

Effect of Varying Hematocrit Ratio on the Gastric Acid Secretion*

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ABSTRACT

Effect of varying hematocrit ratio on the gastric acid production was studied in the heart-stomach preparation of the frog. When the hematocrit ratio was raised by injecting packed red blood cells obtained from the same species of frog, the acid production was increased significantly as compared to the low hematocrit group in which hematocrit ratio was lowered by injecting frog's normal saline.

When a small amount of histamine was added to the medium of 25°C, the acid production was increased in all cases, but the difference in the acid production between the high and the low hematocrit groups was abolished. However, when the temperature of the medium was lowered to 15°C, the differences in the acid production between the two groups became significant.

When a large amount of acetazolamide was added to the medium at 25°C, the acid production was decreased significantly in both groups without showing a significant difference between the two groups.

The reason(s) responsible for the increased acid production in the high hematocrit group was discussed.

the peptic ulcer has since been confirmed by many investigators while performing autopsy. However, the underlying pathogenesis of this peptic ulcer has not been well established to this date.

Among various authors who worked on this problem, Boyd(1934) postulated that this peptic ulcer in polycythemic patients is due to the increased incidence of the formation of thrombosis at the gastric mucosa, which induces anoxic degeneration followed by the subsequent ulceration. On the other hand, Dahl et al.(1959), in their recent animal experiments, found that the ulcer formation in the polycythemic rat could be demonstrated, but the formation of thrombosis was found only in the area of ulceration and no other vascular changes such as dilatation and the thrombosis were found in the rest of the area. On the basis of these findings, they postulated that the formation of thrombosis seems to be a secondary effect of the ulcer formation. Furthermore, in their discussion, they speculated that the rate of blood flow through the capillary vessels of the gastric mucosa would be extremely slow in the case of high hematocrit ratio and thus CO₂ would tend to be retained in the gastric mucosa as a result of which the acid production could be enhanced.

However, it is not even known if the gastric acid secretion is actually enhanced when the hematocrit ratio is elevated. In view of this obvious lack of evidence, this investigation was undertaken to investigate the gastric acid secretion when the hematocrit ratio is varied. In particular, experiments were

An earlier report by Friedman(1913) that patients of polycythemia rubra vera were apt to suffer from

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carried out in the heart-stomach preparation in order to minimize the possible interference of various endocrine glands with the gastric acid production.

METHOD

1. Selection of animals: Male frogs (*Rana nigromaculata*) were used in these experiments because of ease with which one can prepare the heart-stomach preparation (see below).

2. Preparation of packed red blood cells: The hematocrit ratio was made to increase by injecting the packed red blood cell suspension of the frog, as prepared by the following procedure. Blood was drawn with a heparinized syringe from the heart of many male or female frogs of the same species. Blood was pooled into a centrifuge tube so that plasma can be discarded after centrifuge. These packed cells were then washed several times in frog's normal saline in order to eliminate heparin and plasma protein in blood. The last supernatant was used for lowering the hematocrit ratio.

3. Procedures for the animal preparation: Frogs were fasted for several days before the experiment, in order to assure that the stomach is devoid of any food residue. The male frog was pithed and weighed. An incision over the abdominal skin was made longitudinally along the mid-abdominal line in order that the abdominal vein is exposed in the field. Another incision was made at the lower abdomen toward the thorax along the course of the abdominal vein, while a special care was taken to avoid the vein itself in order to prevent any bleeding. The heart was then exposed by means of a Y-incision over the thorax, and the pericardium was subsequently incised. At this point, the entire abdominal viscera and the heart are being exposed. A ligature with cotton thread was made at the beginning of the duodenum. A small cotton swab was introduced gently through the mouth into the stomach to absorb the residual gastric juice. Another ligature with cotton thread was also made around the neck before the head portion was cut off, in order to avoid any influence of the hypophysis. A certain amount of packed red blood cells or the supernatant, equivalent to 2 to 3% of body weight, was injected slowly into

the abdominal vein from the inner surface of the abdominal cavity. The abdominal vein was then tied off around the injected point to prevent bleeding. Abdominal viscera was pushed upward with a blunt instrument and another ligature was made between the liver and the kidneys. The portion distal to this ligature was cut off from the body to avoid any influence of the adrenal glands on the gastric acid

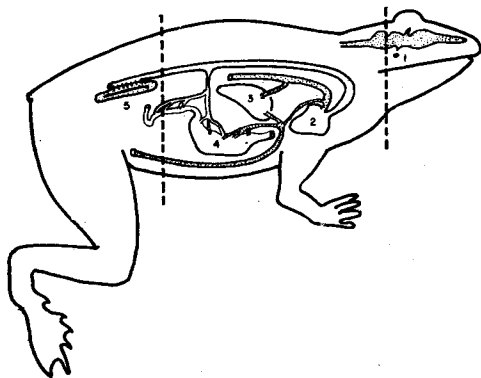


Fig. 1. Schematic representation showing the level of amputation in the heart-stomach preparation.

