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Army Medical Service Field Research Lab., Fort Knox, Ky.
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Continuous Electrophoresis and Ionophoresis on Filter Paper

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20 November 1950

CONTINUOUS ELECTROPHORESIS AND IONOPHORESIS
ON FILTER PAPER*

*Subproject under Studies of Body Reactions and Requirements under
Varied Environmental and Climatic Conditions. Approved 31 May 1946.
AMSFRL Project No. 6-64-12-06-(39).

CONTINUOUS ELECTROPHORESIS AND IONOPHORESIS
ON FILTER PAPER*

by

E. L. Durrum, Major, M.C.

from

Army Medical Service Field Research Laboratory
Fort Knox, Kentucky
20 November 1950

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Varied Environmental and Climatic Conditions. Approved 31 May 1946.
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ABSTRACT

CONTINUOUS ELECTROPHORESIS AND IONOPHORESIS ON FILTER PAPER

OBJECT

It was the object of this investigation to improve the continuous electrophoresis method which has recently been reported by Svensson and Brattsten in order to make it more convenient and generally applicable to separations of minute amounts of biological materials and to make it more easily controllable.

RESULTS

This objective has been achieved by modifying the method of Svensson and Brattsten in light of experience obtained in the investigation entitled, "A Microelectrophoretic and Microionophoretic Technique," (Project No. 6-64-12-06, Sub-project MDFRL 06-(18)) and the investigation entitled, "Two-Dimensional Microelectrophoresis and Microionophoresis," (Project No. 6-64-12-06, Sub-Project MDFRL 06-(38)). Filter paper curtains have been substituted for the lucite box packed with glass powder utilized by Svensson and Brattsten. Capillary forces have been utilized to obtain even constant feed of both background electrolyte and the material being separated. Cells which are suitable for carrying out this process are conveniently made of lucite or other plastic.

This apparatus, in common with the apparatus developed by Svensson and Brattsten permits application of an electrical field at right angles to the flow of fluid downward by gravitational forces. The application of the electrical field causes deviation of charged components passing down the paper to a degree depending upon their rate of flow down the paper curtain and their lateral mobility under the conditions of separation (pH, ionic strength and magnitude of applied electrical field, etc.). A serrated bottom edge of the paper affords "drip points," permitting the collection of various fractions.

The course of separation can be followed by any suitable means of analysis of the fractions collected. Sometimes a convenient method of locating fractions which contain desired components is to remove the filter paper curtain after the run and treat it by some means to follow the paths through which the ions have migrated (for example, in the case of amino acids, by spraying with ninhydrin).

CONCLUSIONS

The method here reported affords sharp separations and as a result of the simplifications permitted by utilizing capillary forces for feeding has proved to be convenient and easily operated even by unskilled personnel with minimum attention.

It therefore promises to have wide applicability, since it has been found that many types of charged substances, including amino acids, peptides, serum proteins, and inorganic ions may be easily separated.

RECOMMENDATIONS

None.

Submitted by:

E. L. Durrum, Maj., M.C.

Approved:

Ray C. Daggs
RAY C. DAGGS
Director of Research

Approved:

Frederick J. Knoblauch
FREDERICK J. KNOBLAUCH
Colonel, M.C.
Commanding.

CONTINUOUS ELECTROPHORESIS AND IONOPHORESIS ON FILTER PAPER

I. INTRODUCTION

Svensson and Brattsten (1) have recently reported a method for carrying out electrophoresis continuously. Their apparatus consisted of a lucite box packed with glass powder as an anticonvection agent. At the lateral edges of the box, vertical filter paper diaphragms separated the glass powder from electrode compartments in such a manner that an electrical potential could be applied at right angles to the downward flow of fluid which was admitted continuously along the top of the glass powder through 24 individually controlled glass tubes. The mixture comprising ionic species of different mobility which was to be separated, was continuously fed through a single tube at some intermediate position at the top. Thus, as the fluid flowed down the box through the glass powder, ions were deviated from their initial horizontal position more or less laterally at some constant angle, depending upon their particular mobility under the conditions of the experiment.

Twenty-eight collecting tubes, distributed in a horizontal line along the bottom of the box permitted various fractions to be withdrawn. The method was shown to be suitable for the separation of a variety of charged substances.

Although this method is admirable in its ingenuity, as a practical matter it demands very careful adjustment and constant attention in order to obtain satisfactory separations. It is the purpose of this report to describe simplifications and modifications of this technique which substantially increase the utility of the method and permit separations to be performed without constant attention.

II. METHOD AND APPARATUS

Svensson and Brattsten (1) recognized that filter paper might be useful for developing a smaller scale preparative apparatus according to their principles and suggested in light of the present author's demonstration that amino acids and proteins could be separated by ionophoresis or electrophoresis on filter paper strips clamped between glass strips (2, 3); that such an apparatus could be made by clamping filter paper sheets between glass plates. Strain (4) has recently made an apparatus according to this principle with which he has successfully separated various inorganic ions.

In the development of the Svensson and Brattsten method, early experiments "were carried out in filter papers hanging over the edge of a container," but they abandoned this procedure "because of difficulties with evaporation and because greater capacity was desired." The fact that experience in this laboratory had shown that sometimes sharper separations can be obtained on paper hanging freely in a vapor space than on paper which is supported on one or both sides by glass strips prompted a reinvestigation of this type operation. There follows a description of an apparatus in which separations according to this principle have been carried out successfully.

Figure 1 illustrates the basic principle of the method. A filter paper sheet is shown hanging from the edge of an electrolyte reservoir. The sheet is cut in such a manner that its lower edge affords tabs which dip into the electrolyte vessels (a detailed diagram of the sheets used in these experiments is shown in Figure 2). Between the electrolyte tabs, the edge is serrated to afford "drip points." At some position at the top of the filter paper curtain, a small tab is produced by making two parallel vertical cuts and one horizontal cut to free the top and bending the tab forward. The mixture to be separated is continuously fed to this tab by means of a filter paper wick. Generally the wick is made of the same filter paper as the filter paper curtain. Background electrolyte continuously "siphons" into the paper from the electrolyte reservoir and passes down the paper. Along with it passes the mixture being separated (in the absence of an applied electrical potential as a narrow band). If an electrical potential is applied across the filter paper curtain, the various mobility species are deviated toward the anode or the cathode as the case may be in accordance with their inherent mobility relationships.

