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THE INTERDEPENDENCE OF GASTRIC SECRETION AND THE CO_2 CONTENT OF THE BLOOD.

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For some years sporadic indications have appeared in the literature that there is a relation between blood CO_2 content and gastric secretion. Maly's theory, first advanced in 1878, postulated the importance of CO_2 in the formation of hydrochloric acid, only, however, secondarily to NaH_2PO_4 which he believed to be the compound directly concerned in the formation of HCl. F. Delhougne [1927] found a decreased acidity in response to the alcohol test meal when the subjects hyperventilated. He found, however, an increase in the CO_2 combining power of the blood under these conditions, which makes the effectiveness of the hyperventilation doubtful. Bakaltschuk [1928] demonstrated an increase in gastric acidity in human subjects after the inhalation of CO_2. An increase was also obtained by Apperley and Semmens [1928] after the rebreathing of the subject's own expired air. In a different type of experiment Szilard [1930] showed an augmented "fasting" secretion following intravenous injection of $NaHCO_3$; the injection of glucose on the other hand caused no such rise. Numerous authors have observed that feeding large quantities of $NaHCO_3$ over prolonged periods of time raises the level of the acid curve during fractional test meals [Linoissier and Lemoine, 1894; Crohn, 1918]. The ingestion of sodium bicarbonate by patients on a Sippy diet in a large number of cases increases the CO_2 content of the blood [Hardt and Rivers, 1923; Koehler, 1927; Gatewood and others, 1928]. Working on dogs, Boyd [1924] observed that, in small doses, sodium bicarbonate tends to increase gastric secretion. Apperly and Semmens [1928] showed, in human subjects, that there was a parallelism between blood bicarbonate content and the response to a standard test meal. They attributed this to a common effect of variation in oxygenation of the blood on both gastric secretion and blood bicarbonate content.

The results reported by the above investigators have been obtained upon human subjects using the gastric test meal. This criterion of gastric secretion is open to serious objection, since the values obtained depend not only on the activity of gastric cells, but also on the emptying time of the stomach, and regurgitation of duodenal contents. Dilution due to salivation and to the ingested meal, and conditioned reflex effects are also sources of error.

Apperly and Crabtree [1931] in a very recent article are cognizant of the above sources of error (as indeed were Apperly and Semmens), and believe that they have taken them into account. In this article, they report a direct relation between the concentration of gastric hydrochloric acid and the plasma bicarbonate content.

In 1929, during an experimental investigation of the production of gastric secretion by vagal stimulation in dogs [Vineberg, 1931], it was observed that secretion was not obtained following excessive artificial respiration, but commenced shortly after the cessation of hyperventilation. The experiments reported in this paper were undertaken in an attempt to elucidate these results. In a preliminary report of this investigation [Vineberg and Browne, 1931] the conclusion at which we arrived was that a definite interdependence existed between the CO_2 content of arterial blood and the character of gastric secretion.

Methods.

Dogs ranging in weight from 8 to 25 kg. were employed. All animals were fed meat and porridge for 3 days, and milk only for the 24 hours immediately preceding the operation. Anæsthesia was induced by means of ether and continued by intravenous injection of a chloralose-urethane mixture (1 g. chloralose, 10 g. urethane in 60 c.c. normal saline: dose, 3 c.c. per kg.). A tracheal tube was inserted, both carotid arteries were isolated and tied, the vagus nerves were carefully separated from the carotid sheath and severed. The œsophagus was tied at the level of the first tracheal ring in order to prevent contamination of gastric juice with saliva. A metal fistula was inserted into the anterior wall of the stomach and firmly secured. The stomach was isolated from the duodenum by means of a ligature secured around the pylorus, thus preventing regurgitation. The anterior abdominal wall was closed around the metal fistula and the animal placed on a stand in the prone position. In one group of experiments, gastric secretion was obtained by electrical stimulation of the vagus nerves; in another group, subcutaneous injection of histamine (ergamine acid phosphate) was the gastric stimulant employed.

Secretion was collected in graduated centrifuge tubes at definite intervals. The volume of each sample was measured, the free and total acidity were determined by titration, using Töpfer's reagent and phenolphthalein as indicators. The total chlorides were determined by the method of Wilson and Ball [1928]. Blood was withdrawn from one carotid artery, the other being used for recording the blood-pressure. All blood samples removed from the carotid were collected and centrifuged under paraffin oil. The time of centrifuging, using the Angle centrifuge, was usually under 2 min., so that loss of CO_2 was minimized. The CO_2 content was determined on the true plasma, without equilibration, by the method of van Slyke [1917]; plasma *p*H was estimated by Cullen's colorimetric procedure [1922]; the chlorides in blood and plasma by the method mentioned above. Lactic acid in whole blood was determined by the method of Friedemann, Cotonio and Shaffer [1927] (expressed as mg./100 c.c. of whole blood).

Experiments.

The experiments have been divided into the following groups, and those described are illustrative of other similar experiments.

(1) *Nerve stimulation:*

Influence of: A. Hyperventilation;
B. Injection of acid (HCl and lactic);
C. Injection of NaCN.

(2) *Histamine stimulation:*

Influence of: A. Hyperventilation;
B. Injection of acid (HCl and lactic, and of base $NaHCO_3$);
C. Injection of NaCN.

(1) *Nerve stimulation.*

A. *Hyperventilation.*

We present two examples of this type.

