ABSORPTION OF RADIOACTIVE IRON AND ITS ASSIMILATION BY ERYTHROCYTES IN ANEMIA CAUSED BY PARTIAL DENERVATION OF THE STOMACH

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Experiments involving the use of radioactive iron have shown that considerable absorption of iron takes place from the digestive tract of dogs suffering from various forms of anemia, and that this process is virtually absent in control animals [11, 12]. The radioactivity of the blood plasma reaches a maximum 1-2 hours after administration of iron, and is totally absent a few hours later. It has been established that the intensity of iron metabolism is determined in the first place by the functional requirements of the organism [1, 6, 9, 10].

We have applied radioactive isotopes to the study of various forms of experimental anemia caused by denervation of certain viscera, and described by V. N. Chernigovskii and his associates [8].

The present paper gives data relating to the absorption of the radioactive iron isotopes Fe^{59} (as $Fe^{59}Cl_3$) from the alimentary tract, and to its utilization for the synthesis of hemoglobin in dogs rendered anemic by partial denervation of the stomach. As a control of the absorptive power of the alimentary tract we also applied the previously obtained data [2] for absorption of radioactive phosphorus (given as $Na_2HP^{32}O_4$).

EXPERIMENTAL METHODS

We examined the absorption of iron and the rate of its incorporation into hemoglobin:

1. In healthy dogs, before operation (dogs Beliak and Malysh).

2. In dogs during the stage of hypochromic anemia supervening after operation for a Pavlov pouch (control, Malysh) or a Klemensiewicz-Heidenhain pouch (partial denervation; the dog Veselyi). The dog Veselyi was further studied during the stage of development of hyperchromic macrocytic anemia.

3. In dogs in which both vagus nerves have been divided below the diaphragm during the stage of hyperchromic macrocytic anemia, and after normalization of the blood picture (Bars, Tarzan, Fokus), and in a control dog in which the esophagus had been exposed below the diaphragm, without injury to the nerves (Beliak). In addition, we examined absorption of P^{32} in the dogs Bars and Tarzan. As controls for these experiments we used the healthy dogs Zhuchka and Rozka.

The radioactive isotopes were administered orally, in 250 ml of milk, at the dosage levels: iron – 1000 cpm phosphorus – 500 cpm per 1 g body weight.

Blood samples were taken 15, 30, 60, 90, 120, 180, 240, and 300 minutes after administration of isotopes, on the first day of administration, and thereafter once daily for the 3-5 days following, and then once in 4-8 days. Each animal was observed over a period of 4-8 months. Radioactivity was measured by means of a Type B counter. The mean error of the measurements did not exceed 3-4%.

We calculated the percentage of the given dose per 1 g body weight found in 1 ml of plasma, and the rate of incorporation of Fe^{59} into hemoglobin from the percentage of the given dose per 1 g body weight found in the erythrocytes of 1 ml of blood (taking into account the hematocrit readings). Serum radioactivity after administration of P^{32} was measured by means of a B-2 counter, and total phosphorus was determined colorimetrically. The specific activity of total serum phosphorus was then calculated. Blood morphology and biochemistry were studied parallel with these measurements [2].

EXPERIMENTAL RESULTS

1. In healthy dogs radioactive iron was found in the plasma 15-30 minutes after its introduction, and reached peak concentration within 120-240 minutes, diminishing gradually thereafter. The radioactivity of the plasma was negligible after 48-72 hours (Fig. 1, 1, 3).

Radioactivity appeared in the erythrocytes on the 4-5th day after administration of iron, and was maximum on the 8-9th day, remaining at this level for a long time, and then falling gradually. After 4 months the Fe^{59} content of the erythrocytes did not exceed 1% (see Fig. 1, 1a, 3a).

2. Post-operation hypochromic anemia developed in the dog Malysh after a Pavlov pouch operation (erythrocytes 3,560,000, hemoglobin 47%); the condition lasted for 1½ months. An analogous form of anemia supervened in the dog Veselyi after operation for a Klemensiewicz-Heidenhain pouch (erythrocytes 2,880,000; hemoglobin 28%). The erythrocyte count and the hemoglobin content rose slightly after 1½ months, but, in contrast to Malysh, reestablishment of the initial levels was not achieved. The erythrocyte count was 1-1.2 million below normal, and the hemoglobin content remained low by 14-18%. After 3½ months the anemia was of the hyperchromic macrocytic type, and persisted throughout the period of observation (8 months).