Figure 1 shows the separation of a hypothetical mixture comprising four distinct mobility species designated I, II, III, and IV. It is evident that the experimental arrangement shown permits collection of the separated materials from the various "drip points." Of course, it is necessary, in order to obtain optimum results with this method, to maintain a steady state, that is, it is important to feed electrolyte at a constant rate and the mixture being separated at a constant rate, to maintain the electrical field constant and to maintain the temperature reasonably constant. To afford this, it is necessary to add certain refinements to the basic principle illustrated in Figure 1.

These include provisions for the maintenance of the fluid levels in the electrolyte reservoir and electrode vessels. This is conveniently accomplished by providing overflow tubes in these vessels and by continuously feeding the background electrolyte to the electrolyte reservoir at a rate slightly in excess of that rate at which it is "siphoned" out by the filter paper curtain. It is convenient from a mechanical point of view to permit the excess electrolyte in the reservoir to overflow and pass by gravity down to one of the electrode vessels.

A second major consideration requires that the rate of evaporation on the paper curtain which is augmented by heating incidental to the flow of electric current be controlled in such a manner that a steady state with respect to this factor be maintained also.

This has been effected by isolating the filter paper curtain from the atmosphere by a cover made of lucite. The cover is designed with a "peaked roof" so that any condensed electrolyte will not drip from the cover onto the paper but will flow down the walls of the cover. An added refinement has been the provision of gutters on the inside of the cover to lead any condensate into the electrode vessels from which it can overflow. By taking these precautions, no difficulty is experienced in obtaining an over-all steady state (equilibrium) even when distillation from the paper takes place at a rapid rate.

In general, however, it is best to choose operating conditions which will result in low distillation or evaporation rates except when volatile electrolyte systems are employed. (For example, aqueous solutions of acetic acid.)

Figure 3 consists of photographs of different views of the cell which has been developed for continuous paper electrophoresis and which permits two filter paper sheets to be run simultaneously. The various parts are labeled in Figure 3B. The cell is made of $1/4$ " and $1/8$ " Lucite cemented with "H-94 cement" and consists of 4 separate parts. These comprise a base and lateral wall section A, a removable electrolyte trough B, a removable shelf C, and an enclosing cover D. The base section is made to contain electrode vessels E and E' which are integral parts thereof. Each electrode vessel is provided with overflow tubes F and F' in order to maintain a constant level therein. Electrolyte overflow is collected in beakers G and G'. Platinum foil electrodes H and H' are shown in the electrode vessels. The platinum wire electrode lead is admitted to the electrode vessel through a small hole above the electrolyte level through the lateral wall of the base section. The electrode vessels are separated by a base plate carrying 2 parallel rows of $5/8$ " holes. These provide a place of exit for the "drip points" (in a more recent model cell, this construction has been somewhat simplified by replacing these holes with 2 parallel narrow slots). A special Lucite test tube rack is shown carrying 14 test tubes in appropriate relationship to the 14 "drip point" exit holes. The lateral walls are further supported by a bracing member I and carry cleats for the support of the electrolyte trough.

The electrolyte trough B has been made removable for convenience in rinsing. It is a simple V trough through one side of which a $5/8$ " hole has been drilled to carry the bent glass overflow tube J which is admitted to the trough through a rubber stopper. The overflow tube maintains a constant level in the trough by virtue of the condition that electrolyte is fed at a rate slightly in excess of the rate at which it is "siphoned" from the trough by the filter paper. The electrolyte is fed to the trough through an adjustable tubing clamp from an overhead siphon bottle (not shown) through a 90° glass jet K carried by a rubber stopper passing through a hole in the lateral wall of the base section. The overflow electrolyte passes downward to electrode vessel E.

A narrow Lucite shelf C is provided for support of the vessel (e.g., watch glass, etc.) containing material to be separated and rests on the end sections of the electrolyte trough. A hole is drilled in one end of the shelf to permit direct entry of the electrolyte to the trough from the jet K.

The cell is completed by a cover section which carries a "peaked roof" so that any condensate will flow down the walls and will not drip onto the paper. Gutters M and M' which are made of small strips of Lucite cemented on the inside of the cover D lead any condensate laterally into the electrode vessels, thus preventing collection of condensate on the bottom base plate where it might dilute the various fractions being collected. The cover is so designed that it can be removed without disconnecting the electrode wires or electrolyte supply tube. This is convenient since it permits photographs to be made directly of the paper during the course of separation (e.g., see Figure 4).

The apparatus is placed on a leveling table N which permits the edges of the trough to be leveled so that flow of electrolyte is uniform along the entire width of the paper.

III. EXPERIMENTAL

The following experiments were all carried out in cells of the type illustrated in Figure 3 and the filter paper sheets were all cut exactly as indicated in Figure 2. The other experimental conditions are enumerated in the various experiments.

Experiment 1: Figure 4 is a photograph taken during the course of a separation of a commercial preparation of acid fuchsin (Coleman and Bell). The acid fuchsin was dissolved in N/4 acetic acid to result in a concentration of 4 mg./ml. This solution was fed by a 1 mm. wide filter paper wick to a ~~tab~~ located 10 cm. from the cathode edge of a sheet of S & S 598 filter paper. The background electrolyte was N/4 acetic acid. A potential of 800 volts was applied across the platinum foil electrodes. After about 1 hour, a steady state had been established and a current of 4 milliamperes was observed to flow constantly throughout the remainder of the experiment. After the potential had been applied for 20 hours, the cover of the cell was removed and the photograph (Figure 4) was taken. When the sheet was inspected under visible light, 7 distinct zones corresponding to various mobility species present in the acid fuchsin are evident. (Acid fuchsin is known to be a mixture of polysulfonic acids of pararosaniline.) The zones were distributed as follows: component 1 migrating into the cathode compartment; component 2 being withdrawn from drip points 2 and 3; component 3 being withdrawn from drip point 7; component 4 being withdrawn from drip point 8; component 5 being withdrawn from drip points 10 and 11; component 6 being withdrawn from drip points 13 and 14; component 7 migrating into the anode compartment. The sheet was removed from the apparatus and dried in the oven at 110°C. for 10 minutes, supported on a glass drying rack. Inspection of the dried sheet under an ultraviolet lamp showed the presence of 3 additional fluorescent zones. The first of these was of blue fluorescence and located lateral to component 1 and accompanied it into the cathode vessel. The second zone was of yellowish fluorescence and was located medial to component 1 and also accompanied it into the cathode vessel. The third zone was of a more marked degree of blue fluorescence and was withdrawn from drip points 5 and 6.

