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ALKALINE SECRETION PRODUCED BY INTRA-ARTERIAL ACETYLCHOLINE

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There have been suggestions that the mediator of vagal impulses to the gastric mucosa is acetylcholine (ACh) (Babkin, 1950). The fact that it can be recovered from the venous effluent of the stomach after vagal stimulation (Dale & Feldberg, 1934), is not in itself evidence for this view, since the ACh found might come entirely from preganglionic endings or from the post-ganglionic nerve terminals which control the muscle fibres of the stomach and blood vessels. It is significant, however, that ACh injected intra-arterially produces a gastric secretory response. Moreover, from differences in viscosity, pH and Cl^- content (Stavraky, 1945; Morton & Stavraky, 1948; and for other references Babkin, 1950) it appears that ACh may stimulate the different epithelial cells of the gastric mucosa; however the experimental conditions that elicit a distinct secretion from the various types of cells are not known, nor is the complete composition of the fluid or fluids obtained.

In the present work, the effect of ACh injected into the artery which supplies an isolated piece of mucosa of the greater curvature of the stomach of dogs has been re-studied. The type of secretion obtained, i.e. whether acid or alkaline, can be controlled experimentally. This report will deal solely with experiments in which alkaline secretion was obtained. The principal ionic constituents, the total protein and the osmotic concentration of this alkaline secretion were determined. The probable mechanisms by which the secretion is produced, and their relation to the so-called 'alkaline component' of the gastric secretion, will be discussed.

METHODS

All experiments were performed on the stomachs of mongrel dogs anaesthetized with sodium pentobarbital. An isolated piece of the mucosa of the greater curvature, connected to the animal only through the blood vessels, was mounted in a plexiglass chamber as described previously (Altamirano, Chian & Bravo, 1960). The experiments in which counter pressure was applied were performed in the modified chamber illustrated in Fig. 1. A piece of mucosa was placed between two square blocks of plexiglass, 1 and 2, having a circular

opening 6·2 mm in diameter. The two blocks were pressed together by four screws; thus, bleeding from the cut edges of the mucosa was completely stopped. As is illustrated in Fig. 1, care was taken to leave the blood vessels, usually one artery and one vein, undisturbed at the centre of the opening. A plate of plexiglass with two semilunar plastic sponges attached, 3, was fixed to the lower block 2; these sponges maintained the mucosa

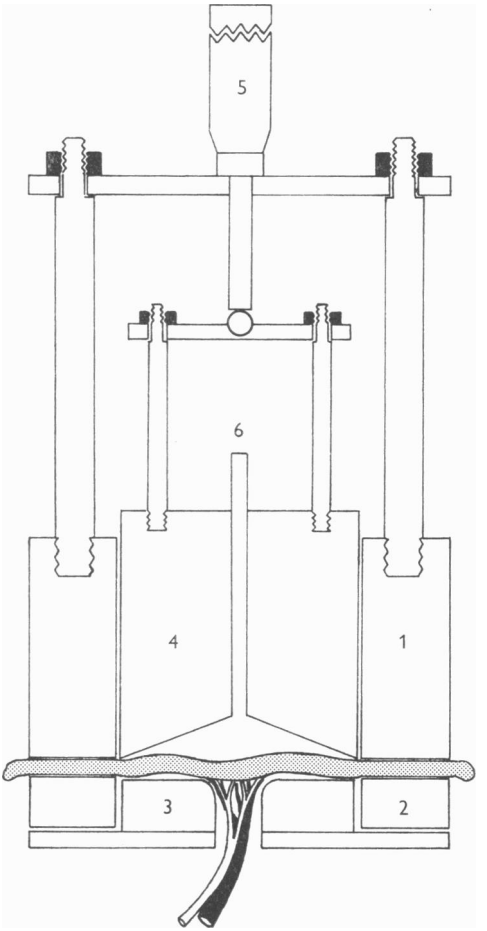

Fig. 1. Chamber used for the counter-pressure experiments.

in position when counter pressure was built up. A precisely machined piston, 4, fitted the opening of the upper block 1. The position of the piston was controlled by means of a micrometer caliper, 5, each turn of which corresponded to a change in volume of 0·03 ml. of the cavity of the chamber. A notch at the upper end of the tube 6 was used as a point of reference when volume measurements were performed, and the chamber could be connected to a mercury manometer or Statham transducer by the same tube 6. The temperature of the whole system was kept at 37–38° C by the use of an infra-red lamp.

Acetylcholine chloride (50 μg/ml.) dissolved in saline solution was injected by means of a polyethylene tube introduced through one of the severed branches of the splenic artery. A Sigmamotor Pump, Model TM 11, was used for the slow injection, and the dosage varied

by changes in the rate of injection. The procedure was the same in all experiments. The mucosa, washed two or three times with distilled water, was carefully dried and the injection of ACh started. The total contents of the chamber were aspirated in a volumetric burette every 20 min (called henceforth a 'period') and the volume measured.

