The common nickel used in this experiment was prepared from nickel sheet metal. A spectrographic analysis of this material gave the following approximate composition: nickel > 99%; cobalt < 1%; iron < 1%; manganese < 0.1%; copper < 0.1%; chromium, Trace. A spectrographic analysis of the nickel–dimethylglyoxime complex obtained indicated no impurities except a trace of iron, estimated at less than 10^{-6} gram atom.

Further study of the purification of macro-quantities of nickel by this general method elicited the following facts, all of which are based on a three-liter reaction mixture 50% in alcohol which was refluxed in a five-liter round-bottom flask.

Solvent.—Isopropyl alcohol appeared to be preferable to ethanol as a solvent for the dimethylglyoxime reagent. The higher boiling point of isopropyl alcohol permits more rapid decomposition of urea. Further, while severe "bumping" was encountered when ethanol solutions were used, this problem did not arise to any appreciable degree with isopropyl alcohol.

Purification with Dimethylglyoxime Solutions.—The maximum amount of nickel which can be precipitated by the use of equal volumes of nickel solution and an alcoholic solution of dimethylglyoxime is a function of the solubility of the dimethylglyoxime and the capacity of the reaction vessel. For a five-liter flask, this is of the order of 10 g. of nickel. Under these conditions, the precipitation of nickel is complete at a *p*H of 4.4.

Purification with Solid Dimethylglyoxime.—The reagent may be added as the solid slurried in alcohol. In this case the maximum amount of nickel which can be treated in a vessel of given size is primarily a function of the volume of the nickel complex. For three liters of 50% isopropyl alcohol in a five-liter flask the maximum permissible amount of nickel is more than 40 g. If solid dimethylglyoxime is used in an amount sufficient to keep the reaction mixture always saturated with this reagent, the reaction is complete at about *p*H 2.4. Under these conditions the nickel complex is usually contaminated with a small amount of dimethylglyoxime even after extensive washing with 50% alcohol (ethyl or isopropyl). The dimethylglyoxime can be removed readily by drying the precipitate at 130 to 150°.

Initial *p*H.—If the *p*H of the reaction mixture is sufficiently low at the start of the reaction, true precipitation from homogeneous solution is obtained, that is to say, there is no precipitation of the nickel complex whatever until an appreciable decomposition of the urea has been effected. Using dimethylglyoxime in isopropyl alcohol solution, this *p*H is approximately 2. For solid dimethylglyoxime, this *p*H is about 0.8. Under these conditions the precipitate is highly crystalline, dark and compact. If the initial *p*H of the reaction mixture is slightly higher, some precipitation occurs as soon as the dimethylglyoxime reagent is added. This can be termed "pseudo" precipitation from homogeneous solution. In this case the precipitate is slightly less crystalline, somewhat lighter in color, and slightly more voluminous.

Reflux Time.—The period of time that the reaction mixture must be refluxed to obtain complete precipitation of the nickel is a function of the initial *p*H, the amount of urea used, and the quantity of nickel to be complexed. In a typical separation using approximately ten grams of nickel, an initial *p*H of 2.0, 300 g. of urea, and a total volume of three liters, a *p*H of 6.2 was reached after refluxing for approximately five hours.

Purification of Active Nickel.—The principal radioactive contaminants present in the active nickel sample were known to be Co-58 and Co-60, in millicurie amounts, and microcurie quantities of Mn-54 and Fe-55. In addition, small amounts of Cr-51 and Fe-59 were present. Stable iron also was present in weighable quantities.

The active nickel was dissolved in dilute nitric acid, and iron was removed at low *p*H as the hydrous ferric oxide.

The nickel solution was diluted to approximately 1200 ml. and 100 mg. each of Co(II), Mn(II) and Cr(III) carriers were added. The *p*H was then reduced to 0.4 by the addition of concentrated hydrochloric acid, and the solution was transferred to a five-liter round-bottom flask. A slurry of 226 g. of dimethylglyoxime in 1500 ml. of isopropyl alcohol was then added, followed by 500 g. of urea slurried in 300 ml. of water. The mixture was refluxed at the boiling point until samples of the supernate did not give a test for nickel upon the addition of ammonium hydroxide. The mixture was cooled, filtered with suction through a medium porosity sintered glass funnel, and washed with 1500 ml. of 50% isopropyl alcohol. The washed precipitate occupied a volume of approximately 300 ml. It was dissolved in concentrated nitric acid, the resulting solution was diluted with water, filtered to remove dimethylglyoxime and oxidation and nitration products thereof, fresh carriers added, and the nickel reprecipitated with dimethylglyoxime as indicated above. This procedure was carried out a total of six times. The final precipitate was dried at 140° to remove excess dimethylglyoxime, and was then converted to nickel oxide.