1) Hypophysis, 2) Heart, 3) Liver, 4) Stomach, 5) Kidney and adrenal gland.

secretion (see Fig. 1). This animal preparation now mainly consists of the heart, the liver and the stomach as illustrated in Fig. 2.

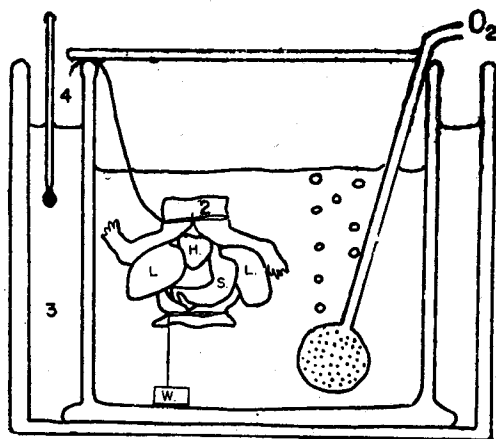


Fig. 2. Schematic representation of the heart-stomach preparation placed in medium.

1) Forster medium, 2) Heart-stomach preparation
L: Lung, S: Stomach, H: Heart, W: Weight,
3) Water bath, 4) Thermometer

4. Experimental procedures: This animal preparation was suspended in Forster medium (Forster, 1948) in a glass jar into which oxygen had been bubbled previously; this jar was covered with a glass plate to prevent aeration of the inside medium. This jar had been previously placed in a glass water bath the temperature of which was maintained at any desired level. The majority of the experiment was performed at 25°C.

The heart rate was checked every 10 minutes from the outside of the water bath during the one hour experimental period. The preparation in which the heart beat was too weak or too slow was discarded. In the present preparation, the stomach was perfused, by its own heart beat, by the blood with either high hematocrit or low hematocrit ratio. An hour later, the preparation was taken out and blood was drawn from the heart into capillary tubes for the measurement of the hematocrit ratio. The upper end of the esophagus was then tied off following which the whole stomach was extirpated along with esophagus and these were immediately incised. The stomach content was washed out in 3 ml of distilled water in a small beaker and the total acidity in the beaker was determined by N/200 NaOH solution. The stomach tissue was separated from the esophagus and was dried in an oven. The dry weight of the stomach was subsequently measured so that the acid produced during the one hour experimental period could be computed per mg of dry weight of the stomach tissue.

RESULTS

1. Control secretion of acid as a function of hematocrit ratio (at 25°C): When the stomach was perfused by blood with high hematocrit, the acid production was increased (Table 1). The difference in the acid production between the high hematocrit group and the low hematocrit group was highly significant ($P < 0.005$). However, the heart rate was practically the same in both groups.

It should be recalled that the stomach has been gently swabbed before the experiment was undertaken. However, the amount of residual acid which is still left in the stomach even after this preliminary procedure could contribute to a certain extent to the values obtained in this series. In order to estimate

this residual acidity, a series of measurements were made in a group of frogs and it was found that only $0.0435 \mu\text{M}$ of acid per mg of dry weight could be detected in the summer (Table 1). Since this residual acidity is so low that the difference in the acid production observed between the high and the low hematocrit groups is very significant.

This correlation between the acid production and the hematocrit ratio becomes more obvious when one plots the rate of acid production as a function of hematocrit ratio, as shown in Fig. 3.

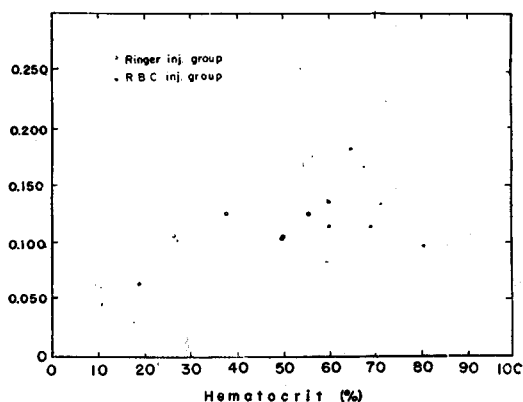


Fig. 3. Gastric acid production as a function of hematocrit ratio in absence of histamine (at 25°C).

