STUDIES OF THE CHEMICAL MECHANISM OF HYDROCHLORIC ACID SECRETION

II. Observations on the Blood Passing through the Stomach of Dogs

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The chemical mechanism by which the stomach secretes hydrochloric acid has been the subject of little experimental investigation. Some doubtful hypotheses have been offered dealing chiefly with the state of equilibrium in a mixture of various electrolytes and the impermeability of tissues for some of the ions (1). Harvey and Beasley (2) suggested the formation of a chloride of an organic base which could set free hydrochloric acid after leaving the gland. Most of the experimental work concerning the production of hydrochloric acid relates to the histology of the gland during secretion, and consists essentially of elaborating the original work of Claude Bernard (3). Information bearing indirectly on the problem is furnished by the studies of Gamble and McIver (4) who investigated the chemical changes which occurred in the secretion of Pavlov pouches in dogs. Similar studies on human gastric juice were reported in the preceding paper of this series. The observations indicated that during the production of acid the chloride concentration in the chyme remains about the same as in serum while the base falls in proportion to the increase in acidity. It appeared that water and chloride ions leave the blood in the same relative concentration as in serum and that hydrochloric acid is liberated by a retention of base.

The following experiments were planned to demonstrate more directly the mechanism by which HCl is secreted. Serum electrolytes of the arterial blood and of the venous blood from the stomach were determined before and during gastric secretion in dogs. In all but one experiment anesthesia was produced by isoamylethyl-barbituric acid intra-peritoneally in amounts of about 60 mgm. for each kilogram of body weight. Histamine was used subcutaneously to stimulate secretion. In each experiment it was designed to collect venous blood draining from the region of the cardia and lesser curvature where a maximum acid secretion would be expected. Post-mortem examinations in each case showed this had been accomplished. The following protocol of a typical experiment will indicate the procedure adopted in all observations.

Dog number 5. Fasting 24 hours. Weight 22 kilograms.

12:45 p.m. 1.4 grams of isoamylethyl-barbuturic acid was injected into the peritoneal cavity.

1:05 p.m. The dog was sleeping quietly, only a little mucus, neutral in reaction, was obtained by aspirating through a stomach tube.

1:10 p.m. A median incision was made from the xiphoid to the lower half of the abdomen. The spleen was delivered, care being taken to avoid trauma to the stomach. The splenic vein was then followed backward and upward towards its junction to the portal vein (fig. 1). The coronary vein from the cardia of the stomach was identified as it emptied into the splenic vein. Two ligatures were placed around the splenic vein, one distal and one proximal to that portion into which the blood from the cardia was flowing. The needle of a 20 cc. syringe was then carefully inserted into the splenic vein between these ligatures and both ligatures drawn taut to prevent any inflex of blood from the spleen or from the portal vein. Thus only blood from the cardia was obtained from the isolated segment of the splenic vein.

1:23 p.m. 30 cc. of venous blood from the cardia was secured without exposure to air and transferred to centrifuge tubes under oil.

1:25 p.m. A specimen of arterial blood was drawn from the previously exposed femoral artery and placed under oil.

1:30 p.m. By mistake another sample of arterial blood was obtained. Venous blood from leg was intended.

1:31 p.m. 10 mgm. of histamine was given subcutaneously.

1:40 p.m. 100 cc. of water placed in stomach. Reaction of stomach contents was found to be neutral.

1:42 p.m. 2 mgm. histamine was given subcutaneously.

1:50 p.m. 2 mgm. histamine was given subcutaneously.

1:55 p.m. 1 mgm. histamine was given subcutaneously. Stomach contents slightly acid.

2:00 p.m. 1 mgm. histamine was given subcutaneously.

2:05 p.m. 1 mgm. histamine was given subcutaneously.

2:10 p.m. 1 mgm. histamine was given subcutaneously. Reaction of stomach contents strongly acid.