Radioactive iron was administered to both dogs during the postoperational phase of hypochromic anemia. Radioactivity measurements revealed pronounced intensification of absorption. On the first day of administration the plasma Fe^{59} level exceeded the preoperational one by 4-4½ times, and remained at a high level up to 5-6 days (see Fig. 1, 4, 5). The radioactivity of the erythrocytes was also 7-12 times higher than before the operation, reaching a maximum on the 6-7th day. It remained at this level for a very long time (over 80 days; see Fig. 1, 4a, 5a).

A second dose of radioactive iron was administered to the dog Veselyi during the phase of hyperchromic macrocytic anemia. As is evident from curve 6 (Fig. 1), absorption of iron was much lower, although still higher than in normal dogs. A similar effect was observed with respect to radioactivity of erythrocytes (see Fig. 1, 6a). It follows that incorporation of iron into hemoglobin proceeded more intensively than in healthy dogs, but less so than during the postoperational phase of hypochromic anemia.

3. After subdiaphragmatic division of the vagus nerves of the dogs Bars, Tarzan, and Fokus hyperchromic macrocytic anemia developed, lasting for 3-4 months. The erythrocyte count fell by 1.5-2 million, and the hemoglobin content by 15-22%.

Radioactive iron was administered to these dogs, at the height of the anemic phase. The radioactivity of the plasma was found to be 3-4 times lower than in normal dogs; neither on the following day nor thereafter did we find even traces of activity (Fig. 2, B, 1-3). The erythrocytes displayed activity on the 3rd and 5th days after administration of iron, and, as with the preceding dogs, the activity rose to a maximum on the 6-7th day, although its level was considerably lower (see Fig. 2, B, 1a, 2a, 3a). It may be concluded that, despite the development of anemia, division of the vagus nerves is associated with a sharp fall in absorption of iron and in its assimilation by erythrocytes. A similarly pronounced retardation of absorption of P^{32} from the alimentary tract was observed for these same dogs. This is clearly shown by comparison with the data for control dogs see Fig. 2, A, 1, 2, and 3, 4). After a second dose of radioactive iron we again found inconsiderable absorption from the alimentary tract (see Fig. 2, B, 4, 5).

Measurements of the radioactivity of feces collected during 3 days (a representative sample was taken, and an aliquot of its suspension in water was taken for radioactivity measurement) showed that 88% of the administered iron was excreted.*

* The actual figure is probably still higher, as the radioactivity of the contents of the intestines is not included in it.