Although the various mobility species were not further identified, this experiment clearly showed that at least 10 distinct mobility species are present and perhaps some of these fractions could be further resolved under other experimental conditions.

The dye acid fuchsin has proved to be very useful as a test substance during the developmental phases of the present technique because most of the individual mobility species are not adsorbed on filter paper in acid electrolyte solutions.

Because considerable difficulty was encountered in previous investigations (2, 3) when attempts were made to separate ions which are strongly adsorbed on filter paper it was thought worthwhile to investigate whether strong adsorption would interfere with the present technique. The following

experiment was carried out with acridine orange to test the applicability of the method in the case of strongly adsorbed ions.

Experiment 2: Figure 5 is a photograph of a curtain resulting from a separation of a commercial preparation of acridine orange (purchased from Eimer and Amend, N.Y.C.). The acridine orange was dissolved in N/4 acetic acid to result in a concentration of approximately 1 mg./ml.

The dye solution was fed by a 1 mm. wide filter paper wick to a tab located 6 mm. from the anode edge of a sheet of S & S 598 filter paper. The background electrolyte was N/4 acetic acid. A potential of 1,000 volts was applied across the platinum electrodes. After a steady state had been established, a current of 6.6 milliamperes was observed to flow constantly throughout the remainder of the experiment. The photograph was taken after the current had been applied for 96 hours.

This preparation of acridine orange was observed to contain a non-fluorescent red impurity which was even more strongly adsorbed than was the acridine orange. During the course of the experiment, a period of almost 24 hours was required for the acridine orange zone to extend down to the bottom of the paper (drip points 6 and 7). However, during this period, the red impurity had progressed only about one-third of the way down the sheet. At the end of 96 hours, the red impurity was just beginning to reach drip points 5 and 6. The significance of this experiment will be discussed hereinafter.

Experiment 3: A test mixture comprising 9 amino acids in equimolecular proportions (arginine, glycine, alanine, valine, serine, threonine, phenylalanine, proline and aspartic acid) was prepared by dissolving them in distilled water with the aid of a few drops of ammonium hydroxide to result in a concentration of 0.04M. each. This mixture was fed by a 1 mm. wide filter paper wick to a tab located 3 cm. from the anode edge of a sheet of Whatman No. 3 MM filter paper. The background electrolyte was 1 N. acetic acid. A potential of 900 volts was applied across the platinum foil electrodes. After about two hours a steady state had been established and a current of 8 milliamperes was observed to flow. Fractions were collected from various drip points for a period of approximately 16 hours after which time the filter paper curtain was removed, dried in an oven and sprayed with ninhydrin to develop color in the usual manner.

Figure 6 is a photograph of the resulting sheet. Examination of collected fractions after concentration by the technique of unidimensional paper ionophoresis as described by the author (2) established that the material migrating into the cathode vessel was arginine. The material collected from drip point 3 and 4 consisted of glycine with traces of alanine while the material removed from drip points 5 and 6 consisted of alanine with traces of glycine. Although direct visual inspection of the ninhydrin-developed sheet showed more distinct zoning in the areas feeding drip points 7-11 than is evident in the photograph, more pronounced overlapping of the remaining components was observed in this section although neither glycine nor alanine was detected in these zones. The material collected from drip point 7 consisted of valine, serine and threonine while the fraction collected from drip point 8 contained principally

threonine and phenylalanine with lesser amounts of serine and proline. The fraction collected from drip point 9 consisted of phenylalanine and proline while the fraction collected from drip point 10 consisted of proline and aspartic acid. The fraction collected from drip point 11 consisted of aspartic acid with only traces of proline.

Experiment 4: This experiment illustrates the separation of a serum protein mixture. When a normal human serum sample is separated by this method the zones corresponding to albumin and gamma globulin are most prominent (because of their greater concentration) on the resulting patterns prepared by the coagulation and dyeing technique described by the author (2). Because it was considered desirable to illustrate that serum protein fractions of intermediate mobility are separated as well as the fractions of the extremes of the mobility range of serum proteins, a test mixture containing more than the normal concentration of these intermediate components was prepared by mixing 1 ml. of each of two abnormal serum samples and adding 1/2 ml. of a commercial preparation of human "immune globulin." This mixture was dialyzed against 500 ml. barbiturate buffer pH 8.58 (0.025 M. in sodium diethyl barbiturate and 0.005 M. in diethyl barbituric acid) for 48 hours at 1°C. Some coprecipitation of proteins occurred during dialysis, therefore, the mixture was centrifuged and the supernatant taken as the test mixture for separation in the following manner:

This mixture was fed by a 1 mm. wide filter paper wick to a tap located in the center of a sheet of Whatman No. 2 filter paper. The composition of the background electrolyte was the same as used for dialysis with respect to barbiturate but had been prepared with 10% by volume glycerol to increase its viscosity. A potential of 600 volts was applied across the platinum foil electrodes and after two hours when a steady state had been achieved, a current of 3.7 milliamperes was observed to flow. After 6 hours the sheet was removed, dried in the oven and dyed with bromphenol blue by the author's method (2).

Figure 7 is a photograph of the significant portion of the resulting sheet. Inspection of this figure reveals four distinct colored zones. An examination of the fractions collected at the drip points (after concentration by partially lyophilizing these fractions) by unidimensional paper electrophoresis by the author's method (2) showed that the fractions collected at drip points 6 and 7 consisted of gamma globulin. Drip point 8 gave a mixture of beta with traces of gamma globulin. Drip point 9 provided a mixture of beta and alpha₂ globulins. Drip point 10 provided a mixture of alpha₂ and alpha₁ globulins and albumin. Drip point 11 yielded a mixture of alpha₁ globulin and albumin.

IV. DISCUSSION

Svensson and Brattsten (1) have given a theoretical treatment for separations in systems without evaporation and with uniformly distributed electrical fields. Theoretical treatment of the present system is made more complex by two conditions which are present. These are:

1. The electrical field distribution is not uniform owing to the configuration of the paper curtains. (It increases in strength as the bottom of the paper curtain is approached.)

2. The electrolyte concentration (except in the special case of azeotropic mixtures) is continuously increased as the bottom edge of the paper curtain is approached as a result of distillation. A secondary factor is the uneven distribution of electrolyte on the curtain as a result of factors 1 and 2. In view of these difficulties, no rigorous theoretical treatment will be attempted. However, certain remarks with regard to practical aspects of the method are in order.