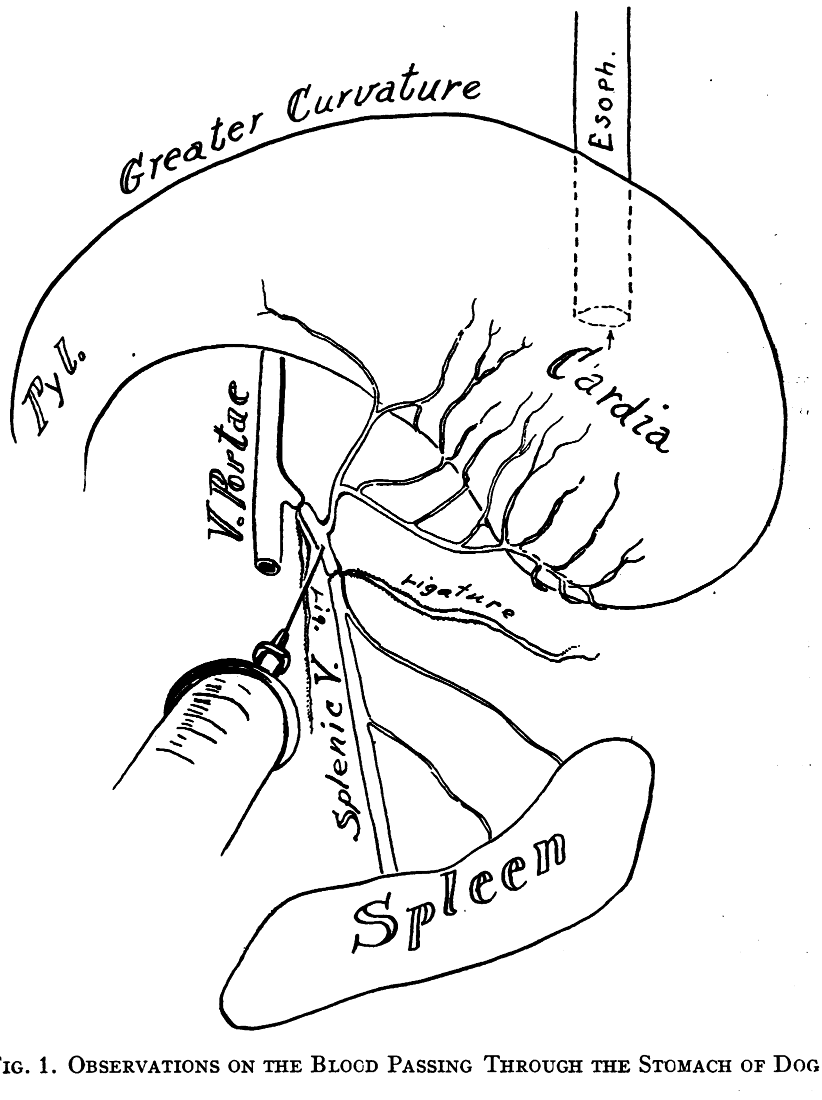

FIG. 1. OBSERVATIONS ON THE BLOOD PASSING THROUGH THE STOMACH OF DOG

2:14 p.m. Sample of venous blood was obtained from the cardia as described above.

2:16 p.m. Sample of arterial blood was obtained from femoral artery.

2:18 p.m. 1 mgm. histamine was given subcutaneously.

2:24 p.m. 1.5 mgm. histamine was given subcutaneously.

2:35 p.m. 1.5 mgm. histamine was given subcutaneously.

2:37 p.m. 30 cc. of venous blood was obtained from cardia.

2:39 p.m. 30 cc. of blood was obtained from femoral artery.

2:44 p.m. 20 cc. of blood was obtained from femoral vein.

4:15 p.m. 30 cc. of venous blood was obtained from cardia.

4:17 p.m. 30 cc. of blood was obtained from femoral artery.

The dog continued to be in good condition. The secretion was still acid, but obviously quite small in amount.

The animal was killed by opening the chest wall. There was no food in the stomach. The veins from which the venous blood of the stomach was obtained drained the lessure curvature and the cardia.

Blood was collected under oil in pyrex centrifuge tubes and defibrinated by stirring gently with a glass rod. The tubes were carefully closed with rubber stoppers and centrifuged. The serum was transferred to sampling bulbs without exposure to the air. Carbon dioxide was determined by the method of Van Slyke and Stadie (5), chloride by the method of Van Slyke (6) and phosphate by the method of Benedict and Theis (7). Serum protein concentration was calculated from the total nitrogen determined by the Kjeldahl method, after correction for the non-protein nitrogen in the trichloracetic acid filtrate. Some details concerning the procedure used for determining the total base are included in the preceding article (8). For the total base of serum, the protein free trichloracetic acid filtrate was used in amounts the equivalent to 1 cc. of serum for each determination.