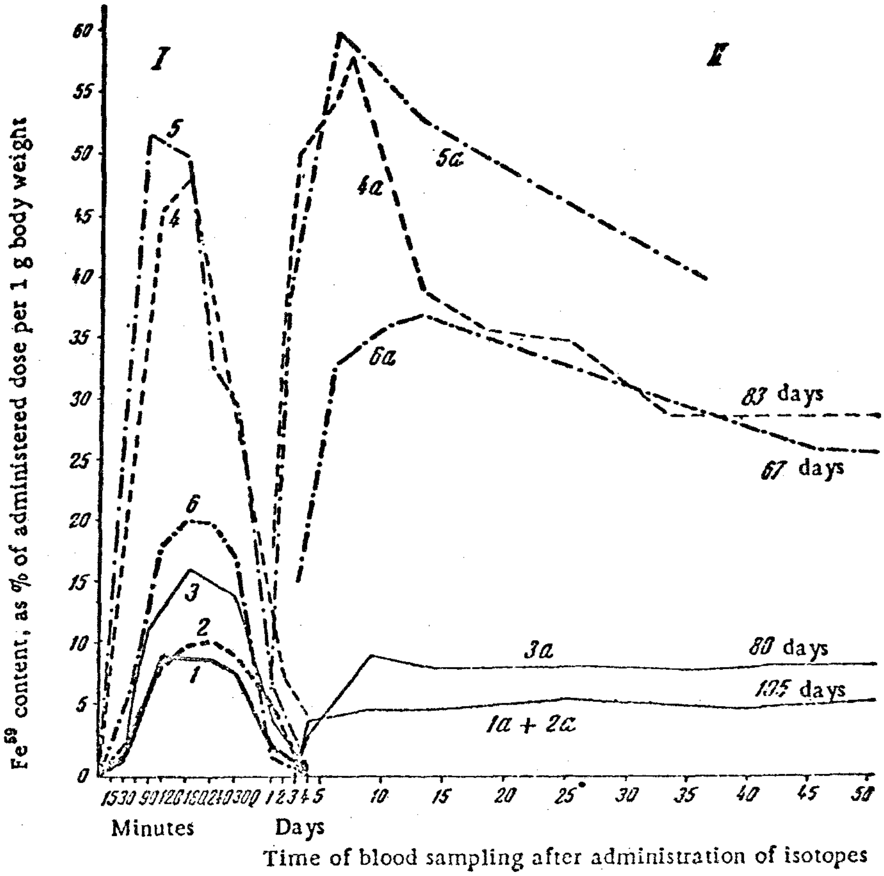

Fig. 1. Rate of absorption and assimilation of Fe^{59} in dogs, before and after partial denervation of the stomach.
I) Velocity of absorption of Fe^{59} from the alimentary tract; 1) Beliak, before operation; 2) Beliak, after laparotomy; 3) Malysh, before operation; 4) Malysh, in the postoperational phase of hypochromic anemia (Pavlov pouch); 5) Veselyi, in the postoperational phase of hypochromic anemia (Klemensiewicz-Heidenhain pouch); 6) Veselyi, in the hyperchromic anemia phase. II) Rate of incorporation of Fe^{59} into hemoglobin: 1a, 2a) Beliak, before and after operation; 3a) Malysh, before operation; 4a) Malysh, in the postoperational phase of hypochromic anemia; 5a) Veselyi, in the postoperational phase of hypochromic anemia; 6a) Veselyi, in the hyperchromic anemia phase.

The dogs were killed, and the iron content of the liver and spleen (homogenates) was determined. The liver was found to contain about 2% of the iron administered, and the spleen 0.7%. Thus vagotomy caused a pronounced lowering of absorption of iron.

Our findings show that absorption of Fe^{59} from the alimentary tract is considerably enhanced during the postoperational phase of hypochromic anemia, as is also its assimilation for synthesis of hemoglobin. Absorption falls somewhat with transition from hypochromic to hyperchromic anemia, but still remains higher than normal. The high levels of radioactivity of erythrocytes persists for a long time in these forms of anemia.

