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Electrophoresis[1]

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The significance of proteins and proteids in physiological processes, in which they play a vital part both as enzymes and as substrates, has led to their increasing study in modern biochemical research. In keeping with their reactivity under physiological conditions, they possess, from the chemical point of view, an extremely pronounced sensitivity or lability, which must be carefully taken into account when they are being investigated or prepared in a pure state. Many of these substances are only stable in solutions of a definite hydrogen ion concentration and at low temperatures, and precipitation by increasing the salt concentration of the solution often alters the physical properties or causes even more fundamental changes.

It is, therefore, of very great importance that the investigator who enters the extensive field of the study of proteins and proteids to-day has at his disposal the analytical methods of physical chemistry. These are best employed in combination, and enable substances of high molecular weight to be characterized and their most important properties determined without risk of their being changed in the experiments. The most important of these methods are: the ultracentrifuge, which gives information regarding the weights of colloid particles; electrophoresis, which permits characterization according to the electric charge; diffusion measurements, which provide a second means of determining particle size; and the measurement of streaming birefringence from which important inferences regarding the form of the particles can be drawn. The especial significance of the first two methods is apparent from the fact that they can always be applied even to crude solutions or mixtures, from which they provide data regarding the individual components. The value of the two latter methods, on the other hand, lies rather in their application to the investigation of pure substances. The methods of the ultracentrifuge and of electrophoresis can therefore also be employed to govern and control the isolation and preparation of proteins and proteids, and in their combined application to such problems ample proof of their value has been given.

[1] Originally delivered by the author in the form of two lectures one on the 20th November 1946 (Naturforschende, Chemische und Medizinische Gesellschaft Basel) and the other on the 16th January 1947 (Chemische Gesellschaft, Biochemische Vereinigung und Ärzteverein Bern).

Thanks to the famous work of SVEDBERG, who has made possible the use of stronger and stronger gravitational fields, and of TISELIUS, who has adapted the electrical fields to the requirements of electrophoresis, these two methods, founded upon long-known principles, were developed to such an extent that further improvements could scarcely be envisaged. This pioneer work was not achieved by constructive development alone, but necessitated also the devising of the most sensitive optical methods and equipment for making visible and recording the experimental results, which can now be obtained with great precision on very small quantities of solution.

In the following, an attempt is made to describe briefly the methods and applications of electrophoresis, as well as to sum up the results of experience obtained in the scientific laboratory of Sandoz Ltd., Basle, which in 1940 was the first in Switzerland to make use of the method of electrophoresis.

The principle of electrophoresis[1] is based upon the movement of electrically charged particles in an electric field. Electrophoresis is an extension of iontophoresis in so far as it is no longer limited to ions, and particles in general are observed to move under the influence of an electric field provided they carry an electric charge. Perhaps this extension of iontophoresis was most beautifully demonstrated using hæmoglobin by PICTON and LINDER[2]. These workers gave the impetus to numerous further researches, especially on proteins, in the course of which HARDY, PAULI, MICHAELIS and other research workers furthermore observed that the direction and rate of travel of these substances were a function of the hydrogen ion concentration, at a certain value of which the velocity became zero. HARDY[3] gave the name "isoelectric point" to this *p*H-value at the isoelectric state.

From the experiments made at the turn of the century it was already evident that the behaviour of different proteins in an electric field differs greatly and is in this way characteristic. It was thus to be expected that a refinement of the method would make possible a much greater degree of characterization and separation of proteins and similar substances. Work in this

[1] The term is derived from ἤλεκτρον = amber and φέρειν = carry.
[2] H. PICTON and S. E. LINDER, J. Chem. Soc. *61*, 148 (1892).
[3] W. B. HARDY, J. Physiol. *24*, 288 (1899).

direction has been carried out by numerous investigators. It is only necessary to mention PAULI and LANDSTEINER[1], MICHAELIS[2], SVEDBERG and SCOTT[3], THEORELL[4] and especially TISELIUS[5], whose experiments published in the years 1937/38 led to an electrophoresis apparatus of which important components (the cell and the schlieren optical observation equipment) are still in general use to-day.