The only active γ-emitting impurity detectable in the final sample was less than four counts per minute (1.5×10^{-9} curie) of Co-60 as measured with a γ-scintillation spectrometer capable of detecting as little as 4.8×10^{-10} curie of this species and comparable amounts of other γ-activities. This represents over-all purification against Co-60 by a factor of approximately 10^7.

The necessity for the large number of reprecipitations of the nickel complex carried out is attributed more to an inability to wash the large quantities of complex efficiently than to any serious retention of active species by the complex itself.

The fact that the dimethylglyoxime separation method described above was used successfully for the purification of an unusually large quantity of active nickel would appear to recommend this general scheme for use with the much smaller quantities more commonly encountered in radiochemical separations. The use of solid dimethylglyoxime would appear to be especially advantageous since thereby one can obtain complete precipitation of the nickel complex at a *p*H so low that carrying of other active cations can, in general, be expected to be minimized.

The authors are indebted to Mr. Bruce Murray for spectrographic analyses of nickel samples, and to Mr. W. F. Harris and Dr. T. R. Sweet for making available their unpublished data on the precipitation of nickel from homogeneous solution.

It is a pleasure to express our appreciation to the Ohio State University Development Fund for grants for research instruments.

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The Single Sucrose Gradient as a Convection Barrier During Descending Boundary Preparative Electrophoresis[1]

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RECEIVED MAY 12, 1954

Boundary electrophoresis as a productive preparative method has been hampered by the occurrence of convection, often long before maximum purity and yield have been attained. When the faster boundaries migrate into the bottom section of the electrophoresis cell, convection due to gravitational instability causes mixing of the descending and ascending solutions. Indistinguishable boundaries then make the isolation of highly purified fractions impossible. The onset of this convection may be postponed by compensating the descending

(1) Supported by grant 228A from the Damon Runyon Memorial Fund for Cancer Research, and an institutional grant from the American Cancer Society.

boundaries upward until the ascending salt or slow protein boundaries enter the bottom section. This situation has been described by Longsworth.[2]

This communication presents a modification of descending boundary preparative electrophoresis which in this Laboratory has routinely permitted the isolation of the slowest migrating proteins in greater percentage yield and potential purity than has been possible by the conventional boundary method. Involved is the interposition of a single, large, steep gradient of an electrically neutral substance (*e.g.*, sucrose) between the bottom section and the fraction to be isolated. This density gradient effectively prevents the passage of convective currents originating from the bottom section, thereby allowing electrophoresis of much longer duration.

The isolation of conalbumin 1[3] (Cl) from chicken egg white serves to compare this procedure with the conventional descending boundary method. Egg whites were diluted with an additional one-third of their volume using sodium phosphate, *p*H 7.8, 0.1 *M* phosphate, and stirred in the cold until a homogenous solution was obtained. Following centrifugation at 15,000 × *g* for ten minutes, aliquots of the clear supernatant fluid were stored at −17° until needed.

For electrophoresis, samples of the diluted egg white were dialyzed against sodium phosphate buffer, *p*H 5.8, 0.1 *μ*. Longsworth, Cannan and McInnes[4] have reported that unfractionated conalbumin 1 in egg white is isoelectric in this medium. For maximum yield and purification, it is desirable to have conalbumin 1 electrophoretically stationary and all other proteins similarly charged (anionic). After dialysis, the precipitates of ovomucin were removed by centrifugation at 15,000 × *g* for five min., and the supernatant solutions, at a protein concentration of 7.4%, were subjected to electrophoresis.

Preparative runs were performed in the 11-ml. cell with divided center section using the Klett electrophoresis apparatus[5] at a potential gradient of 6.3 volts/cm. The new method also has been used in conjunction with the "75" ml. preparative cell for the isolation of larger quantities than here reported.

In control runs, the cell was filled in the conventional manner with protein solution throughout the bottom and descending sections. However, in the modified method, the dialyzed buffered solution of egg white proteins was placed in the descending limb only (Fig. 1). A 0.564 *M* sucrose solution in sodium phosphate buffer, 0.1 *μ*, at *p*H 5.80, was placed throughout the bottom section, ascending limb and entire anode buffer vessel except the saturated sodium chloride electrode solution. (Omission of sucrose in the anode buffer vessel eventually causes a continuous decline in the current, presumably caused by the rarefaction of protein ions, due to mobility differences across the buffered sucrose-plain buffer boundary.) All other characteristics of the control and experimental runs were maintained the same. Compensation at the start of electrophoresis was avoided in order to prevent disruption of the sucrose–protein gradient (at the bottom of the descending limb) caused by its entrance into the bottom section.

