# *In vivo* STUDY OF THE EFFECT OF UREMIC SERUM AND ITS FRACTIONS ON STEM CELLS

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Chronic renal failure (CRF) as a rule is accompanied by anemia, the pathogenesis of which is varied and is still obscure. The principal pathogenetic factors in CRF are a disturbance of erythropoietin production by the kidney, shortening of the life span of the red blood cells, blood loss, a deficiency of iron and amino acids, and so on [1, 15].

Meanwhile many workers have demonstrated that uremic serum has the property of inhibiting erythropoiesis *in vitro:* It reduces the number of mitoses among erythroblasts in culture [3], delays their maturation [4], lengthens the mitotic cycle of erythroblasts [9], and inhibits the incorporation of $^{59}Fe$ into heme in the presence of erythropoietin [6, 7]. The present writers [2] and others [5] showed that the serum of patients with CRF also inhibits the effect of exogenous erythropoiesis in polycythemic mice.

These data suggest that the serum of patients with CRF contains a factor (or factors) inhibiting erythropoiesis, and this may also be one of the pathogenetic factors aggravating nephrogenic anemia.

However, it is not yet clear at what level erythropoiesis is disturbed under the influence of serum of patients with CRF. No data on its possible influence on the hematopoietic stem cells could be found in the literature.

The aim of this investigation was the preparative fractionation of uremic serum and the study of the effect of serum from patients with CRF and of the isolated fractions on erythropoiesis in polycythemic mice and on the pluripotent stem cells.

## EXPERIMENTAL METHOD

Serum from 18 children and adolescents with CRF in the terminal stage of kidney disease (creatinine 11.6 ± 1.3 mg%, urea 77 ± 18.5 mg%), aged from 3 to 17 years, was investigated. All the patients were treated by hemodialysis in the "Charité" Clinic (East Berlin): for 2-4 h three times a week. The NG-AK-4 capillary dialyzer, with a dialyzing surface of 1.3 $m^2$, was used. All the children had anemia: Hb 6.2 ± 0.6 g%, hematocrit index 18.6 ± 2%, which was maintained at a constant level by transfusion (300 ml) of packed red cells (on average 0.9 transfusions per month).

The erythropoiesis-inhibiting properties of the serum of these patients were studied on mice with polycythemia, induced by transfusion of red blood cells [10]. Incorporations of $^{59}Fe$ into red blood cells was studied in the blood of mice and the percentage of inhibition of the effects of erythropoetin in animals of the experimental group compared with the control was calculated [2]. A laboratory preparation of erythropoietin with activity of one unit/mg protein was used.

The effect of uremic serum and its fractions on proliferation and differentiation of stem cells also was studied by the method of Till and McCulloch [14]. Female (CBA × C57BL)$F_1$ mice aged 10-12 weeks served as donors of bone marrow and as recipients. The number of cells transplanted to each animal was 50,000. Test material was injected in a dose of 1 ml twice,

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TABLE 1. Erythropoiesis-Inhibiting Properties of Fractions of Uremic Serum after Ultrafiltration

| Expt. No. | Material | Percent incorporation of $^{59}Fe$ | Percent inhibition | $P$ |
|---|---|---|---|---|
| 42 | Serum of patients with CRF (2 ml)* | — | —40 | <0,05 |
| | Physiological saline (2 ml) | 0,16±0,1 (4) | — | — |
| | Physiological saline (2 ml) + erythropoietin (0.8 units) | 5,25±1,37 (4) | — | — |
| | Concentrate (2 ml)† + erythropoietin (0.8 units) | 7,29±2,68 (6) | +39 | — |
| | Concentrate (2 ml)‡ + erythropoetin (0.8 units) | 5.76±1,97 (5) | +10 | — |
| | Ultrafiltrate (2 ml)† + erythropoetin (0.8 units) | 2,6±1,01 (5) | —51 | <0,02 |
| | Physiological saline (2 ml) | 0,38±0,26 (3) | — | — |
| | Physiological saline (2 ml) + erythropoietin (0.8 units) | 16,67±4,95 (5) | — | — |
| | Ultrafiltrate (2 ml)† + erythropoetin (0.8 units) | 6,83±2,2 (5) | —59 | <0,02 |

Legend. 1) Number of mice given in parentheses. 2)*Mean data for eight patients (taken from the journal "Urologiya i Nefrologiya," 1978, No. 4, p. 40); †Fraction injected in a concentration equal to the quantity of original serum; ‡ Fraction injected in a concentration equal to the quantity of two original sera.