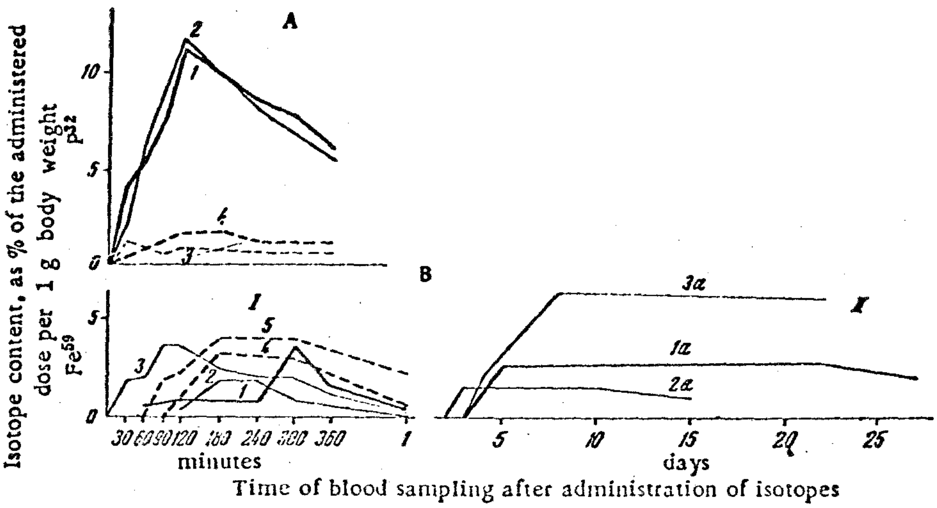

Fig. 2. Rates of absorption and assimilation of radioactive isotopes by dogs after subdiaphragmatic division of the vagus nerves (hyperchromic anemia). A) Rate of absorption of P^{32} from the alimentary tract; 1) and 2) control dogs Rozka and Zhuchka; 3) and 4) operated dogs Bars and Tarzan (vagotomy). B. I) Rate of absorption from the alimentary tract during the phase of hyperchromic macrocytic anemia: 1) and 4) Bars; 2) Tarzan; 3) and 5) Fokus; B. II) Rate of incorporation of Fe^{59} into hemoglobin during the phase of hyperchromic macrocytic anemia: 1a) Bars; 2a) Tarzan; 3a) Fokus.

The onset of hypochromic anemia after stomach pouch operations can scarcely be ascribed solely to loss of blood incidental to them; it is therefore hardly possible to agree with P. Hahn's explanation of the raised absorption of iron [11, 12]. The shape of the curves expressing incorporation of iron into the erythrocytes indicates that this is associated with a fall in the mean survival time of the erythrocytes. Iron is more rapidly utilized for hemoglobin synthesis than normally, but it is also more rapidly liberated from the erythrocytes. This is evidence of enhanced hemolysis. Further evidence of this is afforded by a number of other observations, such as the presence of microspherocytosis, changed osmotic resistance of the erythrocytes, the low color index, etc.

In dogs operated for a Klemensiewicz-Heidenhain pouch, which is considered to involve partial denervation, full recovery from the postoperational anemia does not take place, and it gradually undergoes transition to the hyperchromic macrocytic form. The fragility of the erythrocytes becomes smaller, the color index rises, and maturation of erythrocytes is considerably retarded (bone marrow smears).

Absorption of iron falls during the hyperchromic anemia phase, and approaches the values found before operation. The rate of incorporation of iron into hemoglobin also falls somewhat, although it still remains higher than before operation. The survival time is considerably prolonged, approaching the normal value. There can, therefore, be no question of enhanced hemolysis during this phase. Nor can we consider that there is any lack of iron.

We consider that the gradual onset of hyperchromic anemia is due to disruption of nervous connections between part of the stomach and the central nervous system. This interpretation is based on V. N. Chernigovskii's views [8] on the importance of continuous tonic impulses proceeding from the organs to the central nervous system, for the maintenance of the constant composition of the blood.

Bilateral vagotomy is followed by a considerable fall in absorption from the alimentary tract, and in assimilation of iron by erythrocytes. The same effects are observed with respect to phosphorus. Most of the iron is excreted in the feces, and only a small part of it enters the circulation. The anemia which develops in these circumstances is of the hyperchromic macrocytic form, within the first few days after operation. Pronounced retardation of maturation of erythrocytes is observed. We may, in the given case, postulate a certain lack of iron.

Vagotomy not only interrupts the afferent connections of the stomach, but also leads to considerable disturbances in alimentary tract functions, and to development of structural changes; formation of the intrinsic antianemic factor by the gastric mucosa is interfered with [4, 5, 7]. We cannot exclude the possibility that absorption from the alimentary tract, not only of iron and phosphorus, but also of other products, including the antianemic factor, may be impeded, and this may be one of the causes of the anemia observed.

SUMMARY

Long term experiments were performed on dogs with experimental anemia induced by denervation of the stomach (by Klemensiewicz-Heidenhain partial denervation or by section of the vagus nerves). Investigation was carried out on the absorption of radioactive iron ($Fe^{59}Cl_3$) in the digestive tract and its intake by the erythrocytic hemoglobin.

It was established that absorption and intake of iron is considerably increased during postoperative hypochromic anemia in experiments with the stomach pouch. When hypochromic anemia is changed into hyperchromic the intake of iron by erythrocytes is decreased.

Development of hyperchromic anemia is noted after section of the vagus nerves. At the same time absorption and intake of iron is greatly decreased.

Absorption of radioactive phosphorus (P^{32}) is also diminished.

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* In Russian.

** Original Russian pagination. See C. B. Translation.