A Study of the Gastric Hematopoietic Factor by Hemoculture Methods

By N. A. Feodorov, A. M. Namyatisheva and M. G. Kakhetelidze

THE SUCCESS of liver therapy in Addisonian pernicious anemia and the elucidation of the role of the stomach in blood formation are milestones of modern hematology.

According to Castle’s hypothesis, normal gastric juice contains a special substance, intrinsic factor, produced by the gastric mucosa. This substance reacts with an extrinsic factor contained in the food. The production is an antianemic substance, absorbed via the gut into the bloodstream, deposited in the liver, and gradually utilized by the bone marrow in the process of blood formation. The nature of the intrinsic and extrinsic factors is not altogether clear. It is known that the intrinsic factor is thermolabile, distinct from pepsin and hydrochloric acid, and is absent or minimal in the gastric juice of individuals with pernicious anemia. Lasch regards the intrinsic factor as the third ferment of the stomach and as a proteolytic enzyme when pepsin is inactivated. Other investigators, who obtained a therapeutic antianemic effect by administering pure gastric juice alone, without extrinsic factor, consider the intrinsic factor a hormone. Greenspan regards the stomach as a dual gland, like the pancreas, with endocrine as well as exocrine secretions. Studies conducted by Lester Smith, G. B. Glass and Boyd enabled us to evaluate the nature of the extrinsic and intrinsic factors participating in this process. The extrinsic factor is B12 and the intrinsic one, a special protein (gastromucoprotein) which is formed within the mucous membrane of the stomach. Gastromucoprotein secures the absorbtion of B12 by the intestine. Thus, the antianemic function of the stomach may be considered as a part of the general process of digestion.

This conception, however, cannot completely solve the problem of the participation of the stomach in hematopoiesis. Nobody disputes the facts of the hematopoietic activity of the pure gastric juice or parenterally introduced metabolites and extracts of the human stomach. These data were obtained by the method of rat-reticuloecytic reaction (Singer’s method). It can be suggested that, alongside with gastromucoprotein, the stomach produces another hematopoietic substance which is capable of exerting a direct stimulating effect upon hematopoiesis, acting quite independently from B12.

The present paper deals with a study of pure gastric juice in man and dogs, by means of a new biologic technic, i.e., hemoculture.

The quantitative determination of hematopoietic factor in gastric juice is done as follows: 10 to 15 ml. of blood are drawn and a white cell film is made. The film is cut into shallow pieces of 1 sq. mm. in diameter and cultured in neutralized gastric juice, the latter being previously diluted 8 times with Ringer’s solution and sterilized by straining through a small porous filter.
Cultures of the same white cell film in pure Ringer’s solution serve as controls. The hemocultures are kept at a constant temperature of 37°C for six hours. To determine the rate of migration of cells, the zones of migration of the experimental and control hemocultures are measured planimetrically on paper-projected tracings of the film. The index of migration of the cultures is calculated by the formula $P = \frac{O_2 - O_1}{O_1}$, where $P$ is the index of migration, $O_2$ the circumference of the zone of migration, and $O_1$ the circumference of the film. The percent difference in rate of migration between the experimental and control cultures serves provisionally as the quantitative index of the hemopoietic factor.

The ability to quantitate this factor of gastric juice opens up broad possibilities for experimental and clinical studies on its nature and physiology.

Numerous studies have shown that the neutralized gastric juice of healthy individuals and animals (dogs, cats) provides a marked stimulus for the migration of leukocytes from the film into the surrounding nutrient medium. The stimulating action of gastric juice on cellular migration varies widely from 5 to 10 to 60 to 80 plus.

We have found also that gastric juice brings about basic cytophysiologic changes in marrow cultures, in addition to its stimulatory effect on leukocyte migration. They are: increased mitoses of young cells, mainly the red, plus a speeding up of their maturation; an increased number of macronormoblasts with extruded nuclei; a greater degree of fragmentation and swelling in the nuclei of erythroblasts; an accelerated hemoglobinization of the cytoplasm (figs. 1 and 2).

Of the patients studied, only those with Addisonian anemia showed an absence of hematopoietic factor in the gastric juice. In other forms of anemia, including aplastic, and also in polycythemia, the hematopoietic factor was always found—sometimes in elevated amounts. Our hemoculture method has
also shown that the hematopoietic activity of gastric juice bears no relation to the presence of the pepsin, acidity, or amount of secretion. The hematopoietic factor is readily dialyzable and can be preserved for a long time at low temperatures. Its thermolability is especially noteworthy; on boiling it not only loses its effect on cellular migration but even blocks it.