As stationary conditions are best for measuring the migration of electrically charged particles, the attempt has always been made not only to maintain a constant current in the cell, but also to stabilize all the exterior factors of the electrophoresis experiments.

In this case, the velocity v, with which a (supposedly spherical) particle moves, is given, as in the case of iontophoresis, by the formula

$$v = \frac{eH}{6\pi r \eta}, \tag{1}$$

diminished by a factor which may be calculated from the interaction of the charges on the colloid particles with those of the electrolytic ions present, according to the theory of GUY[6].

Electrophoresis may therefore be defined as a process in which the velocity of the migrating particles is found to be smaller than, or at the most as great as, that in iontophoresis. The effect of the electrolyte ions present does not depend on their chemical nature but principally on their ionic strength[7] μ, where

$$\mu = \frac{1}{2}\sum c_i z_i^2. \tag{2}$$

The effect of the moving particles depends, in the first instance, on their charge which may be considered as a function of the hydrogen ion concentration. The interaction of the electrostatic forces between these particles reduces the mobility $u(v)$ which would be calculated for an independent charged particle in a liquid of infinite volume from the formula

$$u(v) = \frac{QD}{KT}. \tag{3}$$

The observed (apparent) mobility $u(v)$ is therefore given by

$$u(v) = u - u'. \tag{4}$$

The method of calculating the term u' may be found in the relevent literature[1].

Under stationary experimental conditions the apparent mobility $u(v)$, which is expressed as the quotient of the velocity of migration (cm/sec) and the field-strength H (V/cm), is given by the equation

$$u(v) = \frac{q\,s\,\varkappa}{It}, \tag{5}$$

where u is the apparent anodic mobility,
v the apparent cathodic mobility,
q the cross-sectional area of solution in cm^2,
s the distance traversed by the particle in cm,
$\varkappa$ the specific conductivity of the solution in $\Omega^{-1}cm^{-1}$,
I the strength of the current in ampères, and
t the time in seconds.

Of these data, q is known, while s, I and t are determined experimentally; the conductivity $\varkappa$ of the solution under examination must be determined separately at the temperature at which the electrophoresis experiment is made.

In conformity with their definition, electrophoretic mobilities may also be calculated from the velocity of migration W (in cm/sec) divided by the potential difference (in V/cm) across the solution under test. Although the determination of this value is not usually possible without difficulty, it may nevertheless be worth while to undertake it when making a series of experiments which may then be evaluated without the additional measurement of the conductivity.

The values of the anodic or cathodic mobilities must be stated together with the following data:

(1) the temperature at which the experiment was conducted,

(2) the hydrogen ion concentration (pH-value), and

(3) the ionic strength μ of the solution under test, since each of these factors influences the conductivity $\varkappa$ of the test solution on which the mobility depends.

According to SVEDBERG and TISELIUS[2], the behaviour of a substance in an electrophoresis experiment may therefore be characterized, for practical purposes, by the gradient of the mobility curve at the isoelectric point, i.e. by the value $\frac{du(v)}{dp\mathrm{H}_0}$ (at a given temperature). This value can generally be ascertained quite well from a number of experiments conducted under suitable conditions.

Until about 1937, the apparent mobility $u(v)$ was the only value capable of being determined. However,

[1] Wo. PAULI and K. LANDSTEINER, Verh. 25. Kongr. inn. Med. 57 (1908).

[2] L. MICHAELIS, Bio. Z. *16*, 81 (1909).

[3] Th. SVEDBERG and N. D. SCOTT, Am. Chem. Soc. *46*, 2700 (1924).

[4] H. THEORELL, Bio. Z. *275*, 1 (1934); *278*, 291 (1935).

[5] A. TISELIUS, Trans. Faraday Soc. *33*, 524 (1937); Svensk Kem. Tidskr. *50*, 58 (1938); Koll. Z. *83*, 129 (1938); The Harvey Lectures *35*, 37 (1939/40).

[6] G.-L. GUY, J. Phys. *9*, 457 (1910). – M. H. GORIN, J. Chem. Phys. *7*, 405 (1939).

