Simple Purification Procedure for Human Urinary Erythropoietin (37357)

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(Introduced by Howard A. Bern)

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Erythropoietin is the primary inducer of erythrocyte formation in vertebrates (1). Accordingly, physicochemical studies on this compound should help elucidate molecular mechanisms involved in the differentiation of somatic cells. Purification of erythropoietin has not yet been accomplished to the extent that the factor can be subjected to such studies, although isolation of a minute amount of erythropoietin from the plasma of anemic sheep has been reported recently by Goldwasser *et al.* (2).

Urine of human anemic patients provides another possible source of erythropoietin. However, the principle in the crude urine powder shows great instability during purification (3). The present communication describes a rapid procedure for the purification of erythropoietin using electrofocusing.

Materials and Methods. Urine was obtained from 2 patients with aplastic anemia. Crude erythropoietic powder was prepared by precipitation with 80% acetone (3). The crude substance was then purified by electrofocusing at 2°, using a LKB 8101 column and 2% carrier ampholite of pH range 3–4, which was prepared by prefocusing of standard ampholite of pH range 3–5 (LKB). Fifty percent sucrose (25 ml) was added to prefocused ampholite of pH range 3.5–4.0 (30 ml) to make a dense solution. Crude acetone powder (293 mg) was dissolved in distilled water (30 ml) and added to the prefocused ampholite at pH 3.0–3.5 (25 ml) to make a light solution. Both dense and light solutions were introduced into the column, resulting in a density gradient. The anode was set atop the column. Two percent ethylenediamine in 45% sucrose was used for the cathodal solution and 1% phosphoric acid for the anodal solution. Power was supplied at 300 V initially and was raised stepwise to 600 V during the first 3 hr. After 48 hr, power was turned off and the content of the column was collected in 2-ml fractions. The pH of the effluent was measured using a glass electrode.

The biological activity and protein concentration of each fraction were determined after dialysis against distilled water for 24 hr at 2°. Protein concentration was measured (4) using bovine serum albumin as standard. Erythropoietic activity was assessed by determining the ^{59}Fe incorporated into newly formed red cells of starved rats subsequent to the subcutaneous injections of test samples, as described by Fried *et al.* (5). The crude urine powder prepared as described above was standardized against Erythropoietin Standard A (1).

Results. Figure 1 shows results of the electrofocusing experiments on the urine powder.

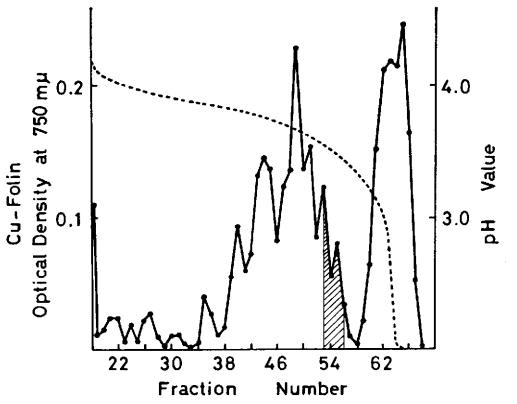

FIG. 1. Pattern of electrofocusing. See text for method. Solid line shows relative concentration of protein, and broken line shows pH value. Erythropoietic activity was located in fractions 53–56.

Erythropoietic activity was located in fractions 53–56. These active fractions were combined and lyophilized. Forty-nine percent of total activity of applied acetone powder was recovered after lyophilization. The specific activity was 183 units/mg protein, whereas that of the applied acetone powder was 0.48 units/mg protein. Therefore, the erythropoietin was purified 380-fold by a single electrofocusing with a good yield. The isoelectric point of the active substance was pH 3.40 ± 0.10 at 2°.

Discussion. Recently Espada *et al.* (6) purified human urinary erythropoietin with high specific activity by several steps including heat treatment, chromatography on DE AE-cellulose and hydroxylapatite, and repeated gel-filtration on Sephadex G-100. However, when we repeated their procedure, a marked loss of activity of urinary erythropoietin resulted. Graham *et al.* (3) also reported that the activity of human urinary erythropoietin was lost during Sephadex or DEAE-cellulose chromatography.

Lukowsky *et al.* (7) subjected sheep plasma erythropoietin (Step I) to electrofocusing over the pH ranges 3–10 and 3–5. Although heterogeneously distributed, all the biological activity was located between pH 3.5 and 4.0. The lower p*I* value of human urinary erythropoietin, as shown in the present paper, might reflect the higher content of sialic acid residue in the molecule.

Summary. Considerable potent erythropoietin with a p*I* of 3.40 ± 0.10 at 2° was purified from the urine of anemic patients by precipitation with 80% acetone and by electrofocusing.

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