Total CO_2 was measured with a Natelson Microgasometer. Na^+ and K^+ were determined with a Baird Atomic Flame Photometer Model KY. A Buchler–Cotlove Chloridometer was used for the Cl^- measurements. Ca^{2+} was measured by a modification of the complexometric method published by Mori (1959). Total inorganic P was measured by the procedure of Fiske & Subbarow (1925). Ammonia and urea were determined as described by Conway (1957). Total concentration of proteins was measured as described by Mehl (1945). A Fiske Osmometer Model G was used for the osmotic measurements. The determinations of pressure were performed by means of a Statham transducer connected to a Grass Polygraph (Model V). A Radiometer Titrator, Model TTT 1c, with a Scale Expander attachment and a micro-electrode chain type E 5020 were used for the pH measurements (performed at 38° C).

RESULTS

Chemical composition of the alkaline secretion

It has been claimed that intra-arterial ACh produces predominantly an alkaline secretion at the greater curvature (Stavraky, 1945; Morton & Stavraky, 1948). We found that the rate of injection of ACh determined to a great extent whether the secretion was acid or alkaline. Small doses usually produced an acid secretion, which rapidly became alkaline if the rate of injection was increased. Secretions at a pH of about 1 were easily obtained. During the work reported here ACh as chloride was injected at rates varying between 5·5 and 27·5 μg of the salt/min. In only one experiment, illustrated in Figs. 3 and 4, was ACh injected at the rate of 50 μg/min.

During the first series of experiments the secretion was collected in contact with air and kept in stoppered tubes. The pH, measured within the first 3 hr after collection, ranged between 7·44 and 8·77 (mean value 8·34), which is in agreement with the reports of Stavraky (1945) and Morton & Stavraky (1948). The total CO_2 content, including bicarbonate, varied between 4·68 and 33·62 m-mole/l. (mean value: 21·75). However, serial analysis of the samples showed that the pH and total CO_2 increased with time from the moment of collection, even in samples protected under mineral oil. Increases of 4 m-mole/l. of total CO_2 and one unit of pH were not unusual. At least in some samples this change was caused by hydrolysis of urea, as shown by serial determinations of ammonia, urea, total CO_2 and pH.

To prevent changes in the samples after secretion, six experiments were performed in which the mucosa was covered at all times with a layer of mineral oil. The secretion was collected in syringes under oil and so was never in contact with air. The total CO_2 and pH were measured immediately after collection of the fluid. Figure 2 illustrates a typical experiment. The columns of section *A* indicate the pH of the gastric secretion during each period; the columns in *B* show the pCO_2 (partial pressure of CO_2), and the columns in *C* illustrate the corresponding concentration of HCO_3^- in the same secretion. The HCO_3^- and pCO_2 were calculated from the total CO_2 and pH data by means of the Henderson–Hasselbalch equation (adjusted to

38° C). It is assumed that the error introduced by the use of a formula derived for plasma is negligible since, as will be shown later, the compositions of the alkaline secretion and blood plasma were closely similar. The circles in sections *A*, *B*, and *C* of Fig. 2 represent the pH, pCO_2 and HCO_3^- concentration, respectively, of the plasma withdrawn from the splenic vein at the middle of each period.

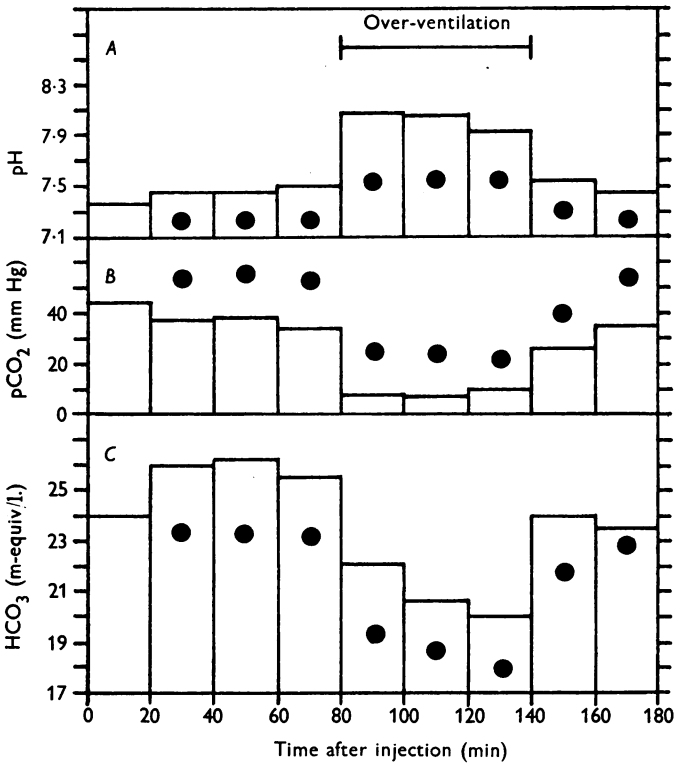

Fig. 2. Relation between pH, pCO_2 and HCO_3^- in the alkaline secretion and plasma. The columns indicate the value of the variables in the sample of secretion aspirated at the end of each period. The circles are the corresponding values for the plasma withdrawn at the middle of each period. Rate of injection of ACh, 22·5 μg/min.

The dog used in the experiment illustrated in Fig. 2 was deeply anaesthetized. Throughout the first four periods its slow breathing caused a rather low blood pH and high HCO_3^- content. During the next hour overventilation was produced by means of a respiratory pump; after the seventh period the breathing was again spontaneous.

