3. G. S. Mazurkevich, I. V. Kreier, A. I. Tyukavin, et al., Problems in Emergency Medical Aid [in Russian], Leningrad (1981), pp. 60-68.
4. F. Z. Meerson, Pathogenesis and Prevention of Stress-Induced and Ischemic Heart Lesions [in Russian], Moscow (1984).
5. E. A. Mutuskina, V. A. Bolyakin, N. V. Kaverina, et al., Anesteziol. Reanimatol., No. 1, 47 (1982).
6. G. I. Mchedlishvili, Patol. Fiziol., No. 2, 75 (1985).
7. B. I. Tkachenko, Physiology of the Circulation; Physiology of the Vascular System [in Russian], Leningrad (1984), pp. 5-38.
8. L. G. Shikunova, Current Problems in Reanimatology [in Russian], Moscow (1980), pp. 127-134.
9. T. Arai, T. Watanabe, T. Nagaro, et al., Crit. Care Med., 9, 444 (1981).
10. M. Bradbury, The Concept of the Blood-Brain Barrier [Russian translation], Moscow (1983).
11. S. E. Gisvold, P. Safar, G. Rao, et al., Stroke, 15, 803 (1984).
12. K. A. Hossmann, Current Problems in Reanimatology [in Russian], Moscow (1980).
13. J. I. Sage, R. L. Van Uitert, and T. E. Duffy, Stroke, 15, 46 (1984).
14. M. M. Todd, B. J. Dunlop, H. M. Shapiro, et al., Stroke, 12, 808 (1981).
15. H. Wagner, R. Cahn, T. Kuroiwa, et al., J. Cereb. Blood Flow Metab., 3, Suppl. 1, 417 (1983).

# EVALUATION OF THE ERYTHROPOIETIN-PRODUCING FUNCTION OF THE KIDNEY AND LIVER DURING CONTROLLED PERFUSION

V. P. Nefedov, V. P. Makarov,
E. K. Tashenov, E. V. Krizhanovskaya,
V. N. Kazakov, A. P. Rupenko,
and I. I. Gitel'zon

UDC 612.111.3-063:[612.46.018 + 612.351.018

KEY WORDS: erythropoietin; perfusion of isolated organs; hypoxia.

Erythropoietin (EP) is the specific hormone of erythropoiesis, the main source of which in man and animals is the kidneys [2, 4, 10]. During the study of erythropoiesis in animals after total and partial hepatectomy, combined with nephrectomy, it was shown that this hormone can also be synthesized by the liver, but in much smaller amounts than when the kidneys are present [10-12]. These findings indicate that the liver is the main source of extrarenal EP formation. In addition EP can be formed in other organs: the spleen, bone marrow stroma, stomach, pituitary gland, and hypothalamus [4, 9, 13]. During stimulation of erythropoiesis, increased EP formation takes place both in the kidneys and in the extrarenal sources. It is not yet clear (because of technical difficulties) what is the relative contribution of individual organs to the total balance of EP production under normal conditions and during intensive erythropoiesis [6, 8].

The aim of this investigation was a comparative study of the contribution of the kidneys and liver to total EP production in animals during excitation of erythropoiesis. For this purpose the method of perfusion of isolated organs (liver and kidneys) obtained from the same animal was used.

## EXPERIMENTAL METHODS

Experiments were carried out on male mongrel dogs weighing 12-16 kg. EP formation was stimulated by the combined action of blood loss and injection of cobalt chloride. Blood equivalent to 25% of the blood volume was removed from the femoral vein of the animals 24 h

Laboratory of Control of Biosynthesis of Animal Tissues and Laboratory of Biophysics, Institute of Biophysics, Siberian Branch, Academy of Sciences of the USSR, Krasnoyarsk.
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 10, pp. 404-406, October, 1986. Original article submitted December 13, 1985.