In this experiment (Fig. 1) the vagus nerves were stimulated with an induction current interrupted 15 times per minute, using the left and right vagi alternately for periods of 10 min. each. Collections of gastric juice were made in periods of 10 min.; these represent corresponding periods of nerve stimulation. Samples were taken in this way in order to follow variations in nerve response. The difference between

right and left nerves is seen in the figures and is especially marked in the volume output. It will be observed that after 120 min. of stimulation, a satisfactory flow of gastric secretion was obtained and showed the values for free and total acidity usual with this type of stimulation. During this period the arterial plasma CO_2 content gradually rose to a

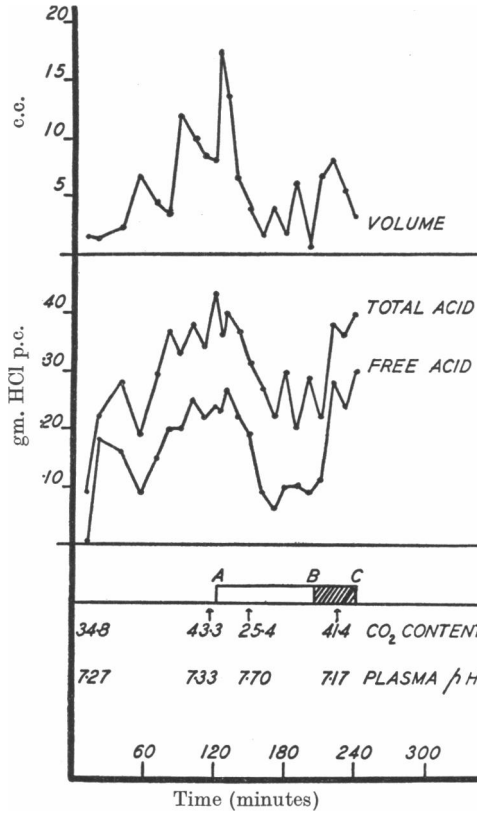

Fig. 1. Dog 9. Wt. 13·3 kg. Vagal stimulation. *A–B*, artificial ventilation at the rate of 64 per min. with air. *B–C*, the same rate of ventilation. 8 p.c. CO_2 in air.

value of 43·3 vol. p.c. (this figure is approximately normal for the dog under these conditions). At the point *A* artificial hyperventilation was applied at the rate of 64 per min. for a period of 80 min. The pump used had a stroke volume of 1700 c.c. There was a rapid fall in the volume output; 20 min. later the free and total acidity also fell. Coincidental with these changes the plasma CO_2 content fell to 25·4 vol. p.c. In other experiments we have found that the CO_2 content usually reaches an approximately constant low level within 20 min. after the application

of hyperventilation. The diminished acidity continued for 60 min. At this stage (*B*) a Douglas bag containing a mixture of 8 p.c. CO_2 and air was connected to the pump inlet. The rate of hyperventilation was kept constant. The acidity rose immediately to control levels; the CO_2 content

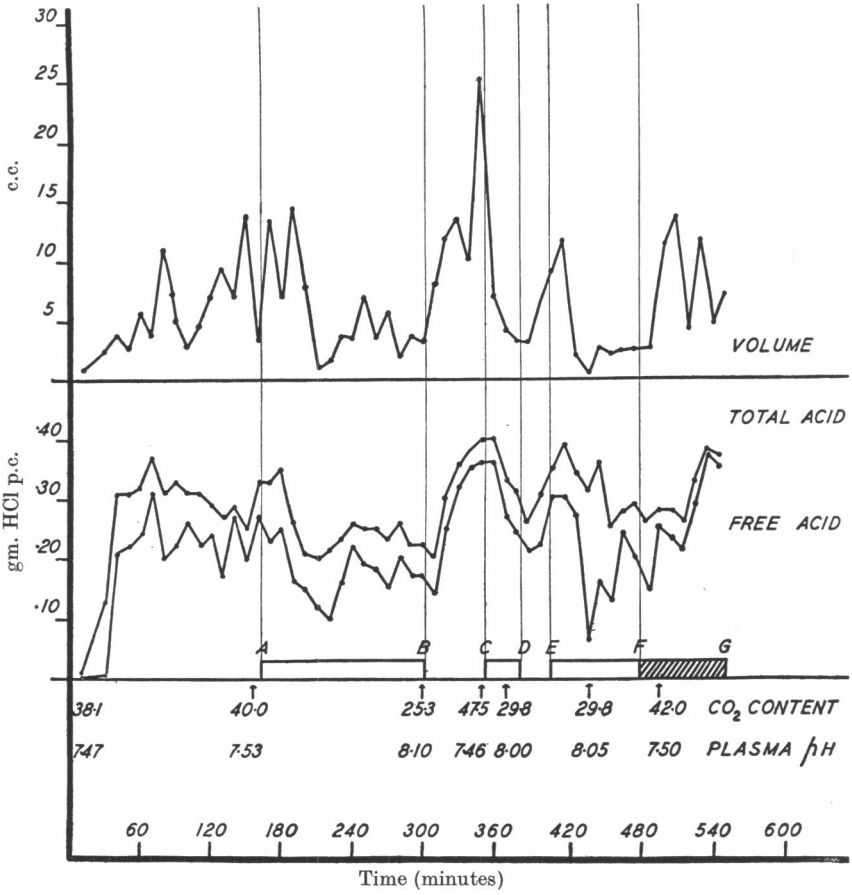

Fig. 2. Dog 10. Wt. 16 kg. Vagal stimulation. *A–B*, artificial ventilation at the rate of 74 per min. with air. *C–D* and *E–F*, the same. *F–G*, the same rate of ventilation. 5 p.c. CO_2 in oxygen.

of the plasma also rose reaching a level of 41·1 vol. p.c. in 27 min.; the *p*H fell below control levels.

A second experiment is presented in Fig. 2. Secretion was obtained following vagal stimulation and maintained for a period of 50 min. Hyperventilation applied at *A* at the rate of 74 per min. reduced the volume of secretion, free and total acidity and plasma CO_2 content.

Secretion practically ceased for a period of 100 min. Hyperventilation was discontinued at *B*, and the animal resumed a normal respiratory rhythm. The volume, free and total acidity rose to and above control values, as did the CO_2 content. This procedure was repeated for a shorter period with similar effects (as may be seen in the figure *C* to *D*). A third application of hyperventilation (*E* to *F*) again reduced all values, and these were restored following the introduction of 5 p.c. CO_2 at *F*. In neither hyperventilation experiment was there any significant change in the total chloride content of the gastric secretion.