In general, allowing for the considerations mentioned above, the position on the filter paper curtain of a given ion at any instant will be the resultant of its rate of flow down the sheet due to gravitational and capillary forces and its lateral deviation due to its charge in the electrical field (mobility). Thus, in selecting proper experimental conditions to effect a proposed separation it is necessary to consider the subordinate factors which determine these two basic factors.

With regard to selecting the optimum field intensities for a given separation, several points must be taken into consideration. In general, for a given flow rate down the paper curtain, the higher the field intensities the better the resolution attainable, for a given set of experimental conditions. However, since heating is incidental to the passage of current, when separating labile substances (for example, protein mixtures) it may be desirable to employ low currents in order to permit separations to be effected at low temperature at some sacrifice of resolution.

The present method when compared to the method developed by Svensson and Brattsten (1) or by Strain (4) which are adapted to utilize conduction for cooling suffers them the disadvantage that the only processes available for cooling the filter paper curtain are radiation, convection, and evaporative cooling. However, the convenience of this method and the fact that it appears to afford higher degrees of resolution than systems in which the paper is supported on one or both sides by a glass or plastic sheets or cooled by an intermediate immiscible hydrocarbon phase (see footnote ^{***}) warrants its use in the separation of even labile mixtures if proper precautions are employed to prevent overheating. Of course for separations of stable materials the problem of cooling is of little practical importance under ordinary experimental conditions.

These heating effects mentioned above may be particularly troublesome where non-volatile background electrolytes are employed. When currents are too high, distillation may become too rapid and the water from the electrolyte may be evaporated so rapidly that the downward rate of flow is not sufficient to "wash the paper free" of the resulting concentrated electrolyte and in extreme cases electrolyte solids even may be deposited, thus destroying the necessary steady state. Nevertheless, under certain circumstances, it may be desirable to operate at currents just short of those causing deposition of solids since, thereby, more concentrated fractions are delivered from the "drip points." In other words, if materials being separated are stable the maximum permissible current is that value which is just short of that resulting in deposition of solids. Under these conditions, it is possible to maintain a steady state and useful separations can be effected.

In cases of separations where volatile background electrolytes are employed, generally higher currents may be tolerated providing components of the mixture being separated do not deposit as solids (and of course are stable).*

* An interesting phenomenon, which sometimes has been observed in these experiments, is a tendency for some electrolyte-paper systems when used in the cell described, to "automatically" adjust the electrical current flow to some fixed value.

This first was observed when a background electrolyte consisting of 1. N. acetic acid in 10 per cent ethylene glycol was studied as a medium for the separation of amino acid mixtures on S & S 598 paper. It was observed that when a potential of 1400 volts was applied that the initial current flow was approximately 8 MA.; however, very quickly the current fell to about 2 MA. and remained constant at this value. It was then determined empirically, by simply changing the applied potential, that there existed a maximum current of 4.2 MA. which could be steadily maintained; this current flowed when a potential of 800 volts was applied. At potentials above or below this value the maximum current which could be steadily maintained fell to lower values.

The explanation for this phenomenon is probably as follows: At a high potential the initial high current results in considerable heating with rapid distillation of the volatile electrolyte from the paper (particularly in the zone between the electrolyte and the base of the paper when the paper is cut as shown in Figure 2). As a result of this loss of electrolyte, the electrical resistance on the paper increases and less current can flow with less heating and less distillation, etc. Since electrolyte is continuously being "siphoned" into this zone from above, a dynamic equilibrium is established which determines and holds the current constant for a given set of experimental conditions.

It is obvious that this principle could be easily adapted to provide a simple method for automatically controlling electrical currents within certain ranges.

The current which flows for a given electrolyte is directly proportional to the applied voltage. However, this may not be a linear function in view of the factors considered above. As a practical matter, it has been found desirable to operate the apparatus described at constant voltage.**

Since electrolytes of low ionic strength permit higher intensity electrical fields to be applied with relatively low current flow, it is often desirable to operate at minimum permissible electrolyte concentrations (as dictated by other factors such as prevention of the precipitation of proteins, required pH, etc.). For the same reason it is desirable to avoid attempting to separate mixtures which are too concentrated. For example, although in unidimensional separations on paper (2,3), it is not necessary to dialyze serum proteins, better resolution of protein mixtures is attained in the present method when the mixture being separated is dialyzed against the relatively dilute barbiturate buffer prior to separation.

Several methods are available for regulating rate of flow down the paper. Many types of filter paper are available in various degrees of porosity and thickness and in general thick coarse papers afford more rapid flow (and poorer resolution) than thinner more compact papers. However, flow of background electrolyte down the paper may be controlled to some degree by adjusting the fluid level in the electrolyte reservoir with respect to the height of the upper edge of the sheet. Also, when it is desired to feed electrolyte to the paper at a rate less than the maximum for a given paper, it is possible to serrate the upper edge dipping into the electrolyte reservoir so that the flow is regulated by the depth to which the tips are immersed in the electrolyte. Sometimes a useful modification for locally controlling the flow rate down the paper is to apply added thicknesses of filter paper to the paper curtain where they adhere by capillary forces as shown in Figure 1 by the dotted lines in areas (A). This permits greater volume and rate of flow of electrolyte down the edges of the paper curtain than in the center. Thus, a rapidly migrating component which might otherwise migrate into one of the electrode compartments would, thereby, be caused to deviate into one of the lateral drip points. Also, this modification may permit higher currents to be employed since the local resistance at the electrode tabs is, thereby, reduced. The point of highest electrical resistance in the system is of course at that part of the paper between the fluid level in the electrolyte vessels and the base of the sheet. The lower dotted lines in Figure 1 encompass these areas (B) in which

** Consden, Gordon and Martin (Biochem. J. 40: 33 (1946)) have pointed out the desirability of utilizing well-filtered full wave rectified alternating current because it permits a greater field strength with less heating than a half wave rectified potential source. In this laboratory full wave rectified power supplies are fed from constant voltage regulators of either the saturable reactor type (e.g. General Electric Co. Voltage Stabilizer, Cat. No. 69 G 852) or the electronic type (e.g. Sorensen & Co. Model 1000 S), both of which have proved to be satisfactory.

it is sometimes desirable to add an extra thickness of filter paper when high currents are caused to flow since, thereby, local heating can be reduced.