Data indicating the changes which occurred in the serum electrolytes during HCl secretion are presented in five tables each representing an experiment on a single animal and exhibiting the differences between arterial blood and the venous blood of the stomach. Values of the anions are expressed in millimoles of base combining capacity, assuming a ratio of primary to secondary phosphate of one to four and calculating the base combined with protein by a formula devised by Van Slyke, Wu and McLean (9). In the latter calculations and in correcting for the dissolved CO_2 a pH 7.35 was arbitrarily assumed. The term total acid is used to indicate the sum of all the determined acids. The difference between the determined acids and the total base is taken to represent the organic acid fraction though it contains a small amount of sulfate which was not estimated.

If, as is indicated by Gamble's experiments and our studies on human subjects, chloride ions leave the blood in a concentration about the same as in serum, little change in the concentration of chloride in the serum from the secreting stomach would be expected. The tables show that this assumption is correct. As serum passed from the arterial to the venous side of the stomach circulation, the chloride changes were not striking. One hour after stimulating secretion in dog number 3, the chloride concentration in the arterial and venous serum was the same. This was also true in the experiment on dog number 5 after 45 minutes. In nine observations the average fall in chloride was only 1.9 mM. an alteration which is comparable to the changes in chloride noted in the venous blood taken simultaneously from the leg. A decrease in chloride in the serum is therefore not a prominent feature of hydrochloric acid secretion. Chemical changes in the gastric juice indicated that as water and chloride ions were secreted acid was produced by a retention of base. In nine observations on the serum from the secreting stomach there was an average rise of 6.8 mM. in the total base of the serum. In

TABLE 1

Data from dog number 1, fasting 24 hours; amytal anesthesia

Fasting contents neutral. Initial 4 mgm. of histamine subcutaneously followed in 10 minutes by acid secretion. Five subsequent injections of histamine, 1 mgm. each, at 10-minute intervals. The second set of blood samples taken one hour after the initial histamine. Although strongly acid only about 40 cc. of secretion was obtained in one hour.

	Before secretion		1 hour after stimulation of secretion		
	Arterial serum	Venous serum, stomach	Arterial serum	Venous serum, stomach	Venous serum leg
	mM	*mM*	*mM*	*mM*	*mM*
HCO_3	22.0	22.7	19.1	23.7	24.0
Cl	113.5	109.7	112.1	109.0	110.8
PO_4	4.0	2.8	4.8	3.9	5.3
Protein	13.3	11.6	12.2	11.1	10.3
Total acid	152.8	146.8	148.2	147.7	150.4
Total base	158.3	157.9	159.0	161.8	159.4
Organic acid	5.5	11.1	10.8	14.1	9.0

the experiment on dog number 1 where the volume of secretion was rather small the least change was observed. The maximum increase of 11.3 mM. was observed in dog number 5 after two and a quarter hours at a time at which the rate of secretion did not appear to be great, but which followed a period of rapid secretion. It is interesting to compare these alterations with similar observations obtained while the stomach was at rest and in the fasting condition. In every instance, with the exception of dog number 8, there was a slight

TABLE 2

Data from dog number 3, fasting 24 hours

In this one experiment the animal was rendered unconscious by a crushing blow on the head under light chloroform anesthesia; anesthesia then discontinued and artificial respiration started. A little mucous in the stomach contained no free acid. Initial histamine 7.5 mgm. subcutaneously. Subsequent histamine 6 mgm. in divided doses. The first acid appeared in 20 minutes. The first pair of blood specimens collected in 33 minutes; the second in 52 minutes. There appeared to be more rapid secretion in this experiment than any other.

	Before secretion		½ hour after stimulation		1 hour after stimulation	
	Arterial serum	Venous serum, stomach	Arterial serum	Venous serum, stomach	Arterial serum	Venous serum, stomach
	mM	*mM*	*mM*	*mM*	*mM*	*mM*
HCO_3	17.1	19.1	18.6	22.5	19.5	22.0
Cl	114.9	113.0	113.0	110.8	113.9	113.0
PO_4	2.5	2.8	2.0	2.4	1.8	2.6
Protein	9.1	9.5	8.6	10.0	6.7	7.2
Total acid	143.6	144.4	142.4	145.7	141.7	144.8
Total base	173.7	171.5	171.3	176.8	165.2	171.4
Organic acid	30.1	27.1	28.9	31.1	23.5	26.6

decrease of base in the venous serum from the stomach. The exception observed in dog number 8 could probably be accounted for by the discovery postmortem of undigested meat in the stomach.