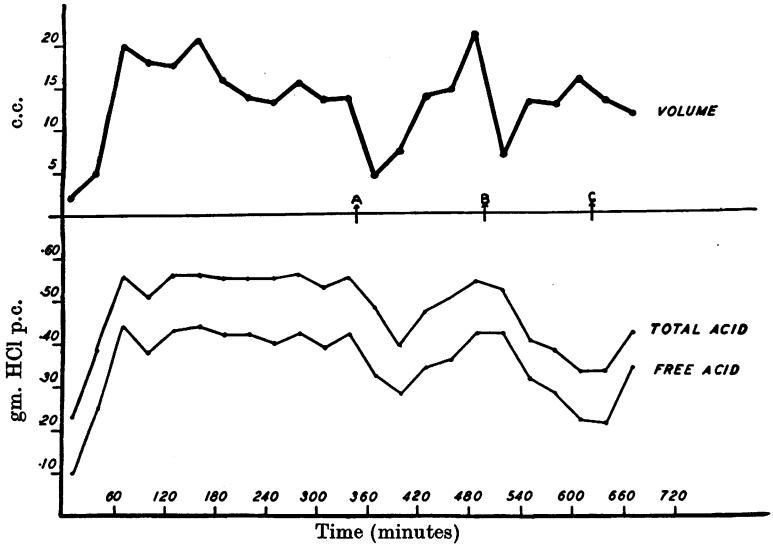

Fig. 3. Dog 36. Wt. 15 kg. Vagal stimulation. *A*, *B* and *C*, vagus ceased acting on heart. Position of electrodes changed.

In a control experiment presented in Fig. 3, stimulation of the vagus produced gastric secretion which gradually rose to reach its height in 1 hour. For a period of 5 hours secretion was maintained at a constant level of volume and acidity. This is longer than the total duration of the hyperventilation experiments. At the end of the 5 hours (point *A* in the figure) the volume of secretion diminished as did the free and total acidity.

This decrease was due to a diminished excitability of the nerve at the point of contact with the electrodes. The values were returned to previous levels by moving the electrode to a fresh position on the nerves. This procedure was repeated on two subsequent occasions. At the end of the experiment the nerves were acting and the dog was in good con-

dition. The total period of stimulation was over 10 hours. We have considered the vagus nerve as acting satisfactorily when a typical effect on heart rate and blood-pressure resulted at each stimulation. In the experiments shown in Figs. 1 and 2 and in Table I, the nerves acted satisfactorily throughout according to this criterion.

TABLE I. Dog 8. Wt. 17·4kg.: nerve stimulation.

Sample No.	Time min.	Volume c.c.	Free acidity g. p.c.	Total acidity g. p.c.	CO_2 content vol. p.c.	*p*H
0	Contents	12·8	0·00	0·10	—	—
1	15	1·7	0·00	0·07	—	—
		Stimulation current: 1 millivolt				
2	20	1·3	0·00	0·10	—	—
3	20	1·5	0·06	0·16	—	—
4	20	4·4	0·09	0·20	—	—
5	20	6·2	0·15	0·23	—	—
6	20	—	0·22	0·31	—	—
7	10	5·0	0·29	0·37	—	—
8	10	4·4	0·25	0·36	—	—
9	10	6·5	0·34	0·40	—	—
10	10	6·0	0·25	0·37	43·3	7·3
11	10	6·7	0·32	0·44	—	—
12	10	5·5	0·26	0·36	—	—
13	10	5·6	0·28	0·40	—	—
14	10	5·5	0·30	0·37	—	—
		Acid injection commenced				
15	10	5·4	0·33	0·42	—	—
		Total of 75 c.c. of 0·5*N* HCl injected				
16	10	4·6	0·35	0·47	43·3	7·0
17	10	3·7	0·33	0·45	—	—
18	10	0·5	0·25	0·43	24·2	7·1
19	10	0·3	0·15	0·33	—	—
20	30	0·2	—	—	—	—

B. *Acid injection.*

In this experiment (Table I) over a period of 80 min. an average rate of secretion of 5·6 c.c. for 10 min. was maintained. The average for the free acidity was 0·29 g. HCl p.c. and for the total acid 0·38 g. HCl p.c. The CO_2 content was 43·3 vol. p.c. and the *p*H 7·33. In a period of 9 min. 75 c.c. of 0·5*N* HCl were injected intravenously; immediately after the injection the plasma CO_2 content was still 43·3, but the plasma *p*H had fallen to 7·00. The respiration of the animal had not yet increased. The secretion continued throughout the injection. The volume gradually fell over the 20 min. period following the cessation of acid administration. In contrast to this the free and total acidity showed a slight rise. When this length of time had elapsed, the secretion suddenly stopped and did not return for the remainder of the experiment. A blood

sample taken 10 min. after the cessation of secretion showed 24·2 vol. p.c. of CO_2 and a pH of 7·10.

The dependence of secretion upon CO_2 content rather than upon blood pH is indicated in the above experiment.

C. *Injection of* NaCN.

Since it has been shown that hyperventilation by changing blood pH has the effect of shifting the dissociation curve of hæmoglobin in such a way that it yields oxygen less readily to the tissues, the possibility that the effect of hyperventilation may be due to interference with the oxygen supply of the gastric mucosa must be considered. It was decided to inject sodium cyanide as a means of interfering with tissue oxidation. Repeated injections of 7 c.c. of $N/100$ NaCN were given intravenously. The total amount given was 41 c.c. in 1 hour. Despite this the gastric secretion was not affected. Although the lethal dose for a 14 kg. dog was 30 c.c. given in a single injection, we do not know the degree of interference with tissue oxidation produced in the above experiment. The changes in the dissociation curve of hæmoglobin are dependent upon those in pH. The significance of the latter will be discussed later.

