THE ERYTHROPOIETIC HORMONE OF THE PITUITARY

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In recent years many researches into the problem of the detection of erythropoietin have been published both in the Soviet Union [1-4] and elsewhere [6, 10, 13, 16]. Erythropoietin production is usually associated with hypoxia [15]. Its site of formation has not yet been finally determined. The most popular view is that it is formed in the kidneys [13, 19]. In the Soviet Union, however, many investigators are of the opinion that the stomach is the principal site of erythropoietin formation [1, 3].

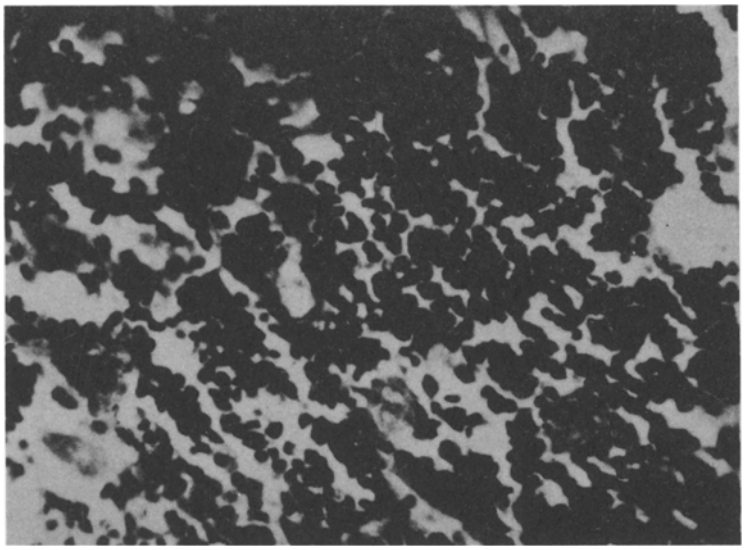
Finally, findings have been described in the literature confirming the importance of the pituitary in the production of the erythropoietic hormone. The connection between the pituitary and the erythropoietic activity of the plasma was first demonstrated by Flaks [10], who fed experimental animals with raw pituitaries and observed the development of a marked reticulocytosis. Later, Van Dyke [20] injected boiled pituitary extracts, deprived of their hormonal activity, into rats and observed stimulation of erythropoiesis in the animals, which he associated with the presence of some hitherto unknown thermostable hormone possessing erythropoietic activity. Contopoulos [7, 8] found that after the simultaneous removal of the thyroid, adrenals, and gonads anemia develops, although it is less severe than that developing after hypophysectomy. Injection of pituitary erythropoietic factors prevented the development of anemia of this type, and in normal rats it caused polycythemia. Recently Matteini and Spigliati [14] used pituitary extracts for the treatment of certain forms of anemia. In more than half the patients these authors noted an improvement in the red blood picture. Crafts [9], however, showed that the erythropoietic activity of the plasma of normal and hypophysectomized animals is identical, and on this basis he denied the existence of a pituitary erythropoietin.

Bearing in mind the conflicting nature of the data in the literature, various authors have postulated the existence of two types of erythropoietins: a plasma erythropoietin, possibility formed in the kidney, and a pituitary erythropoietin. Osnes [15] considers that the pituitary probably exerts a regulatory influence on the production of the renal erythropoietic factor. There are also reports in the literature of the influence of the hypothalamus on the red blood composition [12].

In the course of our studies of the hormonal regulation of hemopoiesis, concerned in particular with the importance of the pituitary in this process, we decided to conduct a series of experiments to determine the erythropoietic activity of pituitary extracts.

METHOD

Experiments were carried out on 38 sexually mature albino rats weighing 150-200 g. For preparation of the extracts we used pigs' pituitaries obtained from the S. M. Kirov Leningrad Meat Combine. The method of preparing the extracts was as follows. After removal of the anterior lobes, these were carefully ground, and distilled water was added in the proportion of 1 ml per pituitary. The mixture was kept on a water bath at 37-40° for 60-70 min, after which it was boiled for 1 min, cooled, and filtered. The resulting solution was again boiled for 1-3 min, when it was considered to be ready for use. Injections of extract (1 ml each) were given daily: to 20 rats for 2 weeks, and to 5 rats for 3 weeks. A control group consisted of 13 rats, of which 5 received injections of distilled water, 5—milk, and 3—muscle extracts prepared as described above. At the end of the experiment all the rats were sacrificed. In some of them the adrenals were weighed and examined histologically.



Bone marrow of rat (femur) receiving injections of pituitary extract. Photomicrograph. Hematoxylin-eosin. Ocular 7, objective 40.

Before the experiment and every 3 days during its course the hemoglobin concentration and the erythrocyte and reticulocyte counts were determined. The bone marrow of most of the animals was investigated histologically.