Some conclusions, substantiated by all the experiments performed on the mucosa covered with mineral oil, can be deduced from Fig. 2.

First, the concentration of HCO_3^- in the gastric secretion is, at most, slightly higher than the HCO_3^- in the plasma. The experiment shown in Fig. 2 was chosen for illustration because it presents the highest concentration of HCO_3^- ever found in a gastric secretion protected from contact with air. Since the total CO_2 of these samples increased after collection by an average of 4·13 m-mole/l. in 3 hr, it is probable that a small but significant rise of total CO_2 occurred during the 20 min that the secretion remained in the chamber. In this experiment the mean ratio of plasma bicarbonate:secretion bicarbonate is 0·914 (excluding periods with over-ventilation). The mean ratio for the whole experimental series is 0·966 (S.D. = 0·034).

Secondly, the concentration of HCO_3^- and total CO_2 of the secretion varies as the concentration of HCO_3^- in the plasma.

Thirdly, the pH of the alkaline gastric secretion is slightly higher than the pH of the blood; at most, 0·25 units when the blood pH approaches normality (7·36).

Lastly, the pCO_2 of the secretion is lower than the pCO_2 of the plasma.

If HCl was to be added to the alkaline gastric secretion here analysed, its pH and HCO_3^- concentration should diminish below the corresponding values of the plasma, and the pCO_2 of the secretion should exceed the pCO_2 of the blood. Since the addition of only 1 m-equiv H^+/l. to alkaline gastric secretion produces measurable changes in the HCO_3^- concentration, the addition of 0·06 ml. of 0·16 M-HCl to every 10 ml. of secretion would be clearly recognizable. Therefore, samples which were not contaminated by HCl could be selected by comparison of the concentration of HCO_3^-, pH and pCO_2 of the gastric secretion and plasma. According to the above-mentioned criterion, 29 samples were obtained in six different experiments in which no significant amounts of acid were added by the secretion from the parietal cells. Samples collected during over-ventilation are excluded. In the remainder of this report it will be assumed that the composition of these samples is characteristic of the 'pure' alkaline secretion of the stomach under ACh stimulation.

The mean concentrations of Na^+, K^+ and Cl^- in this 'pure' alkaline fluid are listed in Table 1, column 1. In column 2 is shown the mean composition of the plasma obtained from blood withdrawn at the middle of each period. The mean of the ratios of the plasma sodium (Na_p^+) to the sodium in the secretion (Na_s^+) is 1·045 (S.D. = 0·021).

The respective mean ratio for Cl^- is 0·976 (S.D. = 0·039). As mentioned before, the ratio for HCO_3^- is 0·966. The possibility exists that minute amounts of HCl were still secreted under our experimental conditions. In this situation Cl^- would increase stoichiometrically as HCO_3^- disappeared, so the ratio of the sum $Cl_p^- + HCO_3^-{}_p / Cl_s^- + HCO_3^-{}_s$ would thus be a better

index of the distribution of these ions. The mean value of this ratio is 0·974 (S.D. = 0·031).

The handling of the samples included in column 1 (Table 1) was rather cumbersome, owing to the mineral oil unavoidably aspirated with the secretion. For that reason other required determinations were performed on samples not protected with mineral oil. This meant that the precise criterion for selecting 'pure' alkaline secretion could not be applied, and an approximate rule was resorted to: samples that contained 20 m-mole/l. or more of total CO_2 were considered free from added HCl. Of the samples collected in 15 different experiments, 81 out of 125 satisfied this condition.

TABLE 1

	Mucosa under mineral oil		Mucosa in contact with air			
Column	1 Secretion	2 Plasma	3 Secretion	4 Plasma	5 Secretion	6 Plasma
No. of samples	29	26	81	—	16	15
No. of experiments	5	5	15	15	5	5
Volume (ml.)	—	—	10·14 ± 5·34	—	11·92 ± 5·56	—
pH	7·42 ± 0·09	7·29 ± 0·05	8·38 ± 0·24	—	8·25 ± 0·10	—
Total CO_2 (m-mole/l.)	24·22 ± 2·34	23·51 ± 1·96	24·26 ± 3·47	—	22·01 ± 4·69	—
HCO_3^- (m-equiv/l.)	23·07 ± 2·15	22·06 ± 1·79	—	—	—	—
Cl^- (m-equiv/l.)	116·66 ± 5·25	113·96 ± 1·79	116·78 ± 5·44	111·61 ± 4·16	117·55 ± 3·65	112·98 ± 2·16
Inorganic P (m-mole/l.)	—	—	1·66 ± 0·60	1·60 ± 0·35	—	—
Protein (g/l.)	—	—	10·28 ± 4·47	—	11·93 ± 6·12	—
Na^+ (m-equiv/l.)	137·79 ± 3·17	143·75 ± 1·46	138·34 ± 4·04	146·64 ± 2·48	140·77 ± 2·34	146·59 ± 0·69
K^+ (m-equiv/l.)	3·52 ± 0·27	3·32 ± 0·25	3·72 ± 0·40	3·59 ± 0·38	3·94 ± 0·32	3·43 ± 0·36
Ca^{2+} (m-equiv/l.)	—	—	4·37 ± 0·59	5·53 ± 0·42	—	—
Osmotic concentration (m-osmole/l.)	—	—	—	—	291·50 ± 11·30	294·47 ± 6·48

Mean ± S.D. of an observation.