With the hemoculture technic we were able to localize more precisely the site of hematopoietic factor formation in the dog's stomach. For this study we utilized gastric fistulae and also isolated pouches on the fundal and pyloric portions of the stomach. Our data showed that hematopoietic factor is produced mainly by the fundus and is absent in the mucosal secretions of the pyloric portion. This refutes the opinion of Meulengracht et al. that the pyloric portion is the main secretor of the substance (see table 1).

Our next task was to clarify the types of stimuli affecting the hematopoietic factor. From Pavlov's experiments we know that the amount, strength and acidity of the gastric secretion is dependent upon the stimulus which evokes it. By experimenting with various substances we were able to demonstrate clearly that the production of hematopoietic factor also depends upon the nature of the stimulus. We employed the Pavlov pouch technic on dogs and used the following substances to stimulate gastric secretion: meat (raw), 200 Gm.; milk, 600 ml.; casein, 200 ml.; milk, 400 ml.; bread, 200 Gm.; histamine, 0.5 ml. (1:1000). Our data showed that the greatest quantity of hematopoietic factor occurs in gastric juice obtained by milk, then meat, and milk serum. Significantly less is obtained by bread, casein, and histamine. Thus, for each type of food there is a corresponding juice of a definite hematopoietic strength. Of all nutrients, the strongest stimulus came from milk—that is, the serum and not the casein. Thus, for the first time, we were able to demonstrate the intimate relation between the quantitative production of the hematopoietic factor and the character of the stimulus evoking the gastric secretion (fig. 3).

We next turned to a study of the hematopoietic properties of blood serum.
GASTRIC HEMATOPOIETIC FACTOR

TABLE 1.—Contents of the Erythropoietic Factor in the Gastric Juice of Dogs, Taken from Different Parts of the Stomach (After Administration of Histamine)

<table>
<thead>
<tr>
<th>Place of Secretion of Gastric Juice</th>
<th>Number of Dogs</th>
<th>Number of Experiments</th>
<th>Erythropoietic Factor Revealed</th>
<th>Erythropoietic Factor Not Found</th>
<th>Average Quantity of Erythropoietic Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>8</td>
<td>37</td>
<td>29</td>
<td>7</td>
<td>19%</td>
</tr>
<tr>
<td>Fundus</td>
<td>5</td>
<td>32</td>
<td>27</td>
<td>5</td>
<td>16%</td>
</tr>
<tr>
<td>Pylorus</td>
<td>3*</td>
<td>11</td>
<td>2</td>
<td>9</td>
<td>82%</td>
</tr>
</tbody>
</table>

*In one dog gastric juice obtained after mechanical irritation of the stomach.

Fig. 3.—Contents of the erythropoietic factor in the gastric juice and its changes under the influence of various stimulants. Dog named “Rex.” Isolated Pavlov stomach on fundal (background) section with count of quantity of gastric juice produced.

In accordance with the view that hematopoietic factor is absorbed via the gut into the bloodstream and carried thence to the liver, it seemed probable that we could find it in the blood.

Angiotomized dogs were used (after the method of E. S. London) and canaliculi were placed in the portal and hepatic veins. Blood was withdrawn simultaneously, under sterile technic, from three vessels—the portal and hepatic veins and femoral artery. By means of hemocultures, a comparative study of hematopoietic factor content was done. We found that the blood serum from each site had a clearly defined hematopoietic index. If the animal’s stomach is empty, the hepatic vein contains much more of the factor than does the portal vein. The latter not only has less, but in isolated cases contains none (table 2). We concluded, therefore, that on an empty stomach the liver brings about a constant flow of hematopoietic factor into the hepatic vein.

We also observed that postcibally, during absorption of digestive products by the gut, the concentration of hematopoietic factor was considerably higher in the portal vein than in the hepatic vein. According to our observation, the liver stores the factor reaching it via the bloodstream and temporarily diminishes or even cuts off the supply of this substance to the hepatic veins (table 3). Therefore, the liver participates in the regulation of hematopoietic factor levels in the blood.
The question arises: what is the effect of postheinorrhagic anemia on the hematopoietic factor, that is, under conditions of compensatory overproduction of red cells by the marrow?

