
Lutwak-Mann, C. (1946). Report to Ministry of Supply by Dixon, No. 32.*
Lutwak-Mann, C. & Barrett, A. M. (1946). *Biochem. J.* **40**, v–vi.
Macfarlane, M. G. & Weil-Malherbe, H. (1941). *Biochem. J.* **35**, 1.
Meldrum, N. U. & Roughton, F. J. W. (1933). *J. Physiol.* **80**, 113, 143.
Parnas, J. K. & Lutwak-Mann, C. (1935). *Biochem. Z.* **278**, 11.
Parnas, J. K. & Wagner, R. (1921). *Biochem. Z.* **125**, 253.
Philpot, F. J. & Philpot, J. St. L. (1936). *Biochem. J.* **30**, 2191.
Rask-Nielsen, R. (1944). *C.R. Lab. Carlsberg*, **25**, 1.
Rosenthal, O. & Lasnitzki, A. (1928). *Biochem. Z.* **196**, 340.

* Information available on application to Ministry of Supply, London.

Appendix. Histological Observations

By A. M. BARRETT, *The Department of Pathology, Cambridge*

Some of the gastric ring sections used in the metabolic experiments were afterwards fixed in Heidenheim's 'Susa' and sent to me in 80% alcohol for histological examination. They were embedded in paraffin, and sections were stained by Ehrlich's haematoxylin and eosin.

Anaerobic experiments with and without glucose (tissue incubated 2–4 hr.). Three specimens from anaerobic experiments without glucose were examined. In two of them the greater part and, in the third, a smaller part, of the epithelium which should line the inner surface of the stomach and the gastric pits were missing. On the other hand, this surface epithelium was intact in all three specimens from comparable experiments in the presence of glucose. Other changes, e.g. separation of the zymogenic cells and focal necrosis in the mucosa, were present in most specimens and appeared unrelated to the presence or absence of glucose.

Aerobic experiments in air and oxygen (2–3 hr. incubation periods). Four specimens from experiments carried out in oxygen were compared with four specimens from comparable experiments in air. Yeast-like organisms were seen on the mucosal surface of all four 'oxygen' specimens, but not in any of the 'air' specimens. Swelling and rarefaction of the connective tissue immediately beneath the surface epithelium, an abnormality not seen in any anaerobic specimens, was present in three of the 'oxygen' specimens but only in one of the 'air' specimens. On the other hand, the surface epithelium was partially absent in two of the 'air' specimens but not in any of the 'oxygen' specimens. The significance of these differences is doubtful and no others were observed. They are commented upon on p. 25.

Two of the 'oxygen' specimens and two of the 'air' specimens were from experiments in which glucose was present; the other two of each from experiments in which glucose was absent. Swollen empty-looking muscle fibres were present in the muscle coat of all the 'glucose-present' specimens but not in any of the others. No other effect of the presence or absence of glucose in aerobic experiments was recognized.

The specimens from experiments in air or oxygen appeared better preserved on the whole than those from anaerobic experiments. In particular, pyknosis of the nuclei of parietal cells and focal necroses were less common in the 'aerobic' specimens.

A Modified Method for the Viscosimetric Assay of Hyaluronidase

By G. I. M. SWYER and C. W. EMMENS
The National Institute for Medical Research, Hampstead, London, N.W. 3

(*Received* 26 *July* 1946)

The method of McClean & Hale (1941) for the viscosimetric assay of hyaluronidase has been in extensive use and is claimed to be accurate to within ±10%; in the hands of the present authors, however, it has not proved entirely satisfactory. We have, therefore, been prompted to investigate some of the fundamental principles upon which the method is based and, as a result, have succeeded in devising a simpler and apparently more accurate procedure. The method of McClean & Hale is based on the work of Madinaveitia & Quibell (1940) who calculated the 'half viscosity time' or 'half-life time of the substrate', which they found to be inversely proportional to the concentration of the enzyme and independent of the concentration of the substrate, both of which conclusions were supported by McClean & Hale and made the basis for their viscosimetric technique. On the other hand, Robertson, Ropes & Bauer (1940) found that the time taken for a fixed amount of enzyme to break down increasing amounts of substrate was proportional to the concentration of the latter. In view of this apparent discrepancy, and since the time taken to reach the half viscosity level of the substrate did not seem to give a satisfactory index of the potency of the enzyme, it was decided to investigate (1) the relationship of the flow-time in Ostwald viscosimeters to the concentration of a simple viscous solution (dextrose); (2) the same relationship for buffered hyaluronate solutions of varying concentration; (3) the curve of flow-times for hyaluronate solutions mixed with hyaluronidase; and (4) the