The average composition of these samples is shown in column 3 of Table 1. The large standard deviations of some determinations arise from the 'non-normal' distribution of the data, so that, in this case, the S.D. is not a good index of the variation of the data. However, it has been considered sufficiently accurate for the purposes of this report. There is no significant difference between the mean values listed in columns 1 and 3 for Na^+, K^+ and Cl^-; the samples included in these two columns therefore probably belong to the same population, at least with regard to the ions mentioned. It is reasonable to assume that the results of other determinations performed on these samples will fall close to the corresponding mean values for the 'pure' alkaline secretion. As mentioned before, the total CO_2 and pH of the samples listed in column 3 changed slowly with time and so are not comparable to the corresponding values in column 1.

The large variation in the volume measurements listed in column 3 arose fundamentally from differences in the rate of secretion from animal to

animal. Section *C* of Fig. 3 illustrates the rate of secretion during the course of seven experiments out of the fifteen listed in column 3 (Table 1), the only experiments in which all the samples contained more than 20 m-mole/l. of total CO_2.

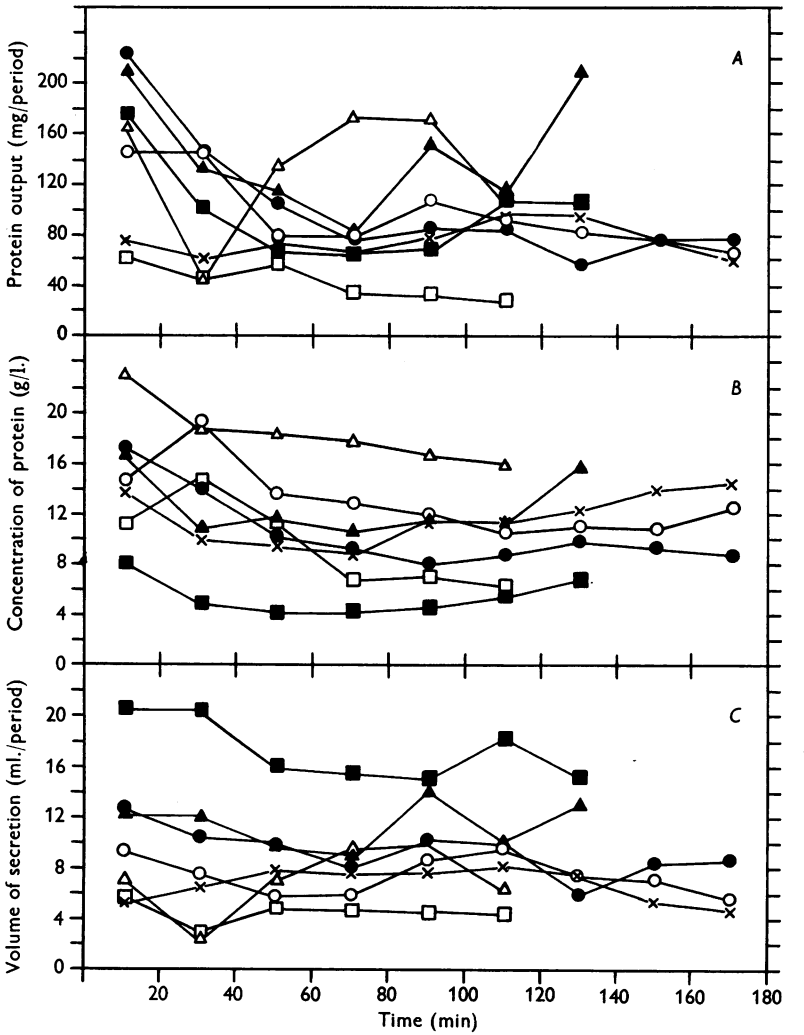

Fig. 3. Variation in the protein output, concentration of protein and volume of secretion during the course of an experiment. Results obtained in seven experiments in which all the samples were considered as 'pure' alkaline secretion (see text). The symbols show the values of the corresponding variables in the samples aspirated at the end of the period defined by the abscissa. Each experiment is represented by the same symbols in sections *A*, *B* and *C*. Rates of injection of ACh (μg/min): ○ 12·3; ● 14·9; × 14·9; □ 25·0; ■ 17·5; △ 50·0; ▲ 17·5.

In column 4 of Table 1 are shown the plasma analyses of the experiments listed in column 3. The concentration of total protein is also shown in column 3. Usually the secretion of the first period had the highest concentration of protein. This concentration remained fairly constant during subsequent periods, even through significant variations in the volume of fluid secreted. On the other hand, the protein concentration varied widely from animal to animal. Figure 3 illustrates these statements. In section *A* is plotted the output per period (mg/period) and in *B* the concentration (g/l.) of protein found in the samples of the seven experiments already mentioned. Especially significant is the approximate constancy of the protein concentration of the experiment represented by ▲, despite the large variations in volume secreted and hence in the output of protein per period.