[7] G. N. LEWIS and M. RANDALL, Am. Chem. Soc. *43*, 1112 (1921).

[1] Cf. e.g. H. A. ABRAMSON, L. S. MOYER, and M. H. GORIN, Electrophoresis of Proteins. Reinhold Publ. Corp., New York 1942. A survey of the theory of electrophoresis can also be found e.g. in the chapter "Elektrophorese" (E. WIEDEMANN) of the book: F. WUHRMANN and CH. WUNDERLY, Die Bluteiweißkörper des Menschen. Benno Schwabe, Basel 1947.

[2] Cf. Th. SVEDBERG and KAI O. PEDERSEN, Die Ultrazentrifuge, Tab. 48, p. 368 seqq. Steinkopff, Dresden und Leipzig 1940.

the introduction of the optical methods of observation and recording employed to-day has added the determination of further values, namely the relative, and also the absolute, quantities of the migrating substances, the significance of which is becoming increasingly great. More will be said regarding this determination during the description of the optical methods.

For several years, the form of the U-tube of W. NERNST, suggested by TISELIUS[1], has been used for carrying out electrophoresis experiments. The following diagram (Fig. 1) demonstrates the building-up of such a U-tube from plane parallel glass plates. The rectangular cross-section thus produced facilitates the removal of Joules heat during the experiment and, at the same time, permits the formation of an optically perfect image of the gradients formed within the

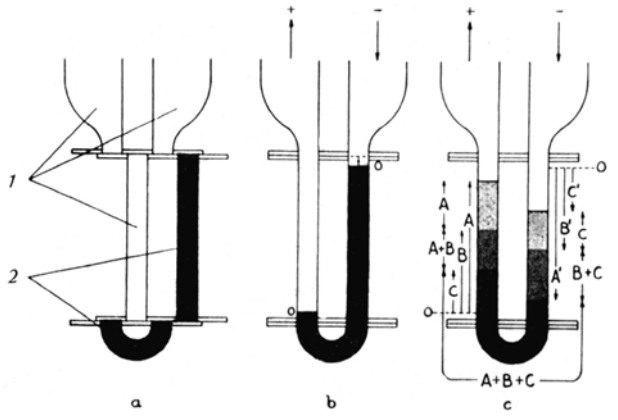

Fig. 1. Schematic representation of the migration of three components in the same direction in an electric field.

1 Buffer solution
2 Protein solution (Same buffer solution, containing 1-2% of proteins)
a Cell filled and closed
b Cell opened, interfaces produced, potential difference set up
c Effect of the electric field after a time *t*

cell which is necessary for the observation and correct recording of the experiments. The horizontal partitions are important not only for filling the apparatus and for removing samples, but they provide also the best means known so far of employing the interface or boundary observation method, which should be used wherever possible on account of its great efficiency.

Fig. 1 also shows how the TISELIUS U-tube is used. After filling the lower segment and one limb with buffer solution containing the dissolved test substances, the cell is closed by pushing to one side the middle section, and is then filled up with pure buffer solution. By careful opening of the cell, the interfaces (boundaries) are then brought into being, and a potential difference subsequently set up. Observation of the interfaces enables the migration of the test substances to be followed, as well as permitting determination of their mobilities and quantities. If the substance examined is not uniform, but is a mixture, it is generally possible to achieve a separation by suitable choice of hydrogen ion concentration even when the components present migrate in the same direction, as depicted in Fig. 1. The boundaries of each component displace to different extents, so that after a certain time the state shown in Fig. 1*c* is reached. Two boundary layers, which are, in principle, mirror images of one another, enable a double determination of the mobility and quantity of each of the components present to be made, as will be further explained later.

From Fig. 1*c* it may also be seen that in such an experiment the components of a mixture separate themselves into different layers, which, although causing some complications, also enables both the slowest and the fastest components to be obtained to some extent in a pure state. Thus, electrophoresis has a preparative application to the isolation of fractions from mixtures in addition to its analytical use; the importance of this has been considerably increased by the construction of larger cells adapted for the removal of samples[1].