Another experimental variable which has been utilized to advantage under some circumstances is the addition of an inert material to the background electrolyte in order to increase its viscosity for the dual purpose of reducing flow rate down the paper curtain and augmenting the anti-convection properties of the filter paper curtain. For example, both glycerol (see experiment 4) and propylene glycol have been successfully utilized in protein separations to reduce the flow rate down the paper curtain. These two substances have been found to be practically without effect upon the pH of many electrolyte systems in concentrations of the order of 10 to 20 per cent.***

Another advantage when utilizing these substances is that the vapor pressure of the electrolyte is reduced and, therefore, a given current results in less distillation from the filter paper curtain.

Factors which are more directly concerned with the amount of material which may be separated under a given set of circumstances in a given interval include the feed rate which is conveniently controlled by altering the width of the filter paper wick. The maximum permissible rate of course depends upon the degree of resolution sought and the thickness of the paper as well as the dimensions of the apparatus. There exists no theoretical reason why apparatuses of larger size could not be built which, under proper operating conditions, would of course give improved degrees of resolution and/or increased through-put.***

*** Abramson, H. A. (Science 110: 716 (1949)) has used glycerol in electrophoretic separations of proteins at room temperature.

**** Separations have been carried out in a cell similar in principle to that described herein but which was adapted to handle curtains of filter paper as large as 60 x 60 cm. The curtains were cut in this apparatus to provide 34 drip points.

Preliminary tests were carried out in another apparatus built according to the principle described herein but adapted to utilize an immiscible hydrocarbon phase (heptane) as a cooling and sealing medium about the filter paper curtain in a manner analogous to that developed by Prof. A. Tiselius and D. Campbell (personal communication) for cooling filter paper strips during electrophoresis. (See also Cremer & Tiselius, Biochem. Zeit. 320: 273 (1950)).

Preliminary results have been disappointing because of the observed tendency for applied test mixtures to spread at the paper electrolyte-hydrocarbon interface with comparatively poor resolution. It is believed that this phenomenon perhaps explains why our results with paper curtains supported by glass or plastic sheets gave relatively unfavorable degrees of resolution. It is possible that the employment of surface active agents will correct these difficulties. Further evaluation of this method is in progress.

Experiment 2 indicated that the method is also applicable to the separation of materials which are strongly adsorbed by filter paper. It is believed that the underlying mechanism peculiar to this type of operation which permits separation to occur, is the condition that the adsorbed ions are continuously fed to the paper and thus gradually "saturate" a path down which subsequently passing ions can proceed at such a rate as they are applied (after the entire path has become saturated). Of course this process is visualized as a dynamic equilibrium situation and the fundamental consideration governing it is the adsorption equilibrium between locally bound and unbound ions and is dependent upon well-known relationships. It is worth noting that under these circumstances an impurity present in even very minute concentration, if it were strongly bound, would build up a relatively large reservoir of bound ions on the filter paper before the entire path became saturated and thereafter would deliver to the drip point concerned excess ions at the relatively low rate determined by its initial concentration.

In this special case it might be desirable to cut up the sheet and remove the reservoir (migration path) of adsorbed ion from the sheet and separate it from the paper by some independent means.

V. CONCLUSIONS

The method here reported affords sharp separations and as a result of the simplifications permitted by utilizing capillary forces for feeding has proved to be convenient and easily operated even by unskilled personnel with minimum attention.

It therefore promises to have wide applicability, since it has been found that many types of charged substances, including amino acids, peptides, serum proteins and inorganic ions may be easily separated.

VI. RECOMMENDATIONS

None.

VII. BIBLIOGRAPHY

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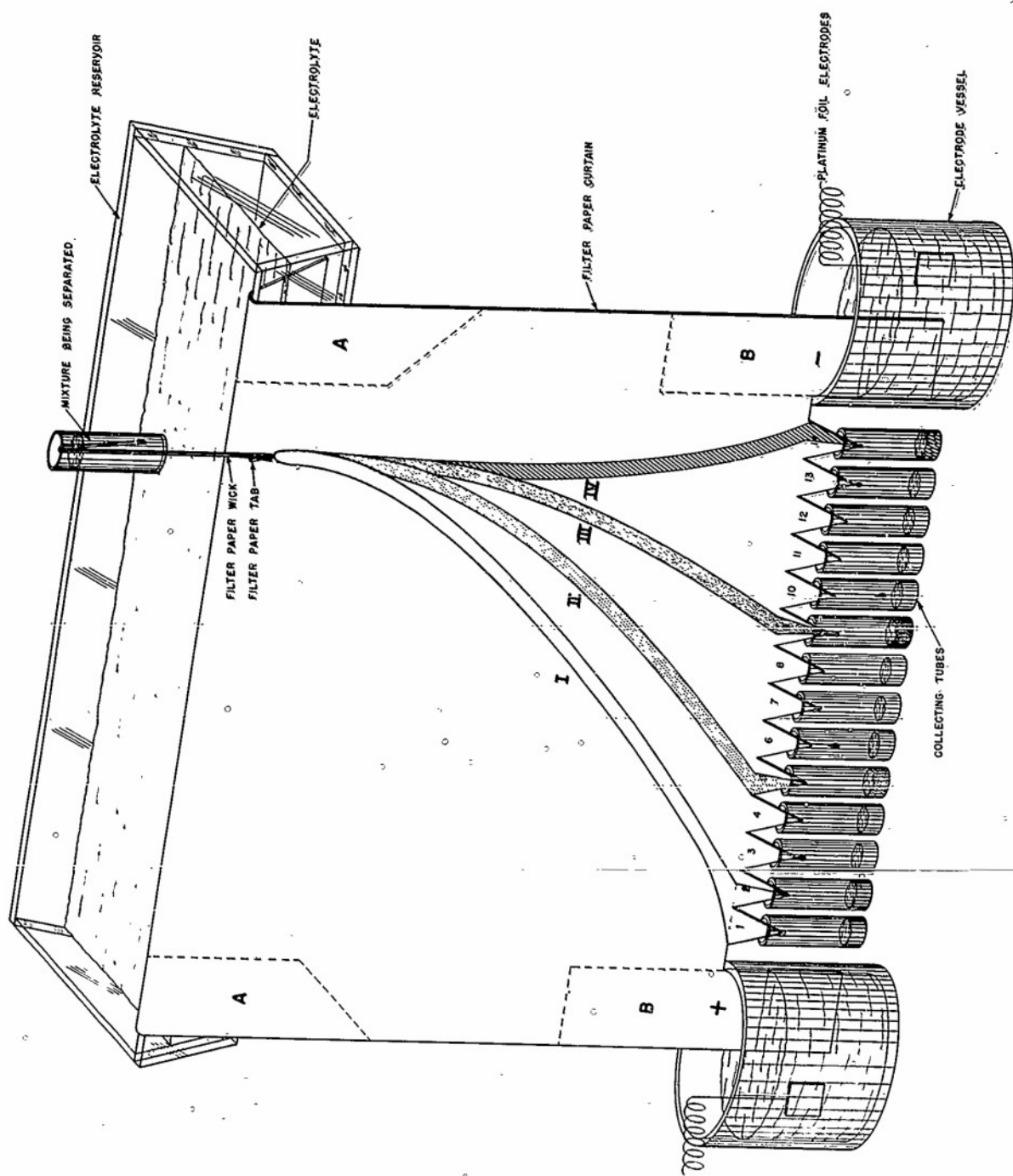