The concentration of Ca^{2+} was measured in only fifteen samples resulting from five different experiments, but it is included in column 3 to avoid excessive lengthening of Table 1. The mean concentration of Ca^{2+} in the plasma of the same five animals is shown in column 4.

As in the case of Ca^{2+}, total inorganic P was measured in only fourteen samples collected in five experiments. In columns 3 and 4 are listed the mean concentration of inorganic P in the secretion and plasma respectively.

The average concentration of ammonia in the secretion was less than 0·24 m-equiv/l. in five samples obtained from three different experiments in which the mucosa was covered with mineral oil. The mean concentration of urea in the five samples was 0·336 g/l., being 0·390 g/l. in the plasma of the same animals.

The appearance of 'pure' alkaline secretion was characteristic. The solution was slightly opalescent and rather sticky just after collection; upon standing it often acquired a jelly-like consistency, if the concentration of protein was high. White streaks, often described as present in gastric juice and as evidence of the secretion of mucus, were present only in samples to which acid secretion had been added. When HCl was secreted in small, distinct areas of the mucosa as shown by their low pH, these could be recognized before measuring the pH by the white, egg-like appearance of the mucus overlying the epithelium.

Osmotic concentration of the alkaline secretion

The relation between the osmotic pressure of the gastric secretion and the osmotic pressure of plasma obtained from the splenic vein was studied in six experiments. The total CO_2 content was measured immediately after aspiration of the samples, which were kept in the refrigerator until the next day, when the osmotic concentration and other analyses were performed. Blood samples were withdrawn at the middle of each period.

Measurements of the osmotic concentration of the gastric secretion were rather poorly reproducible. Differences in successive readings of the same samples amounting to 4 % were not unusual. This inaccuracy could partly be ascribed to the difficulty in obtaining a perfectly homogeneous sample containing mucous secretion.

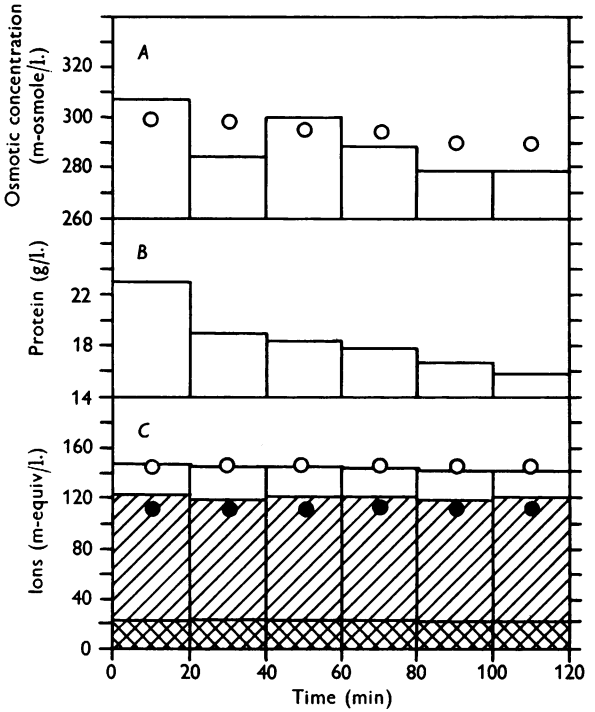

Fig. 4. Relation between the osmotic concentration of the alkaline secretion and of plasma. The columns represent the values of the variables in the alkaline secretion aspirated at the end of each period. The clear columns in *C* show Na^+ concentration (m-equiv/l.); the hatched columns the Cl^- concentration (m-equiv/l.); the total CO_2 (m-mole/l.) is shown by the cross-hatched columns. The circles in *A* represent the osmotic concentration of the venous plasma withdrawn from the gastric vein at the middle of each period. In *C*, the clear circles are a plot of the Na^+ concentration, and the full circles a plot of the concentration of Cl^-, in the plasma. Rate of injection of ACh, 50 μg/min.

Figure 4 illustrates a typical experiment. The histogram of section *A* indicates the average osmotic concentration of the secretion during each period, and the circles show the osmotic concentration of the venous plasma. The values listed are the direct readings in the osmometer used. The columns of section *B* represent the concentration of protein (g/l.) of the secretion. The clear columns in section *C* of Fig. 4 indicate the mean concentration of Na^+ (m-equiv/l.) in the secretion. The obliquely striped

columns show the Cl^- and the cross-hatched columns the total CO_2 in the same secretion, as m-equiv or m-osmole/l. respectively. The open circles show the Na^+ and the filled circles the Cl^- concentrations in the venous plasma (m-equiv/l.).

In column 5 of Table 1 is shown a summary of the composition and osmotic concentration of sixteen samples, collected in six different experiments, which may be considered as 'pure' alkaline secretion. In column 6 are shown the corresponding values for the plasma withdrawn at the middle of each period.

From the results illustrated in Fig. 4 and the data shown in Table 1, it can be concluded that, within the accuracy of the method, the alkaline gastric secretion is isosmotic with the plasma of the venous effluent from the secreting mucosa. As reported in the previous section, and as shown in Fig. 4, the concentration of ions is very similar in the plasma and in the alkaline secretion. This indicates that the proteins of the alkaline secretion (mean value 10·3 g/l.) have an osmotic concentration similar to the proteins of the plasma (about 60 g/l., Prosser, 1952). Thus the average weight of the protein molecules contained in the alkaline fluid must be many times smaller than the average weight of the plasma proteins.