 0007-4888/86/0010-1340$12.50 

TABLE 1. Level of Erythropoietic Activity (in mU) of Perfusion Medium of Kidney and Liver of Control Animals at Beginning (A) and End (B) of Experiment

| Organ | Erythropoietic activity of 1 ml of perfusion fluid | |
|---|---|---|
| | A | B |
| Kidney | 58±7,3 | 59±19,9 |
| | 57±8,3 | 95±9,1 |
| | 60±9,4 | 57±15,3 |
| Liver | 71±8,9 | 76±17,1 |
| | 66±10,1 | 68±7,2 |
| | 96±37,6 | 94±20,5 |

Legend. Here and in Table 2 difference in erythropoietic activity at end of experiment compared with beginning not significant in animals of control group ($P > 0.05$) but is significant in group of posthypoxic animals ($P > 0.001$).

TABLE 2. Level of Erythropoietic Activity (in mU) of Medium during Perfusion of Liver and Kidney of Posthypoxic Animals at Beginning (A) and End (B) of Experiment

| Organ | Erythropoietic activity of 1 ml of perfusion fluid | | Change in erythropoietic activity of 1 ml of perfusion fluid | Quantity of hormone secreted | Total quantity of hormone secreted (average) |
|---|---|---|---|---|---|
| | A | B | | | |
| Kidney | 78±16,3 | 184±29,7 | 106 | 63 600 | |
| | 72±14,5 | 160±31,3 | 88 | 58 800 | 45 600 |
| | 102±11,8 | 168±30,6 | 66 | 39 600 | |
| | 74±14,5 | 108±4,7 | 34 | 20 400 | |
| Liver | 78±4,4 | 160±31,3 | 82 | 123 000 | |
| | 74±10,6 | 136±30,8 | 62 | 93 000 | 111 750 |
| | 76±14,6 | 168±12,7 | 92 | 138 000 | |

before perfusion, and later an equal volume of Ringer–Locke solution was injected into them, and 12 h before perfusion, a subcutaneous injection of $CoCl_2$ in physiological saline was given (250 μmoles/kg body weight). Control animals were not bled, but 12 h before the experiment they were given an injection of the same volume of physiological saline. The perfusion medium consisted of blood from homologous donors with the addition of equal quantities of medium 199 and polyglucin; the hematocrit reading was 8-12% (pH 7.3-7.4). The volume of perfusion fluid was 600 ml for the kidney and 1500 ml for the liver. Controlled perfusion of the isolated organs was carried out on two appliances of the "Gomeostat-3" perfusion complex [3]. The rate of flow of the perfusion fluid was 0.7-1.0 ml/min/g liver tissue and 1.0-1.7 ml/min/g kidney tissue. The pressure in the hepatic artery was maintained at 80-100 mm Hg, in the renal artery at 110-180 mm Hg, and in the portal vein at 15-20 cm water. The partial oxygen pressure was: 200-240 mm Hg in the arterial perfusion fluid of the liver, 180-240 mm Hg in the arterial perfusion fluid of the kidney, in 40-80 mm Hg in fluid flowing from the liver, and 60-80 mm Hg in fluid flowing from the kidney. Each version of the experiment was repeated 3-4 times. The duration of perfusion was 6 h. Erythropoietic activity was determined in the perfusion medium at the beginning and at the end of the experiment by a radioindicator method based on uptake of $^{59}Fe$ into erythrocytes of polycythemic female CBA mice, and expressed in milliunits of the international standard [2]. The results were subjected to statistical analysis by the Student's test.