To determine this, we repeatedly bled five dogs with isolated Pavlov pouches. Bleedings were done once a week at the rate of 20 to 25 ml. per kilogram of body weight. The hematopoietic factor content of the gastric juice was determined immediately before and 24 hours after each blood-letting. Hemoglobin determinations, red cell counts and differential counts of leukocytes were also done.

It was noted that the hematopoietic factor content rose 24 hours after each bleeding, but that later this transitory rise was replaced by a drop in concentration lower than prehemorrhagic levels. It was possible to demonstrate a consistent picture of hematopoietic factor depletion in the course of experimental anemia. When the latter reaches a peak, the factor is totally absent. In separate experiments we were able to establish a quantitative correlation between the degree of anemia, as manifested by peripheral blood indices, and the amount of drop in the hematopoietic factor (fig. 4).

The data suggest that in the normal organism one finds only the excess quantity of hematopoietic factor; under conditions of rapidly developing anemia, when the call on hematopoiesis is great, the amount of factor drops or disappears entirely from the gastric juice. That is, the balance of the substance in a posthemorrhagic organism swings in the direction of heightened need for it. Conversely, repeated blood transfusions of the bled animals produce a consistent rise of the substance, which parallels the rise in hemoglobin and red cell count (fig. 5).

Our clinical observation of an increased concentration of hematopoietic factor in polycythemic patients agrees with this finding. We must assume that when the organism is full-blooded, there is less need to utilize hematopoietic factor. In the face of decreased demand and ample supply, the balance swings over to an excess accumulation of the substance, easily found in the gastric juice.
The role of the nervous system in the formation of the substance is of considerable interest. To determine this, a comparative study was made of the gastric juice obtained from the fundi of the Pavlov and Heidenhain pouches, respectively. (As is known, the latter, unlike the former, is completely devoid of vagus nerve innervation.) We studied 29 samples of juice taken from 9 dogs with the Pavlov pouch, and 60 samples from 5 dogs with the Heidenhain pouch. Two hundred grams of raw meat were used as a stimulant. All the Pavlov pouches yielded hematopoietic factor in the juice. In the denervated pouches, one-half showed a complete absence of the substance; the other
half yielded quantities significantly lower than those of the Pavlov pouches (Table 4).

In a second series of experiments, we studied 3 dogs with double pouches, Pavlov and Heidenhain, each, derived from their gastric fundi. Hence, from one stimulant, we could obtain simultaneous samples of gastric juice from two isolated portions of the fundus, one of which was denervated. Various stimulants were used: raw meat, milk, bread, histamines. After most of these were administered, the hematopoietic factor level reached a high peak in the Pavlov pouch at the same time that it fell sharply or disappeared altogether from the denervated pouch.

We concluded, therefore, that the vagus nerve plays a role in the production of hematopoietic factor by the mucosa of the gastric fundus. The sole stimulant which produced a high level of the factor in the denervated pouch was milk and milk serum. It may be that milk has a different mechanism of action—via sympathetic mediation, or directly on the glandular apparatus of the gastric mucosa.

In a third series of experiments, we investigated the effects of splenic denervation on hematopoietic factor production. Two dogs with normal production of this substance were studied by means of Pavlov pouches. After splenic denervation was carried out, we observed a complete, temporary disappearance of hematopoietic factor (Fig. 6).

Observations on these animals were made over a period of six months. During this time, the substance was often lacking from the gastric juice; at times, it did appear, especially after the administration of milk. We concluded therefore that under normal conditions the spleen has a neurotropic effect on the gastric mucosa. Ablation of this effect may temporarily injure intrinsic factor production. Splenic denervation also affects other aspects of gastric secretory function by way of a fall in both the amount and acidity of the secretion.

These studies confirm our previously published data on the influence of splenic denervation on the blood. Other observers, such as Rozanova and Zhukova, noted an activation of bone marrow hematopoiesis in cats the first week after operation, followed by a sharp and long-lasting depression of marrow erythropoiesis. Likewise, Kan observed persistent hypochromic anemia in cats, following ablation of their splenic innervation.