FIG. 1

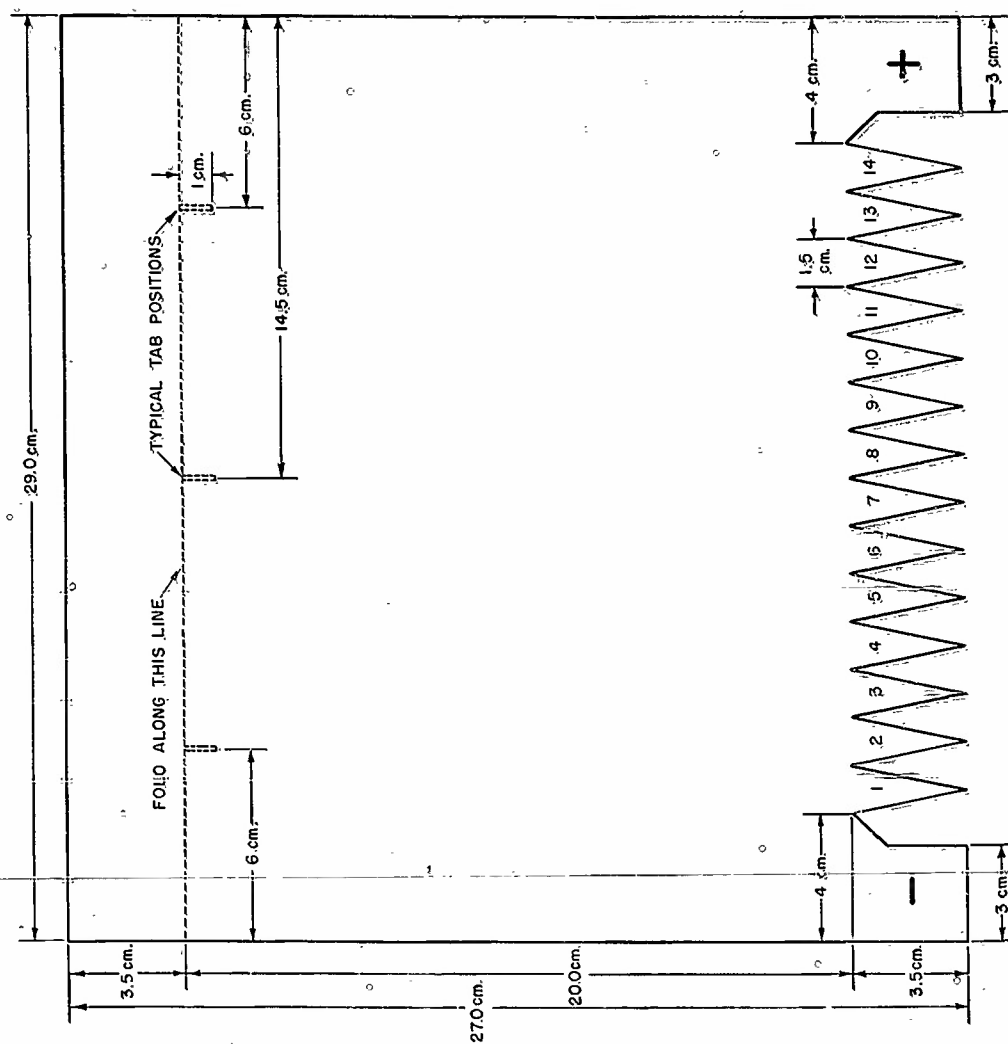
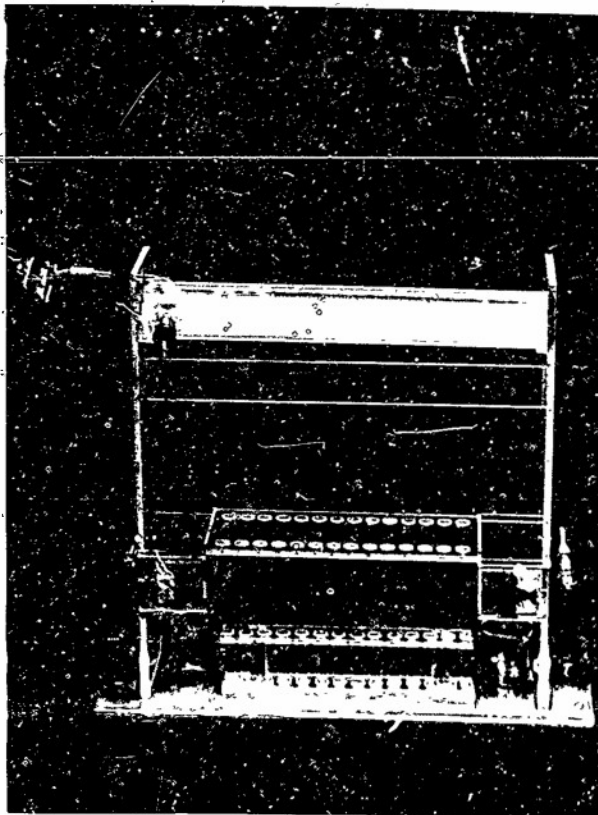
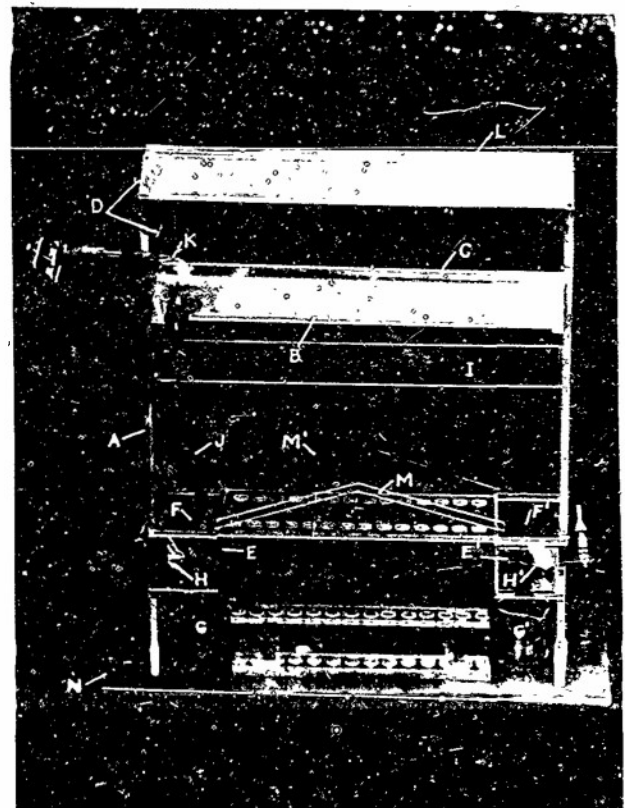