Counter-pressure experiments

The similarity between the gastric secretion obtained under stimulation with ACh and an ultrafiltrate of plasma (see Discussion) raises the question whether the hydrostatic pressure inside the gastric capillaries and small arterioles is high enough to explain the rates of secretion experimentally observed. This question was investigated by two methods. In the first, the chamber of Fig. 1 was completely filled with 0·31 M glucose solution, and the upper tube connected to a Statham pressure transducer. The piston of the chamber was fixed in position by means of the caliper, 5. Considering the relatively small pressures used in this experiment, it might be assumed that the volume of the contents of the chamber, including the gastric mucosa, was constant, and that only the pressure inside the system could change. Therefore, the counter pressure at which no net transfer of solution took place across the blood vessels enclosed inside the chamber could be easily determined.

Figure 5 illustrates one characteristic experiment of the six performed on different animals. The upper graph is a record of the arterial pressure at the root of the coeliac artery and the lower graph is a record of the pressure inside the chamber.

Since the contraction of the gastric muscles produced rhythmic changes of pressure inside the chamber, a value for the average counter pressure was obtained by evaluating the area under the curve with a planimeter.

The numbers under the straight lines indicate average counter pressure measured by this method during the time shown by each line. When the mucosa was not stimulated the average counter pressure needed to reach equilibrium was 15·2 mm Hg, a value which agrees closely with the differential filtration pressure measured in capillaries by other workers (Landis, 1934).

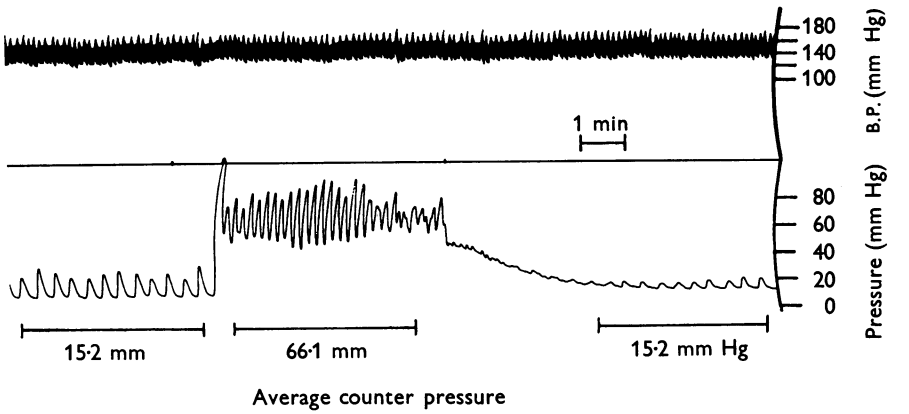

Fig. 5. Evaluation of the effective filtration pressure at the capillaries and arterioles of the gastric mucosa. Upper graph: blood pressure at the gastro-splenic artery. Lower graph: variation of pressure inside the chamber (see text). Between the two marks on the central line ACh was injected at the rate of 16·6 μg/min.

The ACh caused an immediate increase of the tonus of the muscle layer; strong peristaltic waves travelled from the oral to the caudal end of the isolated piece of mucosa. The average pressure inside the chamber increased to 66·1 mm Hg. Since the volume of the contents of the chamber, including the gastric mucosa, did not change, it is concluded that net filtration across the walls of the blood vessels was prevented by the counter pressure. In this situation the hydrostatic pressure exerted upon the blood vessels must be equal to the hydrostatic pressure inside the vessels minus the colloid osmotic pressure of the blood proteins. Therefore the hydrostatic pressure in the arterioles and capillaries reached about 90 mm Hg, i.e. a value as high as that given for the pressure inside the afferent arterioles of the kidney glomerulus (Winton, 1937). Of course, this figure is reached if the average systemic blood pressure is kept sufficiently high; in the experiment of Fig. 5 it was about 150 mm Hg. The equilibrium counter pressure returned to the control level within 3 min after the injection of ACh was stopped.

The increase of the average pressure inside the chamber depended on the amount of ACh injected. Thus, the differential filtration pressure could be precisely controlled by the concentration of free ACh obtaining at the walls of the gastric blood vessels.

The filtration pressure was determined by a second method in two other experiments. The tube 6 of the piston in Fig. 1 was connected to a mercury manometer and the pressure kept constant by manipulation of caliper 5. A constant counter pressure was maintained during each period and changes in volume of the chamber contents were measured. Any increase in the total volume signified that the filtration pressure surpassed the counter pressure maintained during the period. The equilibrium counter pressure was evaluated by extrapolation to zero volume change.

The constant-pressure method is cumbersome, owing to the length of time needed for reaching an equilibrium value, and in only one experiment were both methods used. The equilibrium pressure was 62 mm as determined by the constant-volume procedure, whereas the constant-pressure method yielded an equilibrium value slightly lower than 60 mm. Agreement between the results of the two procedures seems to be good.