## EXPERIMENTAL RESULTS

Toward the end of the 6th hour of perfusion of the posthypoxic organs the EP concentration during perfusion of the kidney and liver was on average doubled in the perfusion fluid compared with initial data (Tables 1 and 2). In the control series the erythropoietic activity of the kidney and liver did not change significantly. To compare the erythropoietin-producing function of the organs during controlled perfusion, we calculated the total quantity of the hormone produced during the experiments: it averaged 45,600 mU for the kidney and 111,750 mU for the liver. These results agree with those in [7], evidence that the kidney can produce EP under normoxic conditions after preliminary exposure to hypoxia, which probably plays the role of trigger factor. The results of the experiments with perfusion of the liver, which confirmed its ability to form the hormone, also showed that this function possesses reserve capacity. Incidentally, the writers showed previously that normoxic perfusion of the liver, taken from an animal bled previously, is capable of forming EP [1].

The method of perfusion of isolated organs which we adopted can be used to determine the contribution of individual organs to EP production. Investigations of this kind have been undertaken on the whole animal, in the case of intact and nephrectomized rats [8]. While using different hypoxic procedures, the authors cited found no significant increase in the extrarenal fraction of the hormone: it amounted to between 12.5 and 18.3% of its total quantity in the plasma. Probably the model which they used (nephrectomized animals) does not ensure adequate triggering of hormone synthesis by the liver after removal of both kidneys. The relative EP production by the kidney and liver in chronic phenylhydrazine anemia was studied in [6]. For this purpose organs were perfused in situ and the concentration of the hormone in the outflowing perfusion fluid was determined. It was found that in the acute period of anemia the kidneys secreted twice as much hormone as the liver, but as compensation of hemolysis was reached, production of hepatic EP increased to become twice the quantity of hormone produced by the kidneys. These data are in agreement with the results of our own investigations which showed the EP production by the liver is 2.5 times greater than the amount produced by one kidney. However, the possibility cannot be ruled out that in these experiments the ability of the organs to produce the hormone was completely mobilized after exposure of the animal to hypoxia.

The liver can thus form EP in the same amounts as the kidney (concentration of the hormone in 1 ml of perfusion fluid), but if EP production is expressed per total volume of perfusion fluid, it exceeds its production by the kidney by 2.5 times. PE production by the liver and kidney, if extrapolated to the intact organism, when both kidneys are functioning, is thus evidently about equal. Incidentally, the conditions of isolated perfusion of organs differ significantly from those of the intact organism, with its integrative control mechanisms. The data given above are only presumptive and do not prove anything, but they do broaden our ideas on the role of the liver in extrarenal EP production.

## LITERATURE CITED


1. I. I. Gitel'zon, V. P. Nefedov, V. V. Mezhevikin, et al., Byull. Éksp. Biol. Med., No. 7, 23 (1977).
2. V. P. Makarov, Erythropoiesis and Energy Metabolism of the Organism [in Russian], Novosibirsk (1984).
3. V. P. Nefedov, G. A. Dorrer, I. V. Yarigina, et al., Parameters of Perfusion of Isolated Organs [in Russian], Novosibirsk (1983).
4. S. I. Ryabov and G. D. Shostka, The Erythron and the Kidney [in Russian], Leningrad (1985).
5. J. Caro, L. J. Zon, R. Silver, et al., Am. J. Physiol., 244, E431 (1983).
6. B. Dornfest, B. Naughton, J. Jonson, and A. S. Gordon, J. Lab. Clin. Med., 102, 274 (1983).
7. A. Erslev, Blood, 44, 77 (1974).
8. A. Erslev, J. Caro, E. Kanzu, and R. Silver, Br. J. Haemat., 45, 65 (1980).
9. W. Fried, Blood, 40, 671 (1972).
10. Z. Kuratowska, B. Lewartowski, and E. Michlar, Blood, 18, 527 (1961).
11. B. A. Naughton, S. M. Kaplan, M. Roy, et al., Science, 196, 301 (1977).
12. C. Peschle, G. Marone, A. Genovese, et al., Br. J. Haemat., 32, 105 (1976).
13. J. N. Rich, W. Heit, and B. Kubanek, Blut, 40, 297 (1980).
14. J. L. Spivak and S. E. Graber, Johns Hopk. Med. J., 146, 311 (1980).