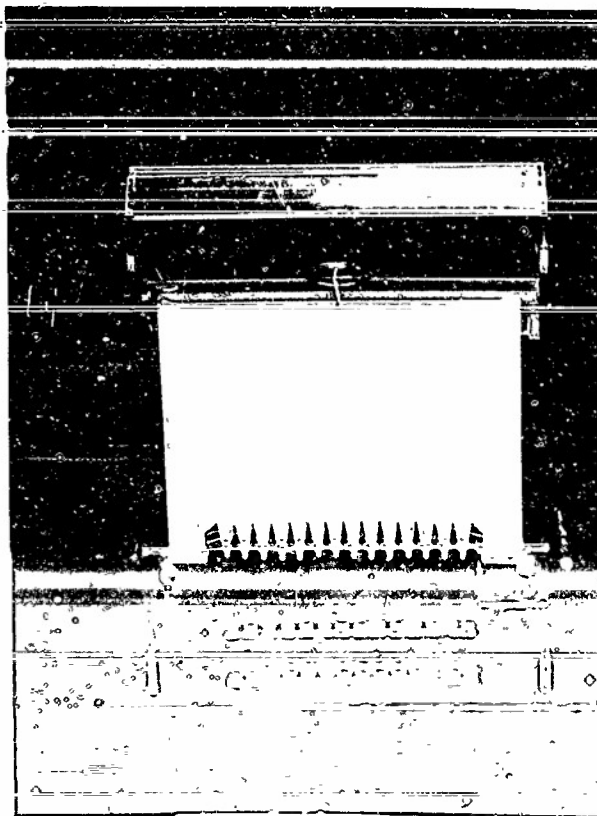
FIG. 2



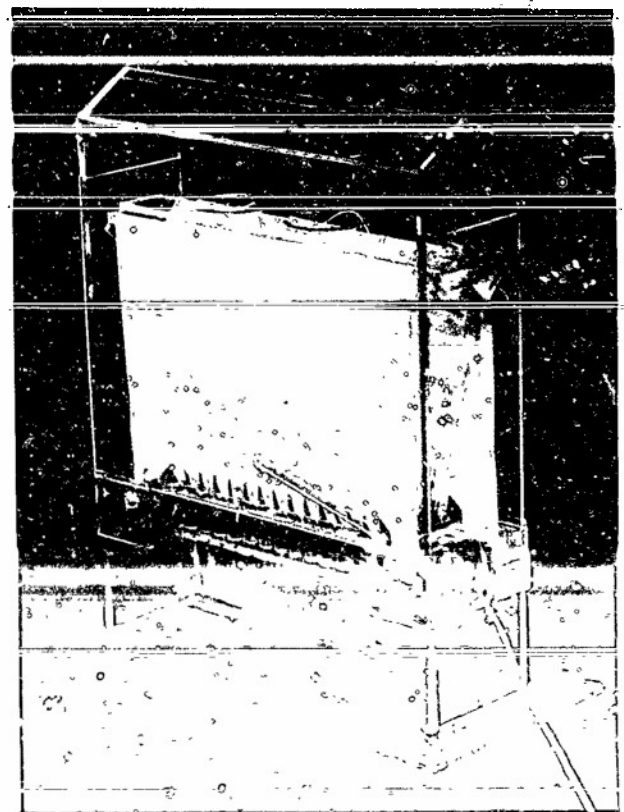
A



B



C



D

FIG. 3

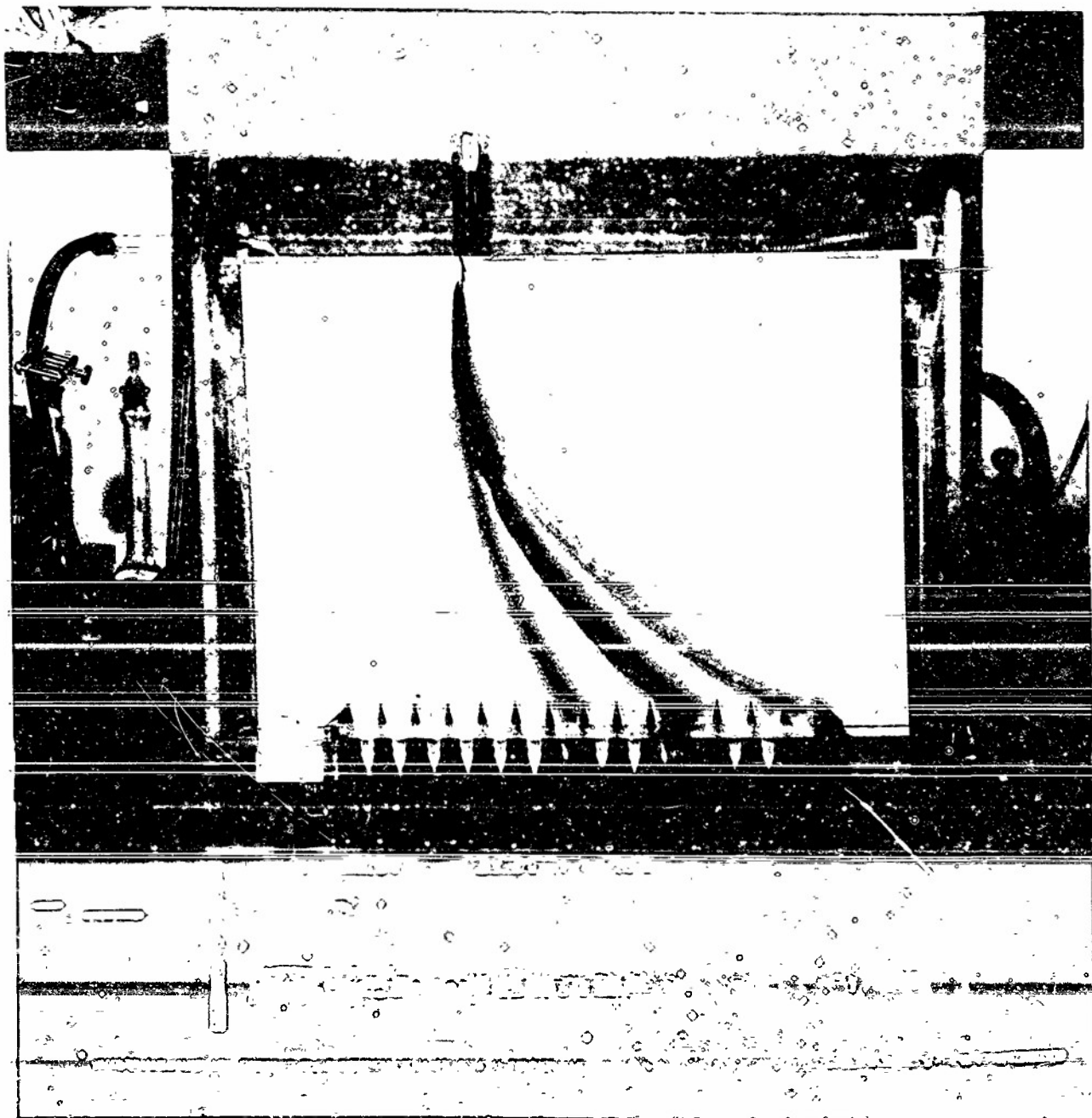