After the above results were obtained, the possibility of an altered plasma CO_2 and pH affecting the vagus nerve endings was considered. This was rendered improbable in view of the continued action of the vagus on the heart during hyperventilation. In an endeavour to exclude this possibility, histamine was used as it is considered to act directly upon the parietal cells [Popielski, 1920; Vineberg and Babkin, 1931]. The operative procedure was identical with that of nerve experiments. The vagi were severed but not stimulated.

(2) *Histamine stimulation.*

A. *Hyperventilation.*

In Fig. 4 it is seen that after a 20 min. period of secretion, artificial respiration was applied and continued for 60 min. (*A–B*) without any effect upon the course of the secretory activity. It was then discontinued for 50 min. without any effect. The dog was again hyperventilated for 100 min. (*C–D*), the volume and acidity remaining practically constant although the plasma CO_2 was lowered to 24·7 vol. p.c. and pH raised to 7·80. 5 p.c. CO_2 was introduced (*D–E*) with the rate of hyperventilation kept constant for a period of 30 min. This raised the CO_2 content of the plasma to 61·0 and lowered the pH to 7·03. The effect of the CO_2 was to diminish the secretion both in volume and acidity. This effect persisted for 45 min. after the CO_2 had been discontinued; hyperventi-

lation was maintained throughout during the following 70 min. (*E–F*). The volume gradually rose; the free and total acidity also rose, but the former did not reach its previous value.

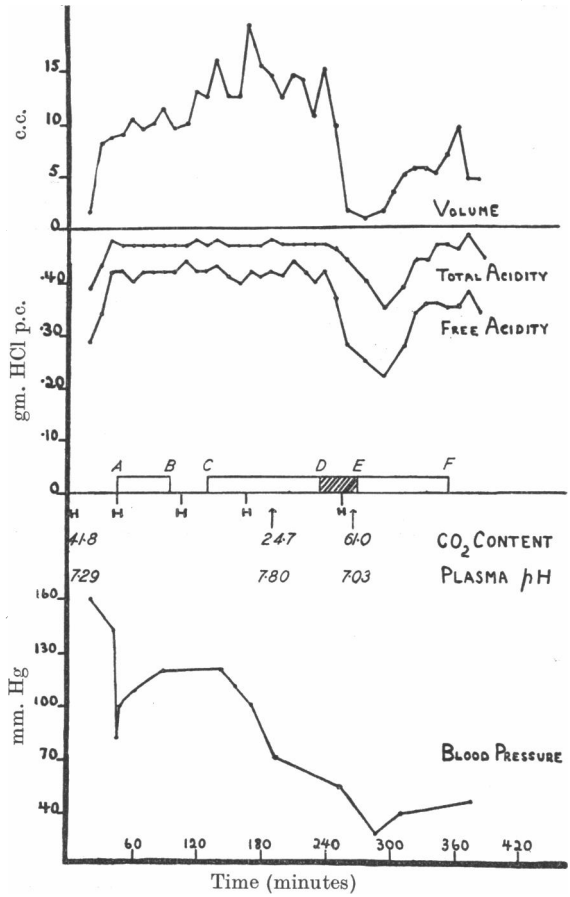

Fig. 4. Dog 12. Wt. 14·3 kg. At *H*, 4 mg. histamine subcutaneously. *A–B*, artificial ventilation at the rate of 76 per min. with air. *C–D*, the same. *D–E*, the same rate of hyperventilation. 5 p.c. CO_2 in oxygen. *E–F*, the same rate with air only.

A second experiment is shown in Fig. 5. In this experiment CO_2 and hyperventilation were applied (*A–B*) as soon as secretion had been established. The volume decreased gradually from the first, but the free acidity rose slightly and the total acidity remained constant for 50 min. and then both fell off abruptly. The CO_2 content of the plasma rose only slightly above control levels. The administration of CO_2 was

then discontinued and hyperventilation was continued at the same rate as before (*B–C*). The volume gradually increased, the free and total acid however remained low. Then 8 p.c. CO_2 was again used in the inspired air (*C–D*). The recommencement of CO_2 was followed by a

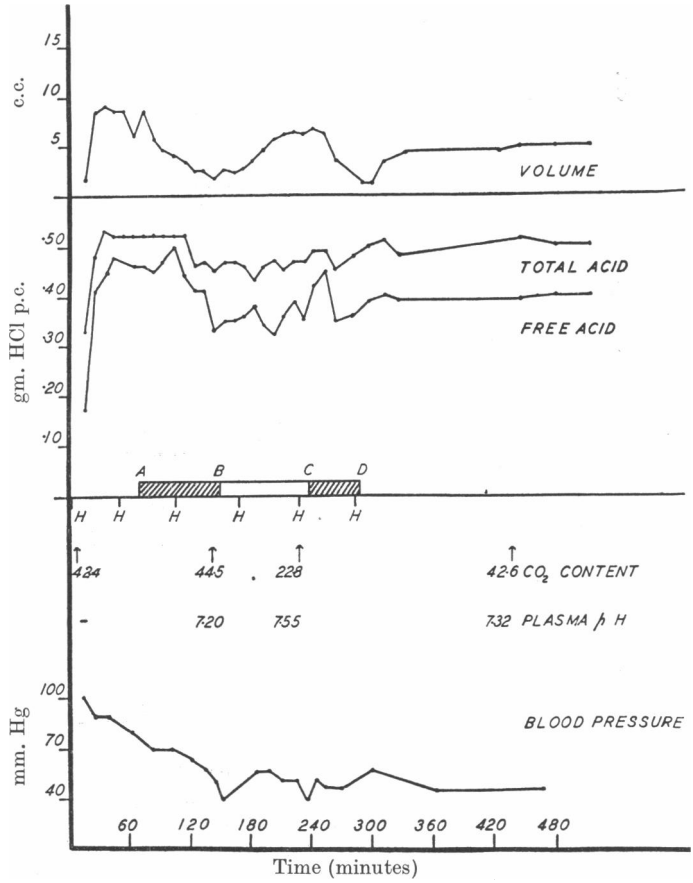

Fig. 5. Dog 14. At *H*, 4 mg. histamine subcutaneously. *A–B*, artificial ventilation at the rate of 74 per min. with 5 p.c. CO_2 in oxygen. *B–C*, same rate of ventilation with air only. *C–D*, with 5 p.c. CO_2 in oxygen.