TABLE 2. Number of Colonies in Spleen of Irradiated Mice and Ratio between Numbers of Erythroid and Granulocytic Colonies after Injection of Test Material

| Material | No. of colonies in spleen | Ratio of erythroid to granulocytic colonies |
|---|---|---|
| Normal plasma | 11,5±1,74 | 4,3±1,5 |
| Uremic plasma | 10,8±1,5 | 5,2±1,7 |
| Fractions: | | |
| $F_1$ | 14,3±3,8 | 4,2±0,67 |
| $F_3$ | 13,3±2,0 | 5,3±0,6 |

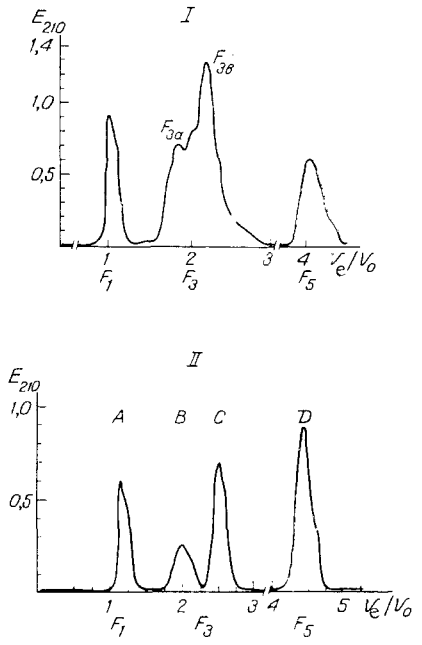


Fig. 1. Chromatograms of fractionation of ultrafiltrate on Sephadex G-25. I) Sera of patients with CRF before hemodialysis; II) marker substances: A) blue dextran, B) bacitracin, C) vitamin $B_{12}$, D) uric acid. Volume of fractions 6.7.

subcutaneously and intraperitoneally, with an interval of 1 h between injections. Each sample was injected into a group of 8-12 mice. The animals were irradiated from a $^{137}Sr$ γ-ray apparatus in a dose of 1200 rads 2 h before bone marrow transplantation. The mice were killed on the 9th day and the spleens were fixed in Bouin's solution and then in 70°C ethanol. The number of colonies in the spleen was counted macroscopically. For microscopic investigation of the composition of the hematopoietic colonies serial histological sections were cut through spleens and stained with hematoxylin-eosin. The relative percentages of the types of colonies were determined in the experimental and control (injection of bone marrow, and also of bone marrow with normal donors' serum) groups of animals.

The results were subjected to statistical analysis by Student's t-test.

The uremic serum was fractionated in two stages: 1) fractionation of XM-50 plates from the firm "Amicon." The concentrate and ultrafiltrate were studied for their content of erythropoiesis-inhibiting factor; 2) the ultrafiltrate was fractionated on Sephadex G-25 (Pharmacia) on a column measuring 2.6 × 100 cm (LKB) using 0.02 MNaCl solution, pH 7.4; the extinction coefficient was measured at 210 nm (with a "Perkin-Elmer" spectrophotometer. The molecular weight of the fractions was calculated from the ratio $V_e/V_0$ and the coefficient $K_d$ by comparison with marker substances: blue dextran (mol. wt. 2 million), bacitracin (1,450), vitamin $B_{12}$ (1,350), and uric acid (195).

Protein was determined by means of a calibration curve plotted for albumin (VEB, Berlin-Chemia) in a dilution of 1-6 mg%, and by measurement of the extinction coefficient in the UV-region at a wavelength of 210 nm [11].

## EXPERIMENTAL RESULTS

The results of a study of the erythropoiesis-inhibiting properties of the serum and of fractions obtained by ultrafiltration are shown in Table 1. These investigations showed that the erythropoiesis-inhibiting properties of uremic serum are concentrated in the ultrafiltrate.

The results of fractionation of the ultrafiltrate of serum from patients with CRF before hemodialysis and data for marker substances are shown in Fig. 1.

The figure shows that during fractionation of the ultrafiltrate three fractions were obtained: $F_1$ mol. wt. 50,000-5,000, $F_3(F_{3a} \pm F_{3b})$ mol. wt. 3,000-800, and $F_5$) mol. wt. under 200.

The results of a study of the effect of the uremic serum and its fractions on proliferation and differentiation of hematopoietic colonies in the spleen of irradiated mice are given in Table 2.

The results of these investigations show that after injection of uremic serum and its fractions the number of macroscopically visible colonies was the same as in the group of control animals; the relative percentage of erythroid colonies was virtually unchanged (the ratio of erythroid to myeloid colonies was the same in all groups of animals).

Our previous investigations of hematopoiesis in intact and polycythemic mice showed that serum from patients with CRF inhibits only cells of the erythroid series. In polycythemic mice the formation of cells of the erythroid series in response to injection of exogenous erythropoietin was inhibited, suggesting that the factor (factors) inhibits erythropoiesis at the stage of undifferentiated erythroid precursors [12].

Some workers [7] have observed a decrease in the number of erythroid colonies (BFU-E, CFU-E) formed in a plasma clot culture after the addition of uremic serum to it. However, these workers suggest that this inhibition may have taken place at the level of the pluripotent stem cells.

The present investigation thus showed that the erythropoiesis-inhibiting properties of uremic serum and of fractions isolated from it, when injected into animals in amounts accumulating in the serum of patients with CRF, are not due to the nonspecific cytotoxic action of the serum of the pluripotent stem cell. It may be that the erythropoiesis-inhibiting action of the serum of patients with CRF is exerted at the same level as the erythropoietin effect.

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