While a sufficiently exact measurement of the current strength '*I*' and the time of the experiment '*t*' presents no difficulty, great precision is required, especially in the construction of the smaller glass cells, to ensure the constancy of '*q*', i.e. the cross-section of the solution. The constancy of the conductivity $\varkappa$ of the solution under examination must be maintained by simultaneous constancy both of the temperature and the properties of the medium. During the experiment, the U-tube and its accessories must therefore be kept in a thermostat, which enables the experiment to be conducted at a low temperature corresponding to the maximum density of the solution under test. In this way, convection disturbances which may arise from the Joules heat produced in the experiment are reduced to a minimum. The optimum temperature range for aqueous buffer solutions is $+0{\cdot}5^0$ to $+2^0$ [2]. The thermostat, like the cell, must be transparent in the direction of observation. In order to prevent misting in the observation field, two glass chambers are built on to the inside surfaces of the thermostat walls. The optical quality of the glass cells as well as the thermostat windows, must satisfy exacting requirements with respect to parallelism and freedom from optical irregularities. The thermostat must, moreover, be so placed as to be free from vibrations as the boundaries are mechanically very sensitive. For this reason, cooling coils, stirring motors and other thermostat accessories must be affixed without contact with the thermostat itself. Constancy in the medium's prop-

[1] A. TISELIUS, loc. cit. p. 342.

[1] A. TISELIUS, loc. cit. p. 342. – H. SVENSSON, Ark. Kem. *15*, B, 19, 1 (1942); *22*, A, 10, 1 (1946).

[2] Cf. L. G. LONGSWORTH, Chem. Rev. *30*, 323 (1942).

erties within the range of migration can be attained by placing the electrodes far from the cell in laterally attached electrode chambers; by this means any products of electrolysis which cannot be prevented from arising on the electrodes can be kept completely away from the U-tube. A constant current strength is maintained by use of a battery of relatively large capacity or by stabilization of the direct current taken from a rectifier.

As the substances subjected to electrophoresis do not leave the region where the medium remains constant during the experiment, they retain their original electric charge, and it is possible to recover them after the test unchanged even in this respect. This circumstance renders electrophoresis a very harmless method and makes it especially suitable for research on labile substances.

With regard to its main field of use: the examination, separation, purification, preparation in a pure state and characterization of proteins and related substances, it must be mentioned that

(1) these substances are mostly readily soluble in the diluted buffers particularly suited to the experiments,

(2) the differences in the electric charges of these substances, with regard to a chosen *p*H-value facilitate their separation, and

(3) the size and sign of their charges, and hence of the velocity and direction of migration are in general variable within wide limits by altering the hydrogen ion concentration.

Electrophoresis is thus seen to be a very adaptable method.

The arrangement of the TISELIUS U-tube, as well as its accessories can be seen from the following Fig. 2. The glass components are held by supports which hang from a common frame. The length of this frame is adapted to that of the thermostat in such a way that the cell and the other glass parts (electrode vessels) are immediately brought into the required positions. The connecting tube visible in the middle above the cell makes it possible, even when closed electrode vessels are used, to equalize the hydrostatic pressure difference while the cell remains closed and the U-tube and electrode vessels are being brought to a steady temperature. This prevents a streaming of the solution, which would disturb the formation of sharp interfaces, when the U-tube is subsequently opened by means of the small pumps which can be seen on the side of the cell segments.

The following modifications which the set-up introduced by TISELIUS in 1938 has undergone may be mentioned: glass cells without a centre partition thus doubling the height of observation for the same volume, in combination with electrode chambers which can be closed (cf. Fig. 2); mechanical (instead of pneumatic) apparatus for moving the middle-section(s) of the cell; partial or complete use of ground-glass joints between the glass components, together with slight alterations in their form and in that of the electrode chambers; electrodes with larger surfaces[1]. All these alterations do not, however, affect the principle of the method, but represent valuable improvements.

