taining in the bone marrow of a mouse because of the uncertainties in atomic composition of bone and bone marrow and because of the lack of homogeneity of composition in volumes encompassing the range (up to about 2 mm) of the recoil protons (the most important and energetic ionizing particles produced by fast-neutron irradiation). In contrast to the fast-neutron case, in typical x-ray irradiations, this ratio in homogeneous bone is perhaps 3 (*8*), and the applicable value of the ratio is subject to the same kinds of uncertainties as for neutron irradiation. Also because of the relative compositions, the ratio of the average linear energy transfer in bone to that in wet tissue is probably less than unity in photon irradiations and greater than unity in neutron irradiations.

In a general way, most acute irradiation deaths have been classed as caused by gastrointestinal or bone marrow damage (*9*). In gastrointestinal damage, deaths in mice tend to occur within less than 7 days after irradiation and in bone marrow damage during the second week after irradiation. Considering the characteristically sharp break in the shape of mortality versus dose curves and the ratios of dose in bone to that in wet tissue, one might anticipate that a change from x-ray to fast-neutron irradiation would markedly increase the percentage of gastrointestinal deaths in irradiations with equal over-all mortality. This has been found by various workers. Furthermore, owing to the increase in gastrointestinal death, against which bone marrow treatment is expected to be ineffective, one might anticipate that bone marrow treatment would be less efficacious against death (from all causes) following fast-neutron irradiation than following x-ray irradiation. At best, the frequency distribution of mortality might be shifted to that of gastrointestinal death alone.

Comparison of the results of the present fast-neutron irradiation experiments, in which bone marrow treatment produced a significant reduction in mortality only after the period assigned to gastrointestinal death, with previous x-ray irradiation experiments (*1*) supports these conclusions.

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4 March 1957

New Principle of Preparative Electrophoresis

Electrophoresis is potentially the most elegant and efficient method for protein fractionation and isolation. Even before the adaptation of the method to analytic purposes by Tiselius, there were numerous attempts to utilize it for preparative purposes. The older literature has been well reviewed by Svensson (*1*) and the more recent upsurge of various apparatus designs has also been partially reviewed (*2*).

Some of the older electrophoretic methods were based on the electrodecantation principle of Pauli (*3*). Proteins, and other charged colloids, when exposed to a direct-curent electric field will migrate toward one of the electrodes and, therefore, will accumulate in the immediate neighborhood of a semipermeable membrane placed in their path of migration. This layer of increased colloid concentration will, by virtue of its higher density, settle to the bottom of the vessel, where it can be withdrawn separately from the bulk of the solution. This principle was also adopted by Kirkwood *et al.* (*4*) in the electrophoresis-convection method, using certain elements of the Clusius column.

Based on the fact of the accumulation of charged colloids in the immediate neighborhood of a semipermeable membrane, a new method of preparative electrophoresis was developed, which is best described by the functional name, *continuous free-boundary flow-electrophoresis* (*5*). The method takes advantage of the laminar flow of liquids, and its principle can be illustrated with the aid of the diagram presented in Fig. 1. An electrophoretic cell is constructed of two outer semipermeable membranes *A* and *A'*, defining the size of the cell, and held stretched in a plastic frame (not shown in the diagram). The cell is immersed in a circulating, cooled buffer, and a direct-current electric field can be established across the membranes. The two membranes are kept parallel, 3 to 4 mm apart, while a third semipermeable membrane *B* is inserted between them part way into the cell. The cell is thus divided into three compartments, two at the top and a common one at the bottom. All three compartments have means for continuous input or withdrawal of the colloid solution.

If it is assumed that the solution to be fractionated contains two proteins, differing in their electrophoretic mobility or isoelectric points, then the *p*H of the solution and of the outside buffer is adjusted close to the isoelectric point of one of the proteins. The solution is continuously fed into one of the top compartments, and the polarity of the current is selected so that the other protein, the mobile one, migrates toward the outer membrane of the compartment, as indicated by the short arrows in Fig. 1. The rate of flow of the vertical column of liquid is adjusted so that the migrating protein can reach the outside membrane by the time the liquid containing it reaches the bottom of membrane *B*. This migrating protein will have formed a layer of increased concentration in the immediate neighborhood of the outer membrane, and will be withdrawn quantitatively in that part of the liquid which is collected through the bottom compartment. The other protein, at, or close to the isoelectric point, will not have been influenced by the electric field, and will therefore be uniformly distributed throughout the liquid. It can therefore be withdrawn from the other top compartment.

The inflowing liquid is thus separated into two fractions. The bottom fraction contains all the migrating proteins, but

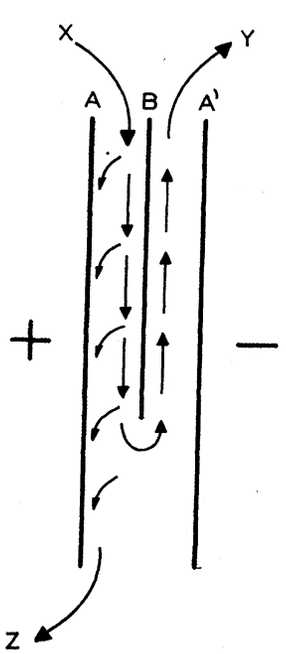

Fig. 1. Continuous free-boundary flow-electrophoresis apparatus. *A, A',* outer membranes; *B,* intermediate membrane; *X,* input of solutions; *Y, Z,* withdrawal of solutions.

is still contaminated with the isoelectric protein in its original concentration. The second fraction, obtained at the top of the cell, contains only the isoelectric protein, in a state of electrophoretic purity, with all the mobile protein eliminated.