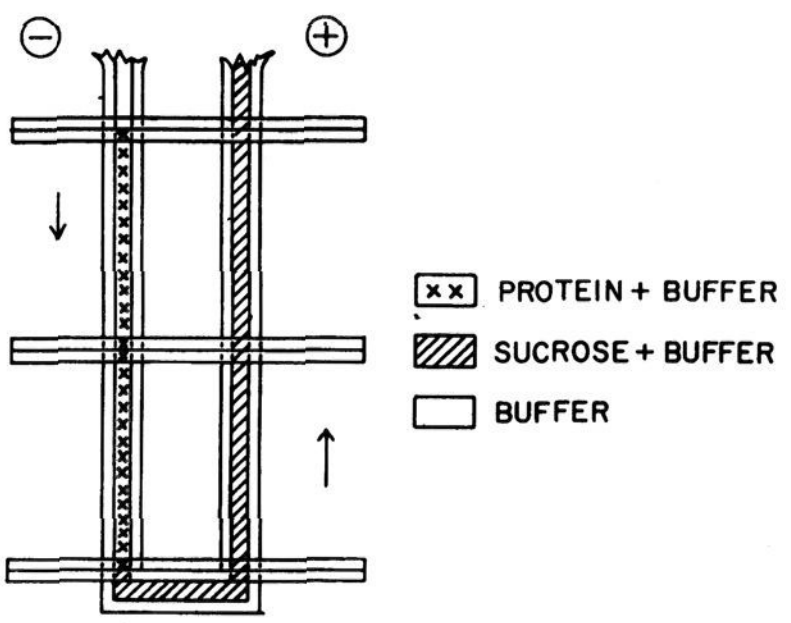

Fig. 1.—Diagrammatic representation of the electrophoretic cell with single sucrose gradient as a convection barrier.

Figure 2 shows the convection in the descending limb which originates from the bottom section in the control run. The increasing number of convection spikes with time is typical of the mixing caused by the entrance of faster descending boundaries into the bottom section. Convection was observed in the descending limb after 530 minutes of electrophoresis (Fig. 2a). Even before 1288 minutes all the descending gradients other than the trailing portion of conalbumin had been so disrupted that detection of the positions of the former boundaries was impossible (Fig. 2b).