2. Effect of histamine on the acid production :

A small amount of histamine diphosphate (0.3 mg/L. of medium) was added to the medium, while a part of this histaminized medium equivalent to 2% of body weight was injected into the dorsal lymph sac of the preparation. In this group, the acid production/hr./mg

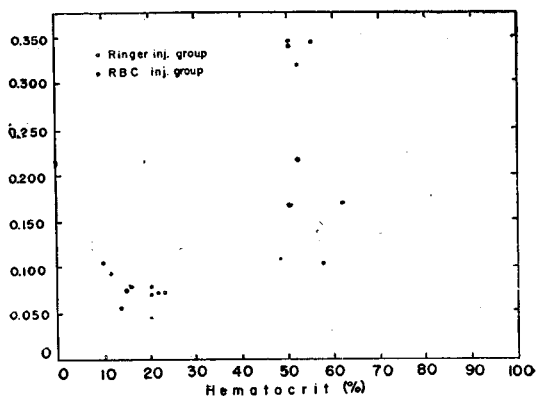


Fig. 4. Gastric acid production as a function of hematocrit ratio in a presence of histamine (at 15°C).

of dry weight was increased in both groups, but there was no difference between the two groups at 25°C ($P > 0.1$; see Table 1). However, when the temperature of the medium was lowered to 15°C, the difference in the acid production between the two groups became significant ($P < 0.005$; see Table 1). As it was demonstrated in the preceeding section, the rate of acid production under the influence of histamine was again well correlated to the magnitude of the hematocrit ratio at 15°C (Fig. 4).

3. Effect of acetazolamide sodium on the acid production (25°C): A series of experiments were first conducted on animal preparations with normal hematocrit ratio in order to study the effect of acetazolamide on the gastric secretion in our heart-stomach preparations. A fairly large amount of acetazolamide (1500 mg/L. of medium) was added to the medium along with histamine (0.3 mg/L. of medium) while a part of this medium containing both acetazolamide and histamine (2% of body weight)

Table 1. Gastric acid production as a function of hematocrit ratio in absence or presence of histamine*

Experimental Series	Experimental Group	No. of Exp't	Acid production $\mu\text{M}/\text{mg}/\text{hr.}$ (Mean \pm S.E.)	P	Hematocrit (%) (Mean \pm S.E.)	Heart Rate min. (Mean \pm S.E.)
Control at 25°C	High hematocrit	12	0.134 \pm 0.011	<0.005	62.0 \pm 3.3	67 \pm 2.2
	Low hematocrit	11	0.078 \pm 0.009		24.0 \pm 3.4	67 \pm 2.6
With Histamine at 15°C (0.3 mg/L.)	High hematocrit	11	0.218 \pm 0.023	<0.005	54.5 \pm 1.3	36.8 \pm 2.0
	Low hematocrit	11	0.092 \pm 0.014		17.2 \pm 5.5	38.0 \pm 0.9
With Histamine at 25°C (0.3 mg/L.)	High hematocrit	9	0.258 \pm 0.148	>0.1	56.0 \pm 4.6	75.0 \pm 1.7
	Low hematocrit	9	0.192 \pm 0.033		19.6 \pm 2.0	78.0 \pm 1.5
Residual Acidity ($\mu\text{M}/\text{mg}$ of dry weight)			0.043 \pm 0.002			

* These experiments were carried out in mid-summer (July-August, 1960).

Table 2. Gastric acid production as a function of hematocrit ratio in presence of acetazolamide with or without histamine*

Experimental Series	Experimental Group	No. of Exp't	Acid production $\mu\text{M}/\text{mg}/\text{hr.}$ (Mean \pm S.E.)	P	Hematocrit (%) (Mean \pm S.E.)	Heart Rate min. (Mean \pm S.E.)
With Histamine at 25°C (0.3 mg/L. of medium)		11	0.059 \pm 0.010	<0.010		61.4 \pm 3.1
With Histamine at 25°C (0.3 mg/L. of medium) and Diamox (1500 mg/L. of medium)		11	0.028 \pm 0.004			64.7 \pm 2.6
Control at 25°C	High hematocrit	5	0.165 \pm 0.025	<0.025	74.7 \pm 4.5	78.0 \pm 2.8
	Low hematocrit	5	0.094 \pm 0.010		27.0 \pm 7.3	71.0 \pm 4.7
With Diamox at 25°C (1500 mg/L. of medium)	High hematocrit	9	0.069 \pm 0.009	>0.1	76.6 \pm 3.1	75.5 \pm 1.5
	Low hematocrit	7	0.056 \pm 0.005		27.0 \pm 4.6	77.0 \pm 2.0
Residual Acidity ($\mu\text{M}/\text{mg}$ of dry weight)		11	0.0202 \pm 0.007			

* These experiments were carried out in early spring (April, 1961).

was injected into the dorsal lymph sac, the gastric acid production was greatly decreased (Table 2).