Information on the mechanisms of secretion of protein and ions was also obtained from counter-pressure experiments. Figure 6 illustrates one of 8 experiments. At the beginning of each period 25 ml. of a solution of 0·31 M glucose was placed in the chamber and the entire contents aspirated at the end of the 20 min. The algebraic difference of the two volumes is plotted in section *A* of Fig. 6. In *B* is plotted the output of protein (mg/period) and in *C* the output of Na^+ (m-equiv/period). During the first three periods the mucosa remained undisturbed; ACh (20 μg/min) was injected from the fourth period on. A counter pressure of 70 mm Hg was maintained during periods 4 and 5, 40 mm during periods 6 and 7, and none during periods 8 and 9. No net volume of fluid was secreted, despite ACh stimulation during periods 4 and 5; the output of protein and Na^+ did not change significantly from the previous periods. As the counter pressure diminished, the volume secreted increased, *pari passu* with the proteins and ions (periods 6–9 inclusive). It was evident that ACh did not determine the appearance of protein in the gastric secretion when fluid with the composition characteristic of the 'pure' alkaline component was not secreted. ACh reached the arterioles during all the periods, as shown by the bright red colour of the mucosa.

Interesting information about the effect of ACh upon the permeability of the gastric mucosa was also afforded by these experiments. A quantitative analysis would be too long and not relevant to this report; it will be offered elsewhere. However, the following considerations seem pertinent. From periods 1–5 inclusive (Fig. 6) there was no significant change in the chamber's contents during each period. It might therefore be assumed that the free energy of water (chemical and hydrostatic components) was about the same on both sides of the gastric mucosa. The permeability of the mucosa to water is rather high under these experimental conditions,

as shown by tritium experiments (unpublished results). On the other hand, there existed an electrochemical gradient of constant value for the ions, with a resultant net diffusion of Na^+ and Cl^- from the interstitial fluid to the gastric lumen. Any change in the permeability of the gastric epithelium to these ions should result in modification of the net ionic fluxes. ACh did not change the flow of Na^+, as is shown in Fig. 6 by comparison of the

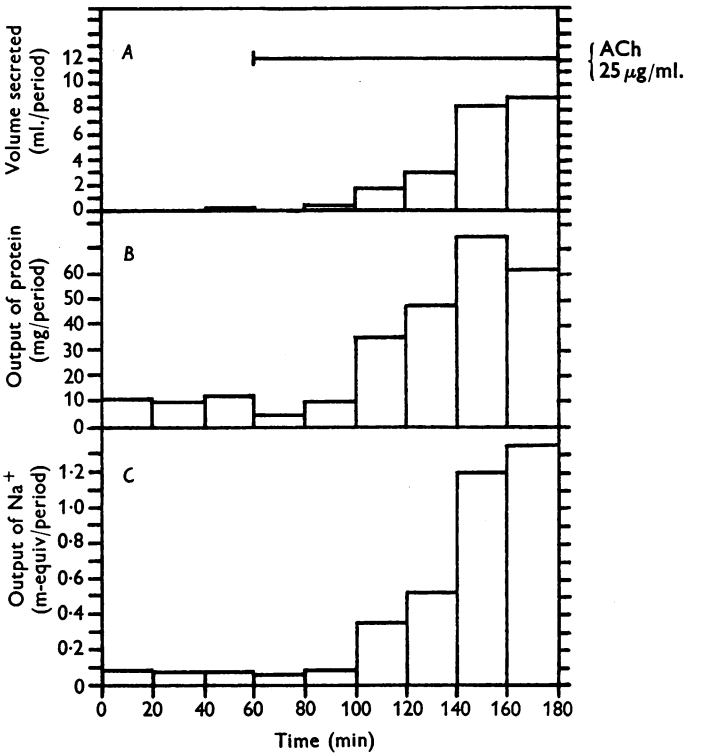

Fig. 6. Output of protein when the volume of secretion is controlled by counter pressure. ACh was injected during periods 4–9. Counter pressure during periods 1, 2 and 3, none; during periods 4 and 5, 70 mm Hg; during periods 6 and 7, 40 mm Hg; during periods 8 and 9, none. Rate of injection of ACh, 25 μg/ml.

Na^+ output during periods 4 and 5 with the previous periods. Also the output of Cl^- (not illustrated) was not modified. Similar results were obtained in the other three experiments in which the counter pressure applied was sufficient to prevent secretion. A logical conclusion is that ACh only affects the flow of ions across the gastric mucosa by increasing the difference in hydrostatic pressure. A bulk flow of interstitial fluid takes place; the permeability of the gastric epithelium to Na^+ and Cl^- remains unaffected.

As described above, the amount of ACh injected was kept constant in these experiments and the volume of secretion varied by changes in the counter pressure applied. There is a highly significant linear correlation between the output of protein and the volume secreted in each period. The value of the correlation coefficient, r, is 0·943 for 73 paired observations.

DISCUSSION

Diffusible cations should be distributed between the plasma and its ultrafiltrate according to a Gibbs–Donnan ratio of 1·04, if the protein-bound base of the plasma is considered to be on the average 15 m-equiv/l. The corresponding ratio for diffusible anions is 0·96 (Van Slyke, 1926). As is shown in results, the distribution of Na^+, Cl^- and HCO_3^- between plasma and 'pure' alkaline secretion agrees approximately with these ratios. H^+ is distributed qualitatively as predicted by the Gibbs–Donnan law, although its concentration in the gastric secretion is somewhat lower than expected. The distributions of Ca^{2+}, total inorganic P, urea and ammonia also appear to be consistent with a Gibbs–Donnan equilibrium. K^+ is the only cation somewhat more concentrated in the gastric secretion than in the plasma.

