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*).

M. BIER

Department of Organic Chemistry and Enzymology, Fordham University, New York

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15 March 1957

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.

While these parallelisms are not conclusive evidence that the material in cobalt plasma is identical with that in anemic plasma, they suggest that both types of plasma contain erythropoietic factors with grossly similar properties (*10*).

EUGENE GOLDWASSER
LEON O. JACOBSON
WALTER FRIED, LOUIS PLZAK
Argonne Cancer Research Hospital and *Departments of Biochemistry* and *Medicine, University of Chicago, Chicago, Illinois*

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5 March 1957

Control of Arrested Fruit Growth in Tomato by Gibberellins

In recent months, several reports have shown that the gibberellins, when applied to higher plants, are effective in promoting the elongation both of dwarf mutants of corn (*1*) and of normal plants of several additional species (*2*). The gibberellins also promote the expansion of etiolated leaves (*3*), reverse the red-light-induced inhibition of pea internode growth (*4*), break the dormancy of red-light-requiring lettuce seed (*5*), and effectively substitute for the cold (*6*) requirement of biennial *Hyoscyamus*.

The afore-mentioned results suggested that the gibberellins might also be effective in alleviating the condition of arrested fruit growth and development (which is essentially a condition of dormancy) in commercial tomatoes. This condition is particularly characteristic of the fruits of the Marglobe variety grown in the field under the high light and temperature of summer or in the greenhouse in early fall at College Station, Tex. (*7*). The condition of "summer dormancy" in tomato in essence amounts to a very marked reduction of growth of both vegetative and reproductive structures. The leaves fold inward toward the petiole; the whole petiole and attached leaflets then fold upward toward the stem, and the internodes of the stem become progressively shorter as the season proceeds. Even though some viable pollen may be produced and fertilization may occur, the fruits remain very small (0.5 to 2.0 mm in diameter) until some external factor breaks their "dormancy." It has been shown that this summer-induced dormancy of tomato fruits can be caused by far red irradiation in the winter (*8*) and that cool temperatures, auxins, or red light are capable of reversing it. However, none of these methods serves as a practical means of control in the field.

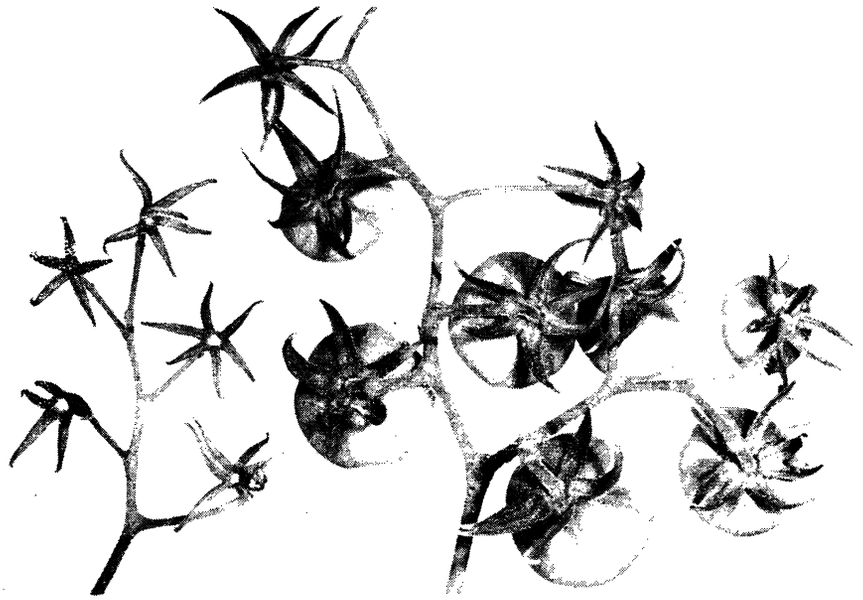

Fig. 1. (Left) Marglobe tomato fruit spur with small, dormant, pollinated fruits. (Right) Spur with enlarging fruits following five applications of a 25-μg/lit gibberellic acid spray to the sepals.

Seedlings of Marglobe tomatoes were potted in individual containers on 15 Aug. 1956 and treated in October and November. The green sepals of the dormant fruits were sprayed until run-off on alternate days for a total of five sprayings with water or gibberellins (*9*) at 25 or 250 μg/lit. The sprayed fruits, approximately 2 mm in diameter before spraying, were allowed to develop for 15 days following the initial spraying before the experiment was terminated.

Both 25 and 250 μg of gibberellin per liter produced an appreciable number of enlarged fruits as compared with controls. Thus, of the 121 fruits on control plants, only nine (7 percent) had broken dormancy and grown to at least 5-mm diameter, whereas 63 of 135 (46 percent) and 30 of 128 (23 percent) of those treated with 25 and 250 μg/lit, respectively, had increased to this size. The higher concentration appeared to be slightly toxic, for a number of young dormant fruits turned brown after application of the spray; this condition was not apparent in either the controls or at the 25 μg/lit level. Figure 1 shows the striking effect of gibberellins on the development of dormant tomatoes.

These results are not in agreement with the negative results on normally developing fruits reported by Marth *et al.* (*2*). However, it is possible that there is no basis for comparing dormant fruit and normally developing fruit. The mechanism of action of the gibberellins in this system is not known. On the basis of experiments with leaf disks conducted in this laboratory (*3*), it would not seem that the gibberellins replace the red light. This is more unlikely when it is considered that auxins and cool weather also break the dormancy of tomato fruits. A more plausible explanation would seem to be that all these factors affect the same biochemical pathway, but at different reaction steps or in a different manner. A full understanding of the action of the gibberellins in this system as well as in other systems reported must await further experimentation (*10*).

J. L. LIVERMAN
S. P. JOHNSON
Departments of Biochemistry and Nutrition and *Plant Physiology and Pathology, Texas Agricultural and Mechanical College System, College Station, Texas*

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