Conclusions

1. A new method of quantitating gastric hematopoietic factor by means of hemoculture has been developed.

<table>
<thead>
<tr>
<th>Place of Secretion of Gastric Juice</th>
<th>Number of Dogs</th>
<th>Number of Experiments</th>
<th>Erythropoietic Factor Revealed</th>
<th>Erythropoietic Factor Not Found</th>
<th>Number of Cases</th>
<th>%</th>
<th>Average Quantity of Erythropoietic Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>The gastric pouch of Pavlov</td>
<td>9</td>
<td>29</td>
<td>29</td>
<td>0</td>
<td>1</td>
<td></td>
<td>+80</td>
</tr>
<tr>
<td>The gastric pouch of Heidenhain</td>
<td>5</td>
<td>60</td>
<td>30</td>
<td>30</td>
<td>50</td>
<td></td>
<td>+27</td>
</tr>
</tbody>
</table>

Table 4.—Contents of Erythropoietic Factor in the Gastric Juice Obtained from Pavlov and Heidenhain Pouches of Dogs: Test Meal 200 Gm. of Raw Meat
2. The neutralized gastric juice of healthy individuals and animals consistently stimulates the migration of leukocytes from a white cell film into a surrounding nutrient medium.

3. In marrow cultures, healthy gastric juice evokes increased mitotic activity in young red cells, an accelerated maturation, and a speeded-up hemoglobinization of the cytoplasm.

4. The main site of formation of hematopoietic factor, both in man and in dogs, appears to be the fundic portion of the stomach.

5. The hematopoietic factor level of the gastric juice depends on the type of stimulant administered, the highest being obtained from milk and raw meat, the lowest from bread and the subcutaneous administration of histamine.

6. With the development of posthemorrhagic anemia, the hematopoietic factor of the gastric juice drops sharply; with multiple blood transfusions, and in polycythemia, it rises.

7. A comparative study of hematopoietic factor levels in Pavlov and Heidenhain pouches shows that the vagus nerve influences hematopoietic factor formation by the fundal mucosa.

8. Denervation of the spleen leads to a complete, though transitory, disappearance of hematopoietic factor from gastric juice.

9. The method of quantitative accounting of the hematopoietic substances of gastric juice which has been worked out is more exact than the method of Singer and opens broad perspectives for the study of the hemopoetic factor of the stomach, and also for other problems connected with this problem.

10. Thus, basing ourselves on the above mentioned experimental data we come to the conclusion that the stomach fulfills manifold functions in the system of neurohumoral control over red blood production. Besides a very important part in producing gastromucoprotein, the stomach, as an erythropoietic center, plays a definite and independent part in regulating hematopoiesis.

**Summario in Interlingua**

1. Esseva disveloppate un nove methodo pro le quantitation de factor intrinsec gastric per medios hematocultural.
2. Le neutralisate succo gastric de normal humanos e animales stimula uniformemente le migration de leucocytos ab un pellicula de cellulas blanc verso le ambiente medio nutritive.

3. In culturas de medulla, normal succo gastric evoca augmentos del activitate mitotic in juvene erythrocytos, acceleration del processo maturante, e un plus rapide hemoglobinisation del cytoplasma.

4. Le sito major del formation de factor intrinsec—tanto in humanos como etiam in canes—pare esser le portion fundic del stomacho.

5. Le nivello del factor intrinsec in succo gastric depende del typo de stimulante usate. Le plus alte nivello resulta de lacte e carne crude, le plus basse resulta de pan e del administration subcutanea de histamina.

6. Experimentos con anemia chronic in canes angiotomisate ha monstrate que le hepate ha tin rob active in le regulation del nivello de factor intrinsec in le sanguine.

7. Con le disveloppamento de anemia posthemorrhagic, le concentration de factor intrinsec in le succo gastric es acutemente reducite. Illo es augmentate per multiple transfusiones de sanguine e in polycythemia.

8. Un studio comparative del nivello del factor intrinsec in saccos de Pavlov e Heidenhain demonstra que le nervo vage exerce un influentia super le formation de factor intrinsec per le mucosa fundal.

9. Disnervation del splen resulta in le complete (ben que transiente) disparition de factor intrinsec ab le succo gastric.

10. Le methodo hematocultural pro le quantitation de factor intrinsec gastric es plus accurate que le methodo de Singer e aperi vaste perspectivas pro studios additional in iste campo.

11. Le methodo elaborate pro le quantitation del substantias hematopoietic in le succo gastric es plus exacte que le methodo de Singer e aperi vaste perspectivas pro le studio del factor hematopoietic del stomacho e etiam de altere problemas de genere affin.

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