It is clear that the hydrostatic pressure gradient between the lumen of the blood vessels and the gastric cavity increases markedly during the injection of ACh in the appropriate direction to displace water and ions from the blood vessels to the gastric cavity. The experiments on counter pressure also suggest that ACh does not greatly modify the permeability of the gastric epithelium, at least to Na^+ and Cl^-.

It seems reasonable, therefore, to suggest that the rate of ultrafiltration at the walls of the capillary and terminal arterioles is increased by ACh. A significant amount of interstitial fluid will flow across the gastric epithelium under hydrodynamic pressure alone; 'pure' alkaline secretion results from the addition of the secretion of the mucous cells to the ultrafiltrate.

The gastric secretion determined by ACh contained an average of 10·3 g protein/l. No attempt was made to classify the protein found, but small amounts of plasma proteins must undoubtedly be present, as shown by Hollander & Horowitz (1960). The addition of mucus apparently does not change the basic chemical characteristics of the ultrafiltrate, thus suggesting that the secretion of the mucous cells is a mucoproteinate of sodium and potassium, practically devoid of other ions. If the ratio weight : charge of the mucoproteins has the same magnitude as the ratio of the plasma proteins, 3 m-equiv of base/l. at most will be added to the ultrafiltrate by the mucus components.

The concentration of protein is fairly uniform among samples collected in each individual experiment, regardless of the volume of the samples. There exists a positive correlation between the output of protein and volume of fluid secreted and, finally, the output of protein surpasses the control values only when a measurable volume of secretion appears in the gastric lumen. All these results suggest that a functional relation exists between the volume of fluid and amount of protein secreted. It seems probable that mucus is eliminated from the mucous cells by the hydrodynamic pressure of the fluid which flows across the epithelium.

The secretion produced by cells other than the parietal cells of the gastric mucosa has been variously called 'alkaline component', 'Verdünnungs-Sekretion', 'contaminating secretion', etc. According to available evidence it seems likely that the alkaline component has the same composition and origin as the secretion determined by ACh. If this is so, Hollander's (1936) contention that the alkaline component contains about 70 m-equiv buffer, mostly HCO_3^-/l., is in error, as is the claim of Fisher & Hunt (1950) that this secretion contains 45 m-equiv HCO_3^-/l. The concentration of HCO_3^- suggested by these investigators would require the active transport of that ion to the gastric lumen; however, the results reported here do not support the idea of any significant transport of HCO_3^-. The composition of the non-parietal secretion estimated by Gray & Bucher (1941) closely resembles the composition of the alkaline secretion given in this report.

It seems pertinent to recall that Hollander (1936), Fisher & Hunt (1950) and Gray & Bucher (1941) extrapolated the composition of the alkaline secretion from data obtained during secretion of *acid* fluid.

The normal amount of alkaline fluid secreted is thought to be small. However, the rate of secretion of alkaline fluid is potentially very large. In fact, the rate of secretion of alkaline fluid by a piece of mucosa stimulated by ACh is as high as the rate of 0·16 M-HCl produced when a similar preparation is stimulated with histamine (Altamirano *et al.* 1960).

It is known that secretion of alkaline gastric juice rich in mucus may be induced by appropriate electrical stimulation of the vagus nerve (Babkin, 1950, pp. 220–224). If the parasympathetic nervous system controls the secretion of the alkaline component, it possibly does so through vasomotor impulses which regulate flow through gastric arterioles. On this hypothesis it is not necessary to assume the existence of a parasympathetic innervation of the mucous cells, a result which would be in agreement with histological evidence on this question (Vial, 1951).

SUMMARY

1. Five–30 μg acetylcholine/min, injected intra-arterially close to an isolated piece of the mucosa of the greater curvature, causes the secretion of an alkaline fluid.

2. Criteria are defined which permit the selection of samples of gastric secretion that do not contain a significant addition of HCl. The samples so selected are considered as characteristic of the 'pure' alkaline secretion.

3. The concentration of Na^+, H^+, Ca^{2+}, HCO_3^-, Cl^-, total inorganic P, ammonia and urea of the 'pure' alkaline secretion are characteristic of an ultrafiltrate of plasma. This 'pure' secretion is considered to be an ultrafiltrate of plasma to which has been added the secretion of the mucous cells. The final fluid contains on average 10·3 g protein/l. and is isosmotic with plasma.

4. ACh injected by the arterial route increases the effective filtration pressure in the arterioles and capillaries of the gastric mucosa to 60 mm Hg or more, augmenting markedly the rate of formation of interstitial fluid. On the other hand, the permeability to Na^+ and Cl^- of the gastric epithelium is not significantly modified.

5. The relation of 'pure' alkaline secretion to the so-called 'alkaline component' of the gastric secretion is discussed.

6. It is suggested that the parasympathetic nervous system could control the rate of secretion of the alkaline fluid through nerve endings situated in the walls of the terminal arteries.

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