Changes such as those observed in the serum would, if not neutralized, yield an exceedingly alkaline blood. Some factors must therefore be operative to compensate for the increase in base and any slight fall in chloride. It is evident that an adjustment is partially effected by a gain in bicarbonate. The magnitude of the increase in bicarbonate during the resting state is often surprisingly small.

During secretion it is more marked. Nevertheless, it is obvious that the bicarbonate supplied fails to accommodate for the entire change. The average increase in base is 6.8 mM., the average decrease in chloride is 1.9 mM., making a total of 8.7 mM. which must be neutralized. The average increase in bicarbonate is only 3.4 mM. Organic acids appear to play an equally prominent part. The relative importance of the two varies considerably in the different experiments. In some of the observations an increase in the organic acid fraction was the chief factor. This was especially true with dog number 5 in which after two and a quarter hours the change in

TABLE 3

Data from dog number 5

See sample protocol in text

	Before secretion			$\frac{3}{4}$ hour after stimulation		$1\frac{1}{4}$ hours after stimulation			$2\frac{1}{4}$ hours after stimulation	
	Arterial serum	Arterial serum	Venous serum, stomach	Arterial serum	Venous serum, stomach	Arterial serum	Venous serum, stomach	Venous serum, leg	Arterial serum	Venous serum, stomach
	mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*
HCO_3	23.8	23.5	23.3	21.5	24.9	21.3	24.9	23.9	24.0	25.6
Cl	113.0	112.2	111.8	111.8	111.9	110.8	109.0	109.9	110.2	108.1
PO_4	3.1	3.0	3.0	4.1	4.1	4.1	3.7	4.1	4.2	5.4
Protein	10.7	11.5	11.5	10.0	9.8	10.4	11.4	11.9	10.6	9.1
Total acid	150.6	150.2	149.6	147.4	150.7	149.0	149.0	149.0	149.0	148.2
Total base	175.0	175.0	171.5	189.8	199.8	195.9	193.0	184.3	186.4	197.7
Organic acid	24.4	24.8	21.9	42.4	49.1	39.2	44.0	35.4	37.4	49.5

bicarbonate was relatively unimportant with a striking increase in base. The data in this particular instance indicate a very marked increase in organic acid.

The last three tables include data contrasting satisfactorily the changes observed in the leg with those of the secreting stomach. A simple comparison is made in the experiment on dog number 7 (table 4), where it so happened that during secretion the alterations in bicarbonate and chloride in the venous serum from both the stomach and leg were the same. But the serum from the leg showed only a slight increase in total base and little indication of an increase in organic acids. At the same time there was a marked rise in the total base of serum from blood flowing through the stomach, the excess presumably neutralized by organic acids. Essentially the same features were presented in the similar observations recorded in tables 3 and 5.

The changes in the concentration of protein during the secretion were not marked. Dog number 3 illustrates the type of variation one would expect, an increase with secretion. But it was surprising that in other cases the protein was unaltered or even decreased. No

TABLE 4

Data from dog number 7, fasting 24 hours; amytal anesthesia

Fasting contents neutral. Initial histamine 8 mgm. subcutaneously. Subsequent histamine 8 mgm. during the next hour in divided doses. The stomach contents remained neutral for 40 minutes, and then gradually became strongly acid. The second sample of blood from the stomach 78 minutes after the initial histamine; the arterial and venous from the leg during the next ten minutes.

	Before secretion			1½ hours after stimulation		
	Arterial serum	Venous serum, stomach	Venous serum, leg	Arterial serum	Venous serum, stomach	Venous serum, leg
	mM	*mM*	*mM*	*mM*	*mM*	*mM*
HCO_3	27.6	27.8	28.0	24.1	26.8	26.6
Cl	111.7	110.7	110.6	108.5	106.8	106.8
PO_4	4.3	4.4	3.9	5.1	4.9	4.9
Protein	8.7	11.0	9.0	10.2	8.4	(10.2)*
Total acid	152.3	153.9	151.5	147.9	146.9	148.5
Total base	195.0	194.4	192.0	185.2	190.7	186.7
Organic acid	42.7	40.5	40.5	37.3	43.8	38.2

* Determination lost; this value assumed.

clear explanation of this can be offered. In experiments on dog number 5, poor checks were obtained in triplicate determinations, averages were recorded and the results may be erroneous. The protein in the secretion would have little influence. In these experiments some water was always left in the stomach. Significant amounts of fluid may have been absorbed, but even this will not explain the dissociation of the base and the protein variations.