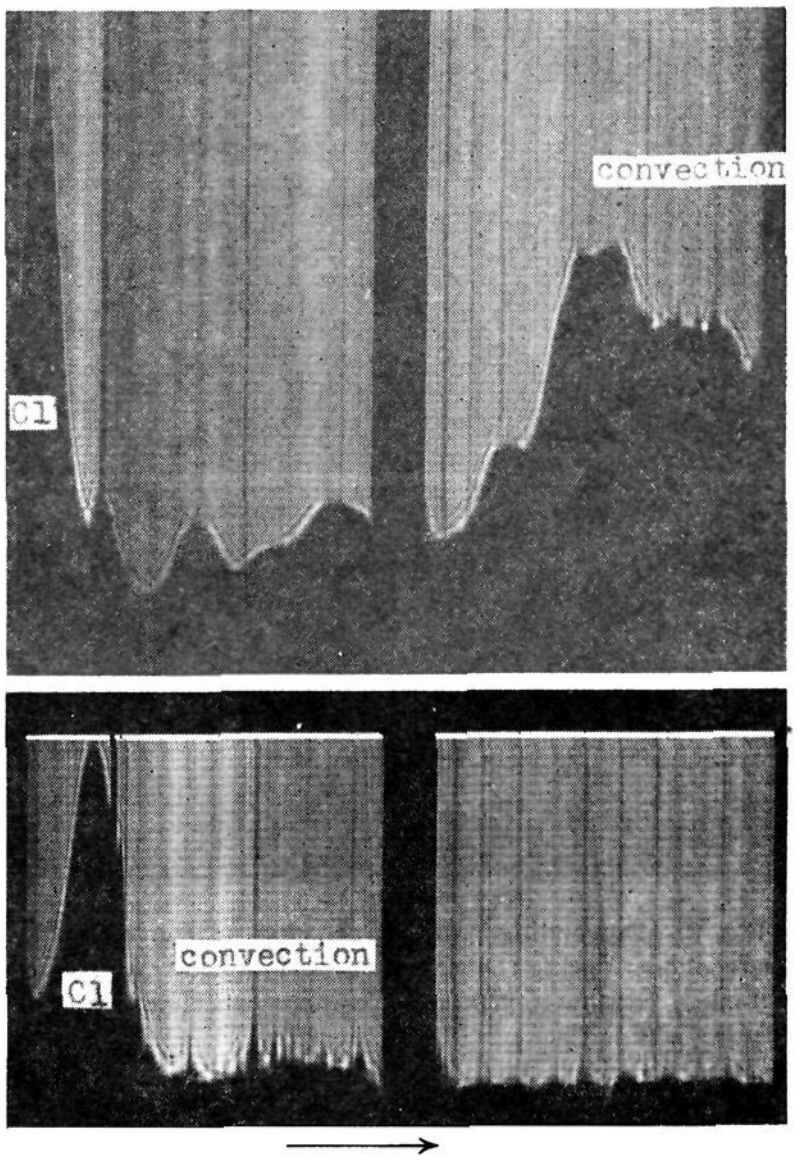

Fig. 2.—Descending patterns showing progressive convection in the conventional type of electrophoresis (without sucrose gradient) at: (a) upper, 530 min.; (b) lower, 1288 min.

However, in the experimental runs using sucrose, no convection spikes in the descending limb were

(2) L. G. Longsworth in A. C. Concoran, Ed., "Methods in Medical Research," Vol. 5, The Year Book Publishers, Inc., Chicago, Ill., 1952, p. 100.

(3) Nomenclature of Longsworth, Cannan and MacInnes.[4]

(4) L. G. Longsworth, R. K. Cannan and D. A. MacInnes, THIS JOURNAL, **62**, 2580 (1940).

(5) Manufactured by Klett Manufacturing Co., New York, N. Y.

observed even after 2706 minutes of electrophoresis[6] (Fig. 3). Hence, it was possible to run the electrophoresis from two to five times longer in time without convection, with a corresponding increased resolution and multiplication of yield. Throughout the experimental runs, the sucrose–protein boundary near the bottom of the descending limb was observed to broaden upwards. Apparently, this is caused by diffusion and the electrophoretic "pile-up" arising from the decrease of the mobilities of the descending anions advancing into the sucrose boundary. In practice, the "pile-up" entering the solution to be isolated limits the duration of the run and the electrophoretic resolution attainable. However, by initially increasing the distance between the sucrose–protein gradient and the starting descending protein boundary, as is possible in the larger preparative cells, this limit in time and resolution may be still further greatly extended.