20 min. period in which a definite rise in free acidity took place. There was no change in volume. This was followed by a decrease in both volume and acidity continuing for 30 min. At this point (*D*) the dog was permitted to resume a normal respiratory rhythm, breathing air only. All values immediately rose and were maintained at an approximately constant level for 3 hours with no further injections of histamine.

Histamine is an extremely powerful gastric stimulant, and it is seen from the above results that once secretion is well established under its influence, hyperventilation has no effect. However, from Fig. 5 there are indications that once histamine secretion has been depressed the hyperventilation tends to keep the values reduced.

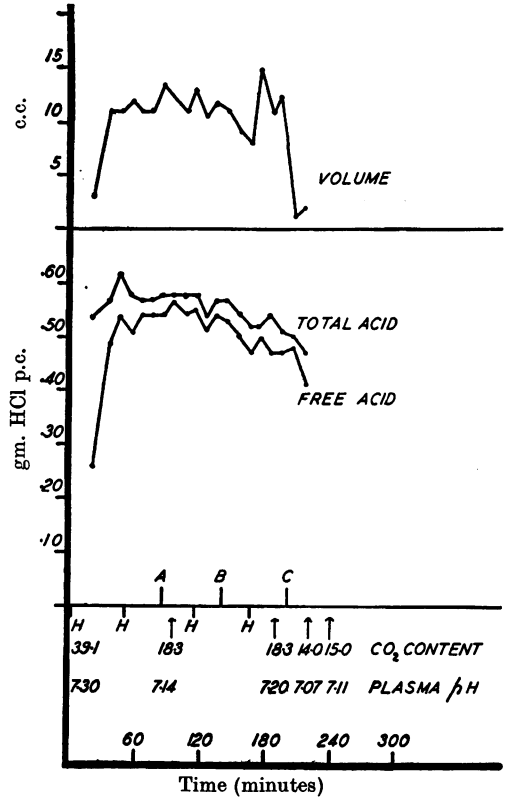

Fig. 6. Dog 16. Wt. 10·3 kg. At *H*, 4 mg. histamine subcutaneously. At *A*, 40 c.c. 0·5*N* HCl intravenously in 5 min. At *B*, 40 c.c. 0·5*N* HCl intravenously in 3 min. At *C*, 23 c.c. 0·5*N* HCl intravenously in 6 min.

B. *Injection of acid.*

In the experiment illustrated in Fig. 6 secretion was maintained by histamine at a constant level for 70 min. Half normal hydrochloric acid was injected intravenously (*A*) (40 c.c. in 5 min.). For the next 40 min. no change in the character of secretion took place. A further 40 c.c. of acid was then injected (*B*) in 3 min. The values decreased only slightly for the next 60 min. A third injection (*C*), 23 c.c. in 6 min., was made.

Secretion stopped abruptly and 55 min. later the dog died. The blood CO_2 content was at the usual level at the beginning of the experiment, and, following the first injection of acid, fell and declined with each subsequent injection of acid. The *p*H also decreased over these periods.

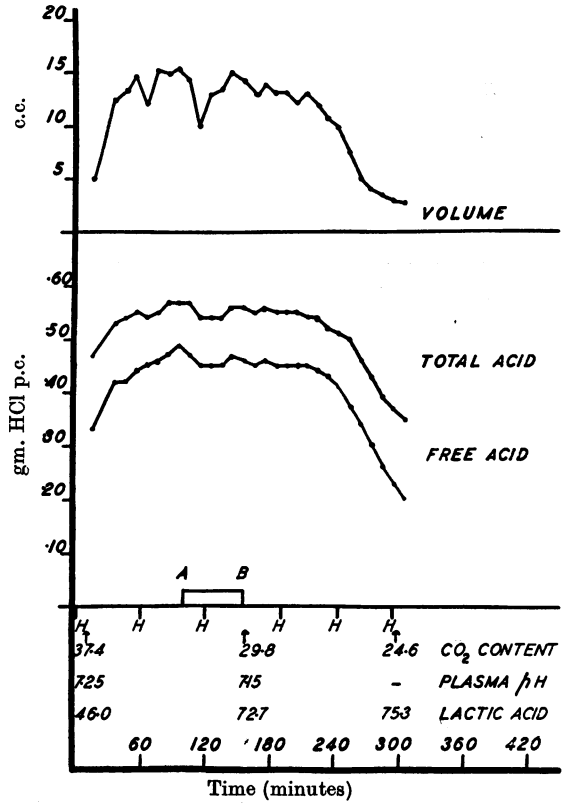

Fig. 7. Dog 21. Wt. 23·3 kg. At *H*, 4 mg. histamine subcutaneously. *A–B*, 120 c.c. 0·5*N* lactic acid intravenously at the rate of 3 c.c. per min.

Because HCl is the acid of gastric secretion, and because it does not enter the blood in physiological conditions, it was decided to use lactic acid which occurs in normal metabolism. Fig. 7 demonstrates the absence of effect of the injection of large quantities of lactic acid on histamine secretion after it has been established. The CO_2 content and *p*H of the plasma were lowered and the lactic acid content of the blood rose. Toward the end of the experiment secretion gradually declined. The death of this animal was due to an overdose of anæsthetic.