In the experiments on human subjects it was shown that the concentration of phosphate in gastric juice is greater than in serum.

Therefore one might have anticipated a decrease in the serum phosphate as the blood flowed through the secreting stomach. But there was little change. In fact, the experiments indicate that there may be a slight increase. An increase in phosphate in both the secretion and serum suggests that a liberation of phosphate may be associated with glandular activity. When the experiments were being planned, it seemed possible that the phosphate ions would be intimately related to the process by which a very acid secretion is formed from slightly alkaline blood, but the magnitude of the phosphate changes which

TABLE 5

Data from dog number 8, under amytal anesthesia and supposedly fasting 24 hours, a little mucus from the stomach showed no free acid

Initial histamine 7.5 mgm. subcutaneously. Subsequent histamines 9 mgm., 1 mgm every 5 minutes. Acid first noted 30 minutes after initial histamine. The first blood from the stomach obtained after 55 minutes, the second after 90 minutes. A little undigested meat was found in the stomach postmortem.

	Before secretion			1½ hours after stimulation			
	Arterial serum	Venous serum, stomach	Venous serum, leg	Venous serum, stomach	Arterial serum	Venous serum, leg	Venous serum, stomach
	mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*
HCO_3	23.7	25.8	26.4	25.9	20.6	25.1	25.7
Cl	112.8	111.1	110.3	108.2	111.1	108.2	107.5
PO_4	3.3	3.1	3.2	3.5	3.2	4.1	3.6
Protein	10.4	11.5	10.7	10.7	10.5	10.6	10.8
Total acid	150.2	151.5	150.6	148.3	145.4	148.0	147.6
Total base	184.0	194.1	186.2	191.4	186.2	186.3	192.8
Organic acid	33.8	42.6	35.6	43.1	40.8	38.3	45.2

are recorded here certainly do not indicate any important relationtionship.

No mechanism is suggested whereby the weak acids, carbonic and possibly lactic, usual products of cell activity could replace the strong hydrochloric acid. In this connection the suggestion of Harvey and Bensley (2) is interesting. They noted the accumulation of secretion in the lumen of the gland in the rabbit's stomach. Microchemical reactions showed this secretion was not acid until it mixed rather slowly with surrounding salt solution. To them it seemed "probable that the chlorine is secreted by the parietal cells in the form of a chloride of an organic base and that hydrochloric acid is only set free after the secretion is poured out of the gland into the foveola." Later, Roseman (10) concluded from his experiments that there were two steps in the secretion of gastric acid, chloride accumulation and the splitting off of acid. Hanke (11) has presented evidence of the presence in gastric and other tissues of a specific enzyme hydrolyzing organic chloride esters.

The results of the present experiments are not in conflict with this concept. Secretion thus liberated into the lumen of the glands would be in contact with a relatively large surface. Osmotic equilibrium with the blood could quickly be established and a secretion could eventually be obtained which might have chemical characteristics noted in the first paper of this series. Under these circumstances a marked change in the total base of serum might occur following the formation of such a hydrochloric acid precursor. This is suggested in the experiment on dog number 5 when after two and a quarter hours the secretion of hydrochloric acid had greatly diminished or possibly stopped. At this time the serum from the stomach presented the most marked changes observed in any of the experiments. Such a process could explain the dissociation of the base and the protein variations in the serum.

CONCLUSIONS

1. As blood flows through the secreting portion of a dog's stomach the alterations in the concentration of serum electrolytes indicate the chemical mechanism by which a strongly acid secretion is produced.
2. There is in the serum an increase in the total base with little change in the concentration of chloride.
3. It appears that chloride ions leave the blood with a proportional amount of water and a strongly acid secretion is produced by a retention of base. This relative increase in base is neutralized and equilibrium in the serum maintained by an increase in the bicarbonate and organic salts.

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