The effective cross-section q of the analytical cell shown in Fig. 2 has been standardized at 3×25 mm $= 0{\cdot}75$ cm^2, the height within which the distance s of migration can be measured is fixed at $(2\times)$ 8·6 cm or, in the cell with a centre partition, $(2\times)$ 4·0 cm. An experiment with these cells needs therefore about

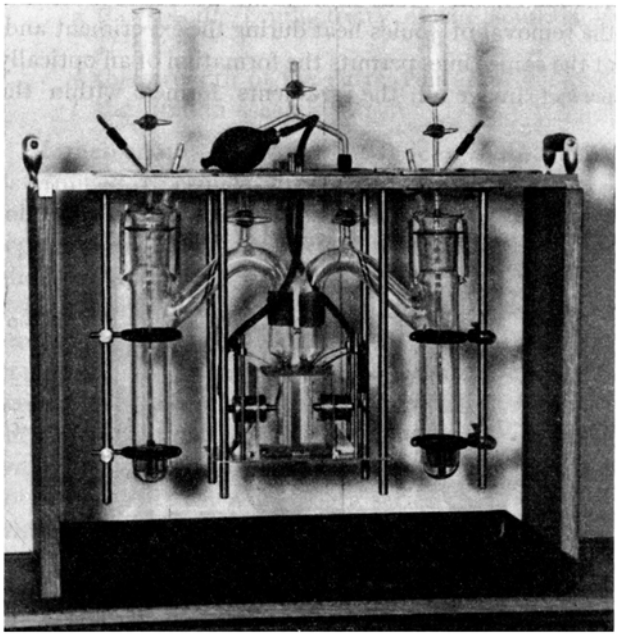

Fig. 2.

12 cm^3 of test solution. The concentration of the substance being examined need not exeed 0·5 to 2%, even in the case of mixtures, so that 60 to 240 mg of a substance are sufficient to carry out an analysis.

The method of boundary observation is naturally only usable without special optical equipment when the substances under examination are coloured or at least opaque. The U-tube can then be illuminated in the same way as a lantern-slide, and a picture of it projected by means of an objective on to a graduated ground-glass screen where it can be observed. Knowing the degree of magnification, the distance s traversed in a time t can then be measured by dividing the observed distance on the screen by the magnification factor.

In by far the majority of cases, however, colourless solutions have to be examined, which exhibit an absorption differing from that of the solvent at most only in the ultra-violet. Formerly, it was very difficult

[1] L. G. LONGSWORTH, Chem. Rev. *30*, 323 (1942). – H. SVENSSON, Ark. Kem. *22*, A, 10, 1 (1946).

to follow the migrations of such invisible, or scarcely visible boundaries[1]. TISELIUS, LAMM, PHILPOT, LONGSWORTH, and SVENSSON have been able, by application and suitable modification of the schlieren method of TOEPLER[2], which depends upon two principles formulated by FOUCAULT[3], not only to overcome these difficulties but also to make the method of electrophoresis considerably more efficient. These modifications of the schlieren method permit, moreover, the determination of both the relative and absolute quantities of substances present.

The schlieren method of TOEPLER depends upon making visible very small light deviations (of the order of one angular minute), by intercepting the deviated pencil of rays. These deviations occur towards the denser of two media when a pencil of light passes tangential to their boundary. The position of such a deviation is marked in the picture by a dark band. The path of the rays in the most usual[4] assembly, according to TOEPLER, is depicted in Fig. 3.

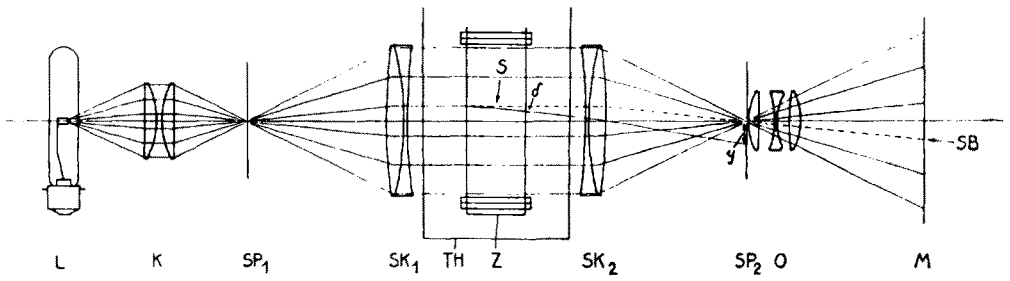

Fig. 3.