From Figs. 6 and 7 it may be seen that under conditions of low plasma CO_2 content and low *p*H, histamine secretion, unlike that

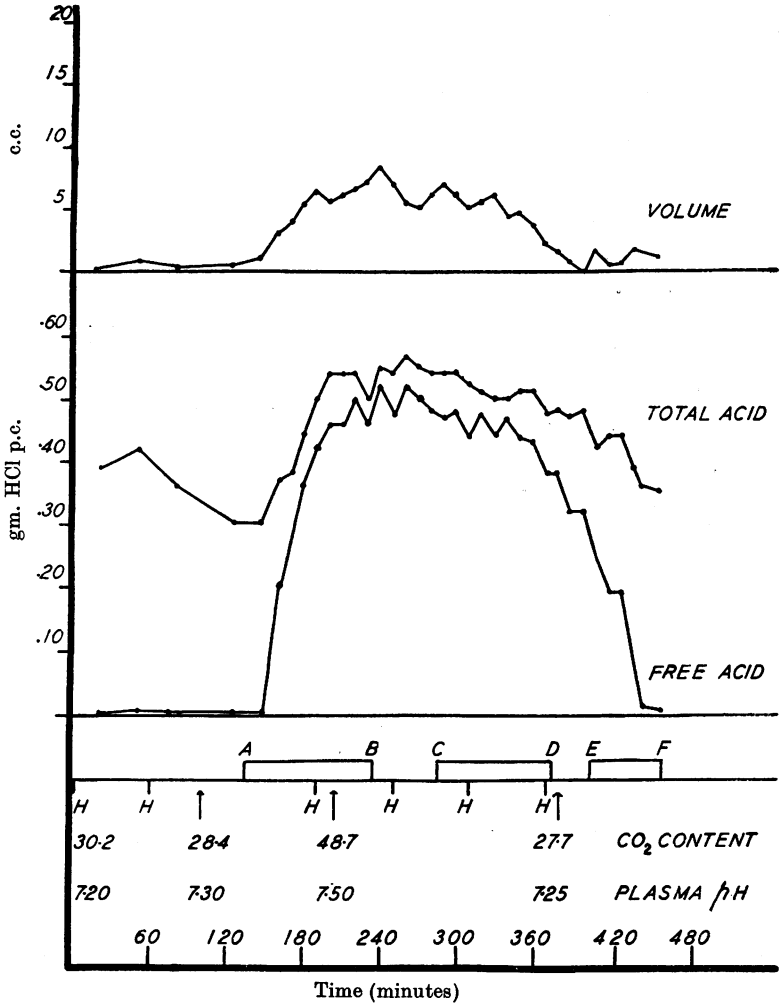

Fig. 8. Dog 17. Wt. 14·5 kg. At *H*, 4 mg. histamine subcutaneously. *A–B*, 47 c.c. 10 p.c. sodium bicarbonate intravenously at the rate of 0·5 c.c. per min. *C–D*, 120 c.c. 0·5 *N* HCl intravenously at the rate of 1·3 c.c. per min. *E–F*, 75 c.c. 10 p.c. sodium bicarbonate intravenously.

produced by vagal stimulation, continues for long periods unaffected. If, however, these conditions of low CO_2 content and low *p*H are in existence before the histamine is injected, it fails to produce a flow of

gastric juice. This occurred in three experiments. The acidosis occurred spontaneously for unknown reasons. One of these experiments is illustrated in Fig. 8. At the commencement of the experiment the plasma CO_2 was 28·4 vol. p.c. and *p*H 7·30. Repeated doses of 4 mg. of histamine were given at hourly intervals. The usual latent period following this dose of histamine is not more than 20 min. In this experiment there was no secretion for 145 min., but it appeared 15 min. after the commencement (*A*) of an intravenous injection of 10 p.c. sodium bicarbonate and was well maintained for a period of 120 min. Hydrochloric acid was then injected (*C–D*), and in this case the secretion gradually diminished and finally ceased. When secretion was well established, the CO_2 content was 48·7 vol. p.c. and the *p*H 7·5. Following the injection of acid when secretion ceased the CO_2 content was 27·7 and the *p*H 7·27. It will be observed that a period of 85 min. elapsed between the last histamine injection and the commencement of secretion after the injection of $NaHCO_3$ intravenously. This indicates that the inhibition of gastric secretion under conditions of initial acidosis is not due to a rapid destruction of the histamine injected.

A similar inhibition of secretion under conditions of initial acidosis occurred in vagal stimulation experiments, and as in the case of histamine a flow of gastric juice was established following the injection of sodium bicarbonate. In another case of initial acidosis where no injection of sodium bicarbonate was given, acidosis progressively increased and repeated hourly injections of histamine failed to stimulate the gastric glands. The animal died 5 hours after the first histamine injection. The injection of 250 c.c. of normal saline failed to induce secretion. This indicates that dehydration is not the cause of the inhibition of secretion.

C. *Injection of sodium cyanide.*

As in the case of nerve stimulation, repeated doses of 7 c.c. of *N*/100 sodium cyanide at 10 min. intervals over a long period of time had absolutely no effect on the functioning of the gastric mucosa. The characteristic effect of sodium cyanide on respiration and blood-pressure was obtained at each injection.

In all the histamine experiments the standard dose used was 4 mg. injected every hour, and in numerous experiments we have found that once a satisfactory flow of secretion has been established, it will be maintained at a constant level of volume, free and total acidity for periods as long as 10 hours.

Discussion.