FIG. 4, EXPERIMENT 1

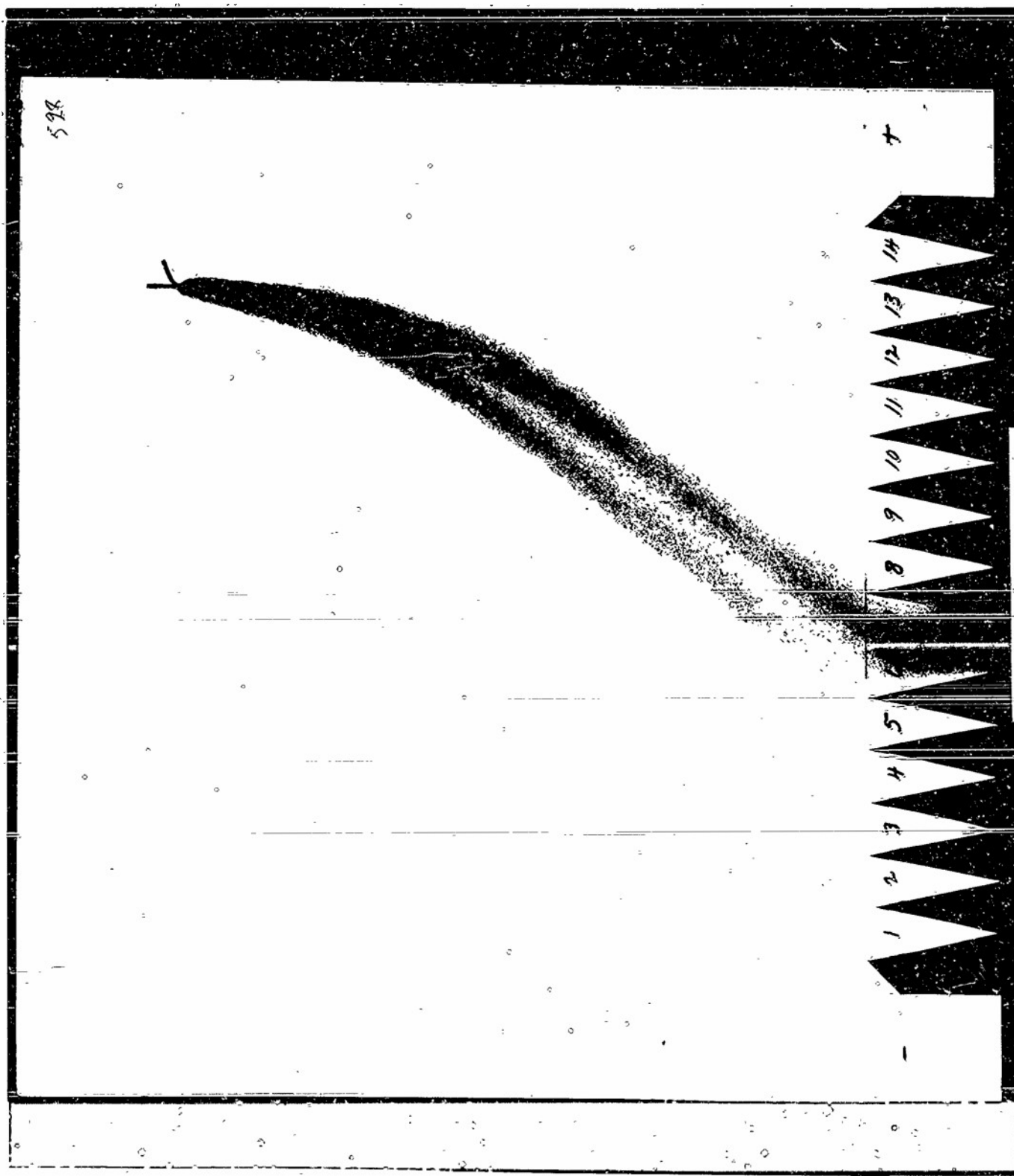


FIG. 5, EXPERIMENT 2



FIG. 6, EXPERIMENT 3



FIG. 7, EXPERIMENT 4