RESULTS

The initial erythrocyte count of the animals examined varied within the range 6,500,000-9,500,000, with a mean value of 7,500,000. Throughout the experiment no significant change was observed in the erythrocyte count. The mean erythrocyte count 1 and 2 weeks after the beginning of the injections of extract was 7,700,000. The hemoglobin level likewise remained unchanged, having a mean value of 14.3 g% (86 units) 1 and 2 weeks after the beginning of the injections of extract, compared with a normal value of 15.3 g% (93 units).

More considerable changes affected the reticulocyte count: in all the animals this rose sharply. For example, at the end of the 1st week of injections of the extract the mean reticulocyte count had risen from 1.88% (ranging from 0.5 to 3.3%) to 3.76% (ranging from 1.1 to 10.6%). Further injections of the preparation led to a still larger increase in the reticulocyte count, the mean value of which at the end of the 2nd week of the experiment was 4.48% (ranging from 2.2 to 10.1%).

Hence, after injection of pituitary extract for 2 weeks the reticulocyte count in the blood was more than doubled. In 18 animals the count rose by 100%, and in 10 by more than 1000%. Only in 4 animals was the increase only 50-90%. In 3 rats, each receiving 1 ml of extract, the reticulocyte count was essentially unchanged.

Observations were made on 4 rats for 1 week after the final injection of extract. The reticulocyte count in these animals remained unchanged, at a high level (4.3%).

In the control animals receiving distilled water, milk, or muscle extract for 2 weeks, all the red blood indices (erythrocyte and reticulocyte counts, hemoglobin concentration) remained essentially unchanged.

Histological examination of the bone marrow of 10 rats receiving pituitary extract in all cases revealed marked hyperplasia (see figure).

To ascertain the mechanism of action of pituitary extract, in 10 rats the adrenals were weighed, and in 5 these glands were investigated histologically. The weight of the adrenals of the experimental rats (mean weight of the right adrenal 22.7 mg, of the left 19.1 mg) and of the controls (20 and 18 mg respectively) was practically identical. The adrenals of the experimental rats showed no structural changes.

The results showed that extract of the anterior lobe of the pituitary obtained from pigs possesses a marked reticulocyte-stimulating action.

Bearing in mind that our extracts were boiled for a total period of 3-5 min, and the weight and structure of the adrenals were unchanged after the injections, it may be concluded that all the tropic hormones (especially ACTH) were destroyed. The erythropoietic action of the extracts was evidently dependent on their containing thermostable substances belonging to the erythropoietin group. For this reason we support the views of Van Dyke, Contopoulos, and other authors [7, 8, 20], who accept the possibility that a pituitary erythropoietic hormone, or a substance regulating its formation, may exist. This does not, of course, rule out the possibility that erythropoietin may be formed elsewhere, notably in the stomach or the kidneys.

LITERATURE CITED

1. M. G. Kakhetelidze and N. A. Fedorov, Pat. fiziol., 1 (1961), p. 14.
2. Ya. G. Uzhanskii, The Role of Destruction of Erythrocytes in the Mechanism of Regeneration of the Blood [in Russian], Leningrad (1949).
3. N. A. Fedorov and M. G. Kakhetelidze, Probl. gematol., 5 (1962), p. 3.
4. S. Yu. Shekhter, Probl. gematol., 12 (1961), p. 17.
5. A. Ya. Yaroshevskii, Ter. arkh., 12 (1961), p. 3.
6. P. Carnot and C. DéFlandre, C. R. Acad. Sci., 143, Paris (1906), p. 384.
7. A. Contopoulos, S. Ellis, M. Simpson, et al., Endocrinology, 55 (1954), p. 808.
8. A. Contopoulos, D. Van Dyke, J. Lawrence, et al., In book: Proceedings of the 6th International Congress of the International Society of Hematology, New York (1958), p. 761.
9. R. Crafts and H. Meineke, Proc. Soc. exp. Biol., 92, New York (1956), p. 222.
10. J. Flaks, I. Himmel, and A. Zotnik, Presse méd., 46 (1938), p. 1506.
11. A. Gordon, Physiol. Rev., 39 (1959), p. 1.
12. K. Hirashima, Acta haemat., 26, Basel (1961), p. 209.
13. L. Jacobson, E. Goldwasser, W. Fried, et al., Nature, 179, (1957), p. 633.
14. M. Matteini and P. Spigliati, Acta endocr. (Kbh.), Suppl. 51, 35 (1960), p. 281.
15. S. Osnes, Brit. med. J., 1 (1960), p. 1153.
16. K. Reissmann, Blood, 5 (1950), p. 372.
17. W. Rosse and T. Waldmann, Ibid., 19 (1962), p. 75.
18. N. Shahidi and L. Diamond, J. Dis. Child., 98 (1959), p. 293.
19. G. Thiel, Dtsch. Arch. klin. Med., Bd. 208, S. 111 (1962).
20. D. Van Dyke, M. Simpson, A. Contopoulos, et al., Blood, 12 (1957), p. 539.
21. P. Ways, J. Huff, C. Kosmaler, et al., Arch. intern. Med., 107 (1961), p. 154.