A definite relation between the CO_2 content of the plasma and gastric secretion is seen in the group of nerve stimulation experiments. Hyperventilation causes a cessation of gastric secretion. That this cessation is related in some way to the coincident lowering of the CO_2 content of the plasma is indicated by the restoration of the flow of gastric juice by the increase of the CO_2 content of the inspired air. The mechanism of the above effects is dependent upon numerous factors. We do not feel that a complete explanation is possible at the present time. We believe, however, that there are certain of these factors which can be excluded. Hyperventilation has been found by previous authors to have a variety of effects upon the vaso-motor system and upon the chemical composition of the blood. Dale and Evans [1922] found a marked lowering of blood-pressure and considerable shock in cats under ether anæsthesia. McDowall [1930] showed that this fall in blood-pressure did not always occur under chloralose. Lowering of blood-pressure if due to splanchnic vaso-dilatation should favour secretion. The blood-pressure effect was not marked in our experiments, being at most 30–40 mm. of mercury. The initial pressure was high owing to the proportions of the chloralose-urethane mixture used. Also, in several experiments nerve stimulation has maintained satisfactory secretion with blood-pressures lower than those produced by hyperventilation. The changes in systemic blood-pressure do not, therefore, appear to be the causative factor in the gastric effect. This does not, however, exclude the effect of variations in blood flow through the gastric mucosa. Vaso-constriction of blood vessels might be expected to cause a diminution in secretion. It has been indicated by Lim and Necheles [1927] that within limits the rate of gastric secretion is independent of the blood flow through the stomach. Furthermore, hyperventilation has been shown to dilate the splanchnic vessels [Dale and Evans, 1922], and this occurs even under chloralose [McDowall, 1930]. We have not studied blood flow in our preparations but feel that its influence may be disregarded in view of the findings quoted above. Mechanical effects of hyperventilation are excluded by the maintenance of a constant rate of ventilation throughout the whole experiment, both during cessation of secretion and during its restoration by CO_2.

The changes in blood electrolytes occurring under hyperventilation have been extensively studied [Henderson and Haggard, 1918; Collip and Backus, 1920; Grant and Goldman, 1920; Davies and

others, 1920; Anrep and Cannan, 1923; Haldane and others, 1924]. The experiments have been made almost wholly upon human subjects. The main changes which take place are a lowering in the CO_2 content of the blood, a rise in *p*H and in lactic acid content, a shift of water and chloride from corpuscles to plasma. Dependent upon the change in *p*H there is an alteration in the dissociation curve of hæmoglobin. Other electrolytes such as phosphates and sulphates also participate in the shift from corpuscles to plasma. The blood picture in our experiments conforms to these findings (Table II). The increased loss of CO_2 through

TABLE II. Changes in blood constituents under hyperventilation of a 5 p.c. CO_2 oxygen mixture.

	CO_2 plasma m.Eq./l.	*p*H	Lactic acid blood m.Eq./l.	Cl plasma m.Eq./l.	Cl blood m.Eq./l.
Before hyperventilation	18·3	7·24	3·67	106·4	86·4
After 50 min. Hyperventilation 76 per min.	9·5	7·60	8·22	105·1	84·2
40 min. 5 p.c. CO_2 oxygen mixture. Ventilation 76 per min.	—	—	—	—	—
After 30 min. Hyperventilation, air only	9·3	7·57	10·1	105·0	81·7
After 30 min. 5 p.c. CO_2 oxygen mixture. Ventilation 76 per min.	18·9	7·10	3·81	104·9	85·3
After 30 min. natural respiration, air only	15·5	7·34	11·9	103·6	83·1

	Cl corpuscles m.Eq./l.	Plasma proteins g. p.c.	Inorganic phosphorus mg./100 c.c.	Total base serum m.Eq./l.	Cell volume
Before hyperventilation	56·3	8·05	6·66	126·2	40
After 50 min. Hyperventilation 76 per min.	50·7	8·65	3·46	131·4	39
40 min. 5 p.c. CO_2 oxygen mixture. Ventilation 76 per min.	—	—	—	—	—
After 30 min. Hyperventilation, air only	44·2	9·04	4·70	132·2	39
After 30 min. 5 p.c. CO_2 oxygen mixture. Ventilation 76 per min.	58·3	8·28	7·20	138·6	42
After 30 min. natural respiration, air only	54·1	—	8·33	159·6	41

Dog 13. Wt. 14 kg. Histamine (4 mg.) injected hourly. Secretion of gastric juice maintained throughout the experiment. Inorganic phosphates determined by the method of Fiske and Subbarow [1925] and total base by that of Stadie and Ross [1925]. Corpuscle chlorides were obtained by difference. m.Eq./l. = milli-equivalents per litre.

the lungs with the consequent fall in the blood CO_2 content is responsible for the other changes in blood electrolytes which are of a compensatory nature.

That the lowered CO_2 content rather than the raised *p*H is the factor involved in the gastric effect is indicated by the experiments in which initial acidosis with a lowered CO_2 and a lowered *p*H prevented the production of secretion. The subsequent injection of sodium bicarbonate raised the CO_2 content of the plasma, raised the *p*H and caused secretion to commence. The above-mentioned experiments of Szilard [1930] and others also indicate that a high *p*H produced by administration of sodium bicarbonate does not inhibit but rather favours the secretion of acid gastric juice. In the case of hyperventilation, no secretion was obtained when there was a low CO_2 content and a high *p*H, whereas in the case of initial acidosis no secretion was obtained when there was a low CO_2 and a low *p*H. Raising the CO_2 content and lowering the *p*H induced secretion in the former instance, and raising the CO_2 and raising the *p*H induced it in the latter. Hence, one may conclude that the observed changes in secretion are not directly due to the changes in plasma *p*H nor to concomitant alterations in the oxygen dissociation curve of hæmoglobin. The experiments with cyanide also indicate that the oxygen tension in the secreting cells may be varied to some degree at least without effect on secretion.

Apperly and Crabtree [1931] have attempted to separate the effects of H_2CO_3 and of "HCO_3." The former they regard as controlling the amount of gastric acidity and the latter its concentration. We feel that neither their results nor ours justify a definite decision on the relative importance of CO_2 present as H_2CO_3 and as "HCO_3." In this connection we may cite the experiment described above where the secretion obtained by vagal stimulation was temporarily increased in free HCl concentration by the injection of hydrochloric acid, while H_2CO_3 concentration increased at the expense of "HCO_3." In the experiments with hyperventilation, the changes in H_2CO_3 are naturally greater than the changes in "HCO_3"; on the other hand, the induction of secretion and a rapid rise in the concentration and amount of free HCl by injecting sodium bicarbonate into dogs in which spontaneous acidosis had prevented the response to vagal stimulation or to histamine, are accompanied by great increases in "HCO_3" with only slight changes in H_2CO_3 concentration (calculated from the Henderson-Hasselbalch equation).