Following the direction of the light pencils, we have: L, a source of light (tungsten lamp with horizontal band-filament, sodium lamp, mercury lamp, etc.); K, a condensor; SP_1, first horizontal slit; SK_1 and SK_2, two (identical) schlieren lenses[5] of extremely high correction on the axis, for which two telescope lenses can be used. The diameter of these lenses must be a little greater than the height of the cell. The thermostat is placed between these lenses so that the light pencils pass through the cell parallel to the (horizontal) optical axis. The schlieren lenses together form a double objective which focusses as sharp an image as possible of the first slit SP_1 on a second slit SP_2. Next comes the objective O, a photographic lens of high and anastigmatic correction immediately behind the second slit, which is focussed on the middle of the cell projecting a sharp picture of it on the screen M or a photographic layer.

This simple optical arrangement was used with success in the earlier electrophoresis experiments. Light deviations at horizontal interfaces (e.g. between solution and solvent) caused by differences in the refractive indices as indicated by S in Fig. 3 prevent the respective light pencils from passing through the second slit. As a result, dark "schlieren bands" (SB in Fig. 3) become visible on the screen M marking the position of gradients in the cell. It may be mentioned that the deviation is always directed towards the denser medium. As it is necessary from other considerations to place the denser medium (solution of proteins) in the lower part of the cell, the deviation is always directed downwards.

If the angle of deviation as caused by a gradient is not too great, it is possible to write

$$\delta = a\frac{dn}{dx},^{1} \qquad (6)$$

where a is the thickness of the solution in the cell and dx the change in the height of the beam of light corresponding to the change in refractive index dn. The apparent downward deviation measured in the vertical plane of the second slit SP_2 of a beam of light traversing a concentration gradient i.e. the distance "y" at the second slit (see Fig. 3) is, in the case of small angles, proportional to the angle δ itself, so that one may write:

$$y = af_{SK_2}\frac{dn}{dx},^{2} \qquad (7)$$

wherein f_{SK_2} is the focal distance of the second schlieren lens SK_2. From this formula it follows that, in order to attain a sufficiently large deviation, the thickness of the solution "a" must not be too small, the focal length f of the second schlieren lens not too short, and the concentration of the substance under examination not too low. In order to give an idea of the order of size, it may be mentioned that for $a = 25$ mm and

[1] Cf. Th. SVEDBERG and N. D. SCOTT, Am. Chem. Soc. *46*, 2700 (1924).

[2] A. TOEPLER, Beobachtungen nach einer neuen optischen Methode, Bonn 1864; Pogg. Ann. *127*, 556 (1866); *128*, 126 (1866); *131*, 33 (1867); *131*, 180 (1867); *134*, 194 (1868).

[3] L. FOUCAULT, Ann. de l'observatoire imp. de Paris *5*, 197 (1859).

[4] Other arrangements vide e.g. H. SCHARDIN, VDI.-Forschungsheft 367, Berlin 1934.

[5] The use of two parabolic mirrors instead of these lenses has also been described by SCHARDIN; cf. S. M. SWINGLE, Rev. Sci. Instr. *18*, 128 (1947).

[1] O. LAMM, Nova Acta reg. Soc. Sci. Upsaliensis *10*, 6 (1937).

[2] O. LAMM, loc. cit. – H. SVENSSON, Koll. Z. *90*, 141 (1940).

$f = 1200$ mm a 1% protein solution gives a value for "y" of at least 5 mm.

If one considers more closely the course of the change in refractive index at a boundary, it is found to be in conformity with the principles of statistic distribution of dissolved substances, as deduced from the occurrence of free diffusion at the interface between a solution and a solvent, increasing from zero to a maximum value, and then decreasing to zero again. This alteration in the refractive index is, in the ideal case, identical with that of the ideal distribution function of GAUSS[1]

$$\frac{2}{\sqrt{\pi}} \int_0^y e^{-y^2} d\,y\,. \tag{8}$$

It is very remarkable that this ideal case can not only be strictly realized in diffusion experiments with pure substances, but also a good approximation may be reached in electrophoresis. The ideal case is demonstrated schematically in the following Fig. 4:

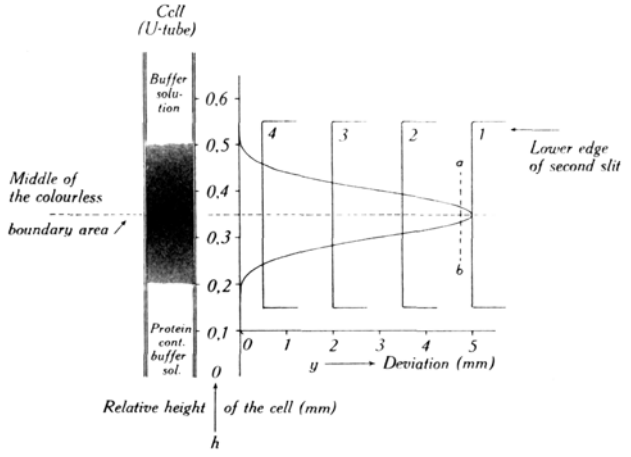

Fig. 4.

With the modern optical methods as derived from TOEPLER'S principle, the course of the change in refractive index, as well as the measurement of the apparent mobilities $u\,(v)$ is recorded as exactly as possible. As the area between the curve reproduced in Fig. 4 and its base line is proportional to the sum of all the refractive index increments $\Delta\,(n_2 - n_1)$ and, as derived above, at the same time to the sum of all the concentration increments $\Delta\,(c_2 - c_1)$ of the substance causing the gradient, this area gives a measure of the concentration of the substance. This determination of concentration from the areas included between the curves and the base line has proved particularly important in the investigation of proteins and similar substances and especially in the analysis of normal and pathological human serum and plasma[2].

Three variants of the schlieren method of TOEPLER have become known and are in use, making it possible to record photographically the course of variations in refractive index over a gradient and hence to determine the concentrations of the substances causing them.

In Fig. 4, it is assumed that a concentration gradient is producing a maximum deviation at the second slit of 5 mm. In this case, according to TOEPLER'S principle, a shadow in the picture will be seen if the lower edge of the second slit SP_2 in Fig. 3 has not been moved downwards more than 5 mm from its zero position. If it is lowered further, no darkening takes place since the deviated light pencil can then pass through the slit also. If it is raised, it cuts off more and more deviated light, in accordance with the progression of the Gauß curve, and the shadow on the screen becomes larger, until, at the zero position of the lower edge, the whole picture begins to darken.

If now, as suggested by LONGSWORTH[1] in 1939, the raising of the lower edge of the second slit is combined with a simultaneous lateral displacement of the photographic plate, refractive index gradients produce, instead of TOEPLER'S schlieren bands, dark gradient areas in an otherwise bright picture. These areas are directly proportional in size to the relative percentages of the substances present when their specific indices of refraction are the same (as is often nearly the case).

The concentration c is given by:

$$c = \frac{k}{a\,b\,K} \int y \cdot d\,x\,,^2 \tag{9}$$

where a is the thickness of the solution,
b the optical distance between the (middle of) the cell and the second slit ($= f$ in [6]),
K the specific refractivity of the substance,
k a proportionality constant and
$\int y \cdot dx$ the surface area in the picture which can be determined, for example, by aid of a planimeter.

This variant of TOEPLER'S schlieren method, which has been called the "schlieren scanning method" is very much used in America, although it is not the most exact of the available methods, does not permit visual observation of the picture and requires a relatively complicated mechanism for taking the photographs.

A second variation of TOEPLER'S schlieren method, which leads to the same result by the use of optical components alone, was originated in principle by PHILPOT[3] in 1938 and has been further developed by SVENSSON[4], in 1939/40 into a method of direct re-

[1] Cf. K. PEARSON, Phil. Trans. Roy. Soc. London, A. *185*, 107 (1894).

[2] L. G. LONGSWORTH, TH. SHEDLOVSKY, and D. A. MCINNES, J. Exp. Med. *70*, 399 (1939).

[1] L. G. LONGSWORTH, Ann. Acad. Sci. New York *39*, 105 (1939).