The method has the added advantage that the rates of withdrawal of the solutions from the top and bottom of the apparatus can be controlled independently, the flow through the top outlet being adjusted, for example, to 4 times the flow through the bottom outlet. Accordingly, in a single circulation of the solution through the apparatus, it is possible to obtain 80 percent of the isoelectric protein in a state of electrophoretic purity at its original concentration, while all of the mobile component is obtained concentrated fivefold, admixed with only 20 percent of the initial amount of the isoelectric protein. The ratio of the two outflows can be varied to an even greater extent, or the bottom fraction can be recirculated, if greater recovery of the isoelectric component is desired. The method can thus be used either for the preparation of electrophoretically pure proteins or for the concentration of protein solutions, as may be desirable, for example, in highly dilute enzymatically active metabolic filtrates.

The rate of flow obtainable in a given system will depend on the electrophoretic mobility of the mobile components —the greater the mobility, the higher the rate. It will also depend on the intensity of the electric field applied, which is limited only by the total power that the cooling system will dissipate. With a constant-amperage input, little is gained by changing the cross-sectional area of the cell, and cells have been used whose cross-sectional areas varied from 125 to 13 cm^2, the latter being preferred at present (*6*). For a given cell and constant power, the field strength increases inversely with the ionic strength of the buffer. This makes buffers of low ionic strength preferable.

Up to the present, the various cells have been employed mainly on two systems. One application was the concentration of hemoglobin solutions, this being a particularly convenient system, as the concentration can be determined colorimetrically. Rates of flow up to 4 ml/min were obtained, using an input of 2 amp and a sodium barbiturate buffer, 0.02*M*, at *p*H 8.6. The other application was the isolation of γ-globulins from half-diluted human plasma. The same buffer was employed, and comparable rates of flow yielded an 80 percent recovery of electrophoretically pure γ-globulins in a single passage through the cell.

The flow rate of 4 ml/min obtainable with the apparatus is of the same order of magnitude as the per day output of some of the hanging-curtain paper electrophoresis instruments employed at present. It can also be favorably contrasted with the electrophoresis-convection method, which yields about 100 ml per batch, the processing of which may require 24 hours or more. The output of the present method can also be conveniently multiplied by arranging a series of cells in parallel. The power consumption for such an arrangement would not be considerably increased, while the output would be multiplied by the number of cells used. Accordingly, flow rates can be achieved which may be of importance for the industrial preparation of electrophoretically pure proteins (*7*).

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Mechanism of the Erythropoietic Effect of Cobalt

It has long been known that cobalt ion increases the rate of erythropoiesis in man and in experimental animals and will eventually produce and maintain a polycythemia (*1*). More recent work (*2*) has demonstrated that the earlier suggestion that cobalt exerted its effect by making the bone marrow anoxic (*3*) is untenable.

With increasing attention being paid to the role of the plasma factor, erythropoietin, in the control of erythropoiesis (*4*), and with the availability of simple, short assays for this hormone (*5, 6*), we have investigated the possibility that cobalt may be effective through erythropoietin production. The data presented in this report indicate strongly that cobalt enhances red-cell production by increasing the formation of erythropoietin.

Male Sprague-Dawley rats (400 to 450 g) were given subcutaneous injections of $CoCl_2$ (3 ml containing 75 μmole plus 2 μc of Co^{60}). Ten hours later, the animals were bled by cardiac puncture, and enough blood was withdrawn so that a minimum of 20 ml of "cobalt plasma" was available for assay. The amount of cobalt remaining in the plasma, determined from the Co^{60} content, was 0.21 μmole/ml. The cobalt plasma was assayed by the Fe^{59} incorporation method in starved rats (*7*) using normal plasma with 0.21 μmole/ml of $CoCl_2$ as the control. The incorporation of Fe^{59} into the red blood cells of rats that had been treated with these preparations of plasma was compared with that of rats that had been treated with plasma obtained from animals made anemic by the injection of phenylhydrazine.

After 1 day of starvation, rats were given two intravenous injections of 2 ml each at 1-day intervals while starvation was continued. On the fourth day of starvation, 1 μc of Fe^{59} citrate was injected into the tail vein, and 18 hours later, 1 ml of blood was removed and counted in a well-type scintillation counter. The percentage incorporation of the injected radioiron was then calculated as described previously (*5*). Average values for four experimental groups of five or more animals are as follows: cobalt plasma (containing 0.21 μmole of Co^{++} per milliliter), 13.9 percent (± 2.6); normal plasma (with 0.21 μmole of Co^{++} per milliliter added), 3.1 percent (± 0.6); anemic plasma, 15.1 percent (± 3.3); and normal plasma, 2.8 percent (± 0.9).

The pronounced increase in the incorporation of Fe^{59} that was produced by the cobalt plasma cannot be the result of the presence of a small amount of cobaltous ion but can be interpreted as being the result of the presence of an increased amount of erythropoietin in the plasma. It is possible, however, that a form of cobalt other than cobaltous ion is responsible for the observed effect. When erythropoietin has been characterized more fully, it will be possible to determine whether cobalt plasma contains the factor identical with that found in anemic plasma.

We have accumulated some evidence showing that certain of the properties of erythropoietin in anemic plasma are also common to those of the active factor in cobalt plasma. The erythropoietic activity of both anemic and cobalt plasmas is retained in the soluble fraction after either denaturation of the proteins at 100°C at *p*H 5.5 (*8*) or precipitation of the bulk of the plasma protein with 5 percent perchloric acid. Although there have been reports (*9*) that erythropoietin is not heat-stable, in our hands the loss of activity with this procedure is appreciable but not complete. The heat-stable, acid-soluble activity of both types of plasma may be dialyzed without marked loss of erythropoietin titer.