The lowered plasma CO_2 content may conceivably act either directly upon the nerve or its terminations or alternatively upon the chemical mechanism necessary for the formation of gastric secretion.

Just how changes in plasma CO_2 content affect nerve conduction

and nerve endings we are unable to state. In our experiments when secretion had diminished under hyperventilation, there was no change in the cardio-inhibitory action of the vagus. It is, however, possible that the nerve endings in the stomach might be affected. The possible effect of lowered CO_2 content on the liberation and destruction of a "vagus substance" should also be considered.

On the view that the action of CO_2 is a chemical one rather than an action on nerve sensitivity, its effect may be a direct one in the reaction for the formation of hydrochloric acid in the parietal cells or an effect upon the ionic equilibria between blood plasma and tissue fluids or between tissue fluids and parietal cells. The lowered CO_2 content may prevent transfer of chloride to the mucosa or parietal cells. This retention of chloride tends to take place to balance the fixed base liberated when the bicarbonate ion content of the blood or tissue fluid is lowered. In connection with this it may be noted that recently Babkin [1932] and Webster obtained a gastric secretion introducing CO_2 gas into the stomach of dogs with different permanent gastric fistulæ.

In the group of histamine experiments it will be observed that the results differ decidedly from those of the nerve stimulation experiments. When histamine secretion was established hyperventilation had little inhibitory effect, even though blood conditions were similar to those of the nerve experiments. The inhalation of CO_2 is seen to tend to inhibit histamine secretion. While this cannot be explained, we feel that it does not invalidate the main argument. In several cases of initial acidosis with low CO_2 and low pH, no secretion was obtained with histamine stimulation and as in the nerve experiments, it was activated when the CO_2 and pH were raised following the injection of sodium bicarbonate. On the other hand, when acidosis is produced by the administration of acid during histamine secretion, no inhibitory effect is observed. The absence of effect after histamine secretion has been established does not invalidate a chemical interpretation of the essential part played by CO_2 in the formation of HCl. It is possible that the level of CO_2 required for secretion may be related to the strength of the stimulus producing that secretion. It is impossible to lower the CO_2 content of the blood and of the tissues beyond a certain value. If the stimulus is an extremely powerful one (such as histamine) the parietal cells may be capable of utilizing the remaining CO_2 which they are incapable of doing under a weaker and more physiological one (such as vagal stimulation). The absence of secretion under conditions of acidosis existing prior to the injection of histamine indicates that a lowered

CO_2 content is capable of inhibiting the action even of this powerful stimulant.

The clinical application of these results appears to us to be of some importance. Apperly and Crabtree [1931] have indicated some conditions in which the lowering of gastric secretion may be correlated with lowered sodium bicarbonate in the blood. We would draw attention to some considerations which may be of significance in any clinical application. We have found that the level of arterial CO_2 content at which inhibition of secretion takes place is about 30 vol. p.c. This is true whether the lowering of the CO_2 is due to hyperventilation or to acidosis. This corresponds to a venous CO_2 content of about 36 vol. p.c. In one experiment where both CO_2 content and the ordinary CO_2 combining power on separated plasma were done, the initial values were: CO_2 content, 34·8 vol. p.c., and the capacity or combining power, 39·5 vol. p.c.; after secretion was well established the values were: content 43·3 and capacity 45·1. Under hyperventilation when secretion had ceased and the CO_2 content had been lowered to 25·4, the capacity was only lowered to 35·8. After the administration of CO_2 the content was 41·4 and the capacity 40·5. These figures are presented to indicate that the CO_2 combining power of the separated plasma is not a suitable index in the case of hyperventilation of the relations between CO_2 and gastric secretion. Complete inhibition of secretion in this experiment took place when the arterial CO_2 combining power was 36 vol. p.c. This corresponds to a venous CO_2 combining power of about 42. A definite decrease in secretion might, therefore, be expected even if the CO_2 combining power were only slightly lowered. Such a slight lowering is not uncommon with mild acidosis and with hyperventilation and might easily influence the character of gastric secretion. It would be difficult to detect these changes in the case of hyperventilation by the use of the ordinary CO_2 combining power determination in venous blood. Changes in CO_2 alveolar air tension and hence of CO_2 content of the blood during sleep and on waking [Leathes, 1919; Endres, 1922] and during digestion [Porges, Leimdorfer and Markovici, 1911; Dodds and Bennett, 1921] do take place under physiological conditions.

It is possible on the other hand that a raised CO_2 content sensitizes the parietal cells so that they respond more readily to a weak stimulus. This may be of importance in cases of hypersecretion where apparently a stimulus which in normal individuals would initiate a secretion of average acidity produces an excessive secretion with abnormally high acid content. For some years there has been a controversy as to the

usefulness of sodium bicarbonate therapy in the treatment of gastric ulcer, with hyperacidity. Our results would tend to support those who hold that its use though producing a temporary neutralization is followed by a secretion of greatly augmented acidity.

SUMMARY.

1. Gastric secretion produced by vagal stimulation in dogs under chloralose-urethane anæsthesia is inhibited by hyperventilation. It is restored by raising the CO_2 content of the inspired air, though the hyperventilation is maintained at the same rate as before.

2. Secretion is also inhibited by the injection of acid intravenously and by the occurrence of acidosis prior to the stimulation of the nerves.

3. The factor involved in the effect of hyperventilation and acidosis on gastric secretion is the lowering of the CO_2 content of the plasma rather than the accompanying changes in plasma *p*H.

4. Gastric secretion in response to vagal stimulation is inhibited when the CO_2 content of the arterial plasma falls below 30 vol. p.c.

5. Secretion in response to injections of histamine is also inhibited by the occurrence of acidosis prior to the commencement of secretion but not by acidosis or hyperventilation produced after secretion is established.

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