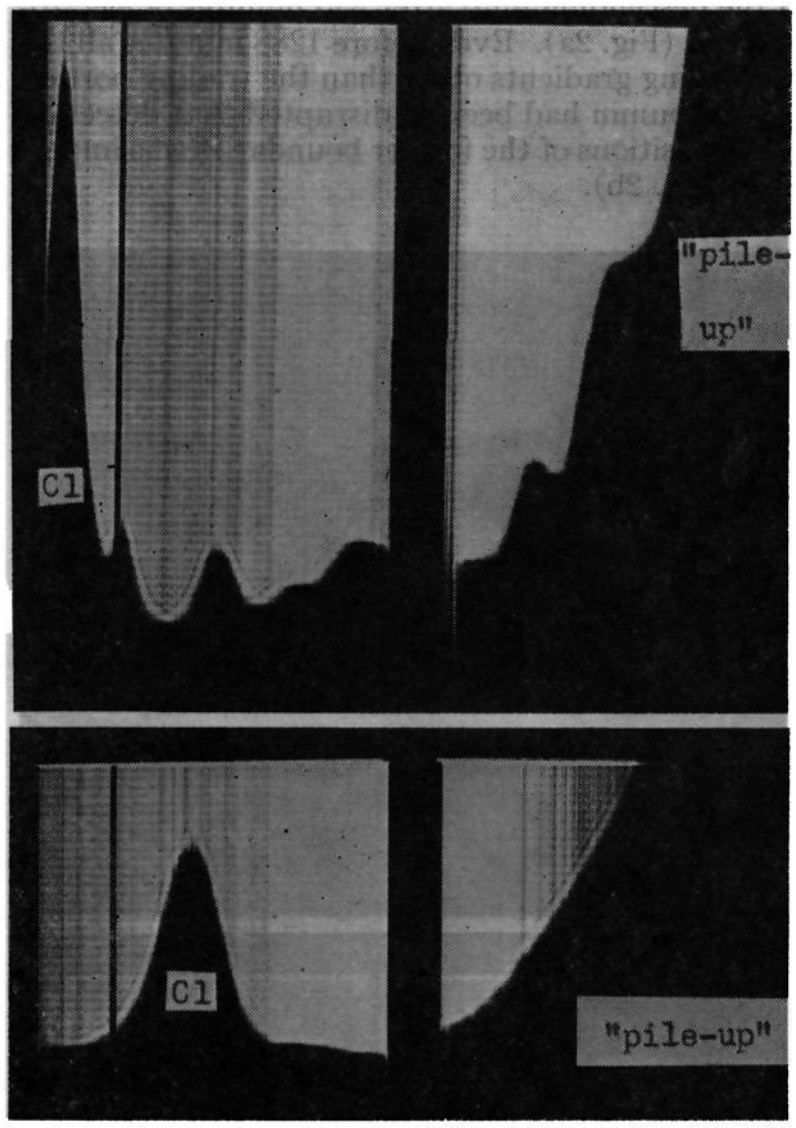

Fig. 3.—Descending patterns showing no convection in electrophoresis using the single sucrose gradient: (a) upper, 530 min.; (b) lower, 2706 min. (compensated boundaries as in text).

At the time of maximum resolution and yield (2706 minutes), the boundaries of the sucrose-containing runs were compensated to exclude all but the lagging portion of the slowest protein (conalbumin 1) from the top half section of the descending limb (Fig. 3b). This isolated solution had a volume of 3.2 ml. and a protein concentration of 0.6%.

In order to test the purity of the isolated conalbumin 1, the removed solution was dialyzed at the same concentration against sodium phosphate buffer, 0.1 μ, pH 5.80 and analyzed in the 2-ml. open cell of the Perkin–Elmer electrophoresis apparatus, Model 38.[7] The isolated conalbumin 1 exhibited an average mobility of -0.24×10^{-5} cm.^{2}/volt/sec. (*cf.* reported values[4,8]), and appeared electrophoretically homogeneous under these conditions (Fig. 4).

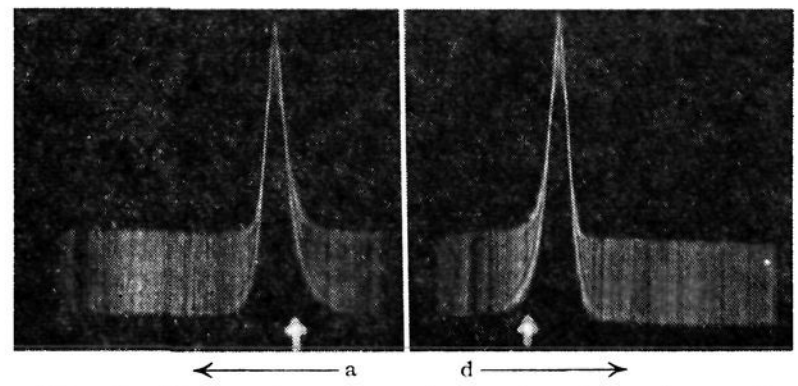

Fig. 4.—Electrophoretic analysis after 240 min. at 6.4 volts/cm. of conalbumin 1 isolated by preparative single sucrose gradient electrophoresis. Vertical arrows indicate starting boundaries.

An interesting example of the role of a large, steep gradient was encountered during the electrophoretic isolation of γ-globulin from human serum, where the β-anomaly spike acted as an effective convection barrier.

In summary, a modified method of descending boundary preparative electrophoresis has been presented. Involved is the use of a single, large, steep sucrose gradient as a barrier to the convection arising from the entrance of descending protein boundaries into the bottom section of the electrophoresis cell. The procedure permits substantially increased electrophoretic resolution, with corresponding gains in the yield and potential purity of the isolated fraction.[9]

(6) In order to avoid the entrance of sodium chloride from the electrodes into the cell, it was necessary at 1400 minutes to move temporarily the cell out of alignment and to refresh the solutions in both buffer vessels.

(7) Manufactured by Perkin-Elmer Corp., Norwich, Conn.

(8) J. A. Bain and H. F. Deutsch, *J. Biol. Chem.*, **172**, 547 (1948).

(9) Recent experiments have attested to the analytical value of the new method. The prolonged duration of the electrophoresis of mixtures thus made possible permits the discovery of slow components which are unresolved by conventional electrophoresis.

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Purification and Manipulation of Samples of Alkali Metals[1]

BY GEORGE W. WATT AND DONALD M. SOWARDS

RECEIVED APRIL 26, 1954

Frequent need for the preparation of a considerable number of standard solutions of alkali metals (or alkali metal amides) in liquid ammonia has led to the development of a simplified procedure for the simultaneous preparation of several samples of twice distilled metal contained in fragile Pyrex glass ampoules. The method is simple, rapid and

(1) This work was done in connection with studies supported in part by the Atomic Energy Commission, Contract AT-(40-1)-1639.