When an identical dose of acetazolamide alone was supplied to animals with either high or low hematocrit ratio, the gastric acid production was again diminished in both groups without showing any significant difference between the two (Table 2).

DISCUSSION

It is obvious from the present investigation that, when the stomach was perfused by blood with high hematocrit ratio, the gastric acid production was increased. The reason(s) responsible for this increased acid production is not clear, although various possibilities will be discussed later.

When a small amount of histamine was added to the medium at 25°C, the difference of the acid production in the two groups was abolished. This finding is somewhat difficult to understand, although it may be postulated that, if the parietal cell is stimulated by histamine in absence of adequate O₂ supply, the difference in acid production in the two groups could be abolished, for inadequate O₂ supply may possibly limit the activity of the parietal cell in both groups if these cells are producing acid at a maximal level under a given condition. Even in this case, if O₂ supply were sufficient, the difference in acidity would be again demonstrated. In order to suppress the cellular activity of the parietal cell, either the histamine dose could be reduced, or the temperature of the medium could be lowered. We have chosen the latter in which case the difference in acid production in the two groups was again realized. On the basis of these findings, it may be said that, in the present preparation, the oxygen supply which seems to be sufficient under the control condition is not adequate when the cellular activity was augmented by histamine at 25°C.

The amount of acid produced in experiments shown in Table 2 was much less than that shown in Table 1, even though the same amount of histamine was added. The only difference is the fact that the former experiments were carried out in early spring while the latter in summer. These results indicate the existence of a seasonal variation in the rate of gastric acid production as well as in their response to histamine.

mine.

The effect of acetazolamide on the gastric acid production has been studied extensively by many investigators both in vivo and in vitro. As to its effect on in vitro preparations, an inhibitory effect of this agent has been consistent among various investigators (Davenport and Jensen, 1948; Davies and Edelman, 1951). However, in the case of in vivo experiments some investigators obtained an inhibitory effect (Janowitz et al., 1952; Texter and Barbaorka, 1955; Janowitz, 1958) on the gastric acid production while others failed to confirm this (Davies, 1951). According to Davies (1951), a small amount of carbonic anhydrase inhibitor would fail to inhibit the acid production in vivo, for the gastric mucosa contains very large amounts of carbonic anhydrase. On the other hand, when a large amount of carbonic anhydrase inhibitor was given to the experimental animal, the carbonic anhydrase in the erythrocyte would be completely blocked prior to the inhibition of carbonic anhydrase in the parietal cell. This would result in the death of animal due to the CO₂ retention in tissues.

In animal preparations used in the present investigations, a large amount of carbonic anhydrase inhibitor can be given without any danger, because the abdominal viscera as well as the heart are exposed to a large amount of the medium which would remove a considerable amount of CO₂ from the body even when all the carbonic anhydrase activity of the red blood cells is completely blocked. Therefore, in our preparation, the possibility of an excess CO₂ retention at least in the cardiac or the stomach tissue can be ruled out. When a large amount of acetazolamide was given, the acid production was decreased significantly in this preparation.

Although the rate of acid production under the influence of acetazolamide was not significantly different between the high and the low hematocrit groups, the high hematocrit group tended to produce more acid than the low hematocrit group (Table 2). If all the carbonic anhydrase activity of both the red blood cell and the parietal cell were blocked completely, then the reaction ($\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^-$) would proceed as a non-catalyzed reaction and would be a function of CO₂ retained locally.

Hence, the observed tendency to produce more acid in the high hematocrit group even in presence of a large amount of acetazolamide suggests that the non-catalyzed hydration of CO_2 proceeds faster in this group as compared to the low hematocrit group. Although it is by no means certain why this could be so, it is tempting to postulate that perhaps there is local retention of CO_2 in the high hematocrit group by virtue of a slow blood flow through the local tissue. In other words, CO_2 produced locally in the stomach is not carried away as fast as it should because of a slow blood flow through the gastric mucosa. However, there is an alternative view which is equally consistent with the observation and that is that, with high hematocrit more CO_2 could be brought to the stomach from the rest of the body by virtue of an elevated capacity of blood to carry CO_2 . However, this alternate view seems to be less likely when one considers the fact that a large amount of acetazolamide could probably block the activity of carbonic anhydrase in the red blood cell. In this case, blood, regardless of the hematocrit level, will carry very little amount of CO_2 to the stomach, with the result that there will be no difference in the rate of acid production between the high and the low hematocrit

groups. However, the observed difference in acid production in presence of acetazolamide between the two groups is too small to favor any of the above two possibilities.

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