[2] L. G. LONGSWORTH, loc. cit. p. 344. – L. G. LONGSWORTH and D. A. MCINNES, Chem. Rev. *24*, 271 (1939).

[3] J. ST. L. PHILPOT, Nature *141*, 283 (1938).

[4] H. SVENSSON, Koll. Z. *87*, 181 (1939); *90*, 141 (1940).

cording of the course of variations in refractive index gradients as bright lines on a dark ground. Further improvements of this method have been published by the author[1].

In this method of recording, an inclined second slit is used, as a consequence of which pencils of light deviated by gradients are not intercepted but can (partially) pass through this slit. As a result of the inclination of this slit, it follows that the points of penetration of pencils of deviated light are displaced not only downwards, but also laterally, with respect to undeviated light. If the lateral displacements of the points of penetration are also focussed on the screen by augmenting the dioptric elements of Fig. 3 by a convex cylindrical lens (whose axis must be vertically orientated), the course of the changes in refractive index will be depicted directly as a bright curve on a dark ground. The surface areas enclosed by these curves and the base line are, in this so called "cylindrical lens method" also, proportional to the concentrations of the substances producing the gradients, assuming their specific refractive indices to be the same.

The concentrations c can be calculated according to the formula proposed by SVENSSON[2]:

$$c = \frac{1}{F G K a b \tan\theta} \int y \cdot dx, \qquad (10)$$

in which the following factors replace the value of $\frac{1}{k}$ in formula (9):

F the horizontal magnification between the inclined slit and the screen,

G the vertical magnification between the cell and the screen,

$\tan\theta$ the tangent of the angle formed between the inclined slit and the perpendicular.

This second variation of TOEPLER'S schlieren method has recently acquired more importance since its sensibility to error[3] has been overcome[4] by improvements in the optical system and a suitable construction of the second inclined slit[5]. The precision of this cylindrical lens method is now at least equal to that of the schlieren scanning method[6], in addition to which the continously visible picture, to which a base line can easily be added, as well as the absence of mechanism is very advantageous. The PHILPOT-SVENSSON cylindrical lens method is the method most in use in Europe.

A third variation of TOEPLER'S schlieren method was already recommended by LAMM[7] in 1937. This variation is the so-called "scale method" which is especially suitable for recording weak gradients with the highest degree of accuracy. This method requires no slit, but instead a suitably graduated glass scale (or its projected picture) is placed in front of the cell, that is between it and the first schlieren lens SK_1. In this case, a sharp picture of the scale is focussed on the screen by means of the objective O in Fig. 3. The light deviated by a refractive index gradient then produces a displacement of the scale graduations in the picture. This can be reduced to a distribution curve and thus leads to the same result as the two methods already described. The magnitude of the displacement Z is given by

$$Z \quad G a b \frac{dn}{dx}, \qquad (11)$$

the corresponding surface area A by:

$$A = \int_{z_1}^{z_2} Z\, dz\; B_0 B_a, \qquad (12)$$

and the concentration c (of a substance) by

$$c = \frac{F A}{B_0 B_a G a b \alpha}, \qquad (13)$$

where B_0 is the magnification factor along the ordinate,

B_a the magnification factor along the abscissa,

α the increment of refraction of the substance, and

F the projection factor $\frac{1}{G}\frac{l-b}{l}$ in which l is the distance between the scale and the nearer main-plane of the objective O.

The scale method of LAMM requires a not inconsiderable amount of calculation work for the evaluation of the picture, so that its use for series of experiments is, to a great extent, beset with difficulties if no calculating office is to hand. It is, however, the most exact of known methods, as the single scale line displacements, which can be measured with great precision, correspond to points on the distribution curve, while in the schlieren scanning method one must select the line of 50% darkening in the transition from light to dark, and in the PHILPOT-SVENSSON direct diagrams the middle of a bright line of not quite constant width must be determined. The scale method of LAMM is always adopted when the greatest accuracy is desired and its use is facilitated by the fact that it can be employed at will with all commonly used apparatus.

It will be seen from the foregoing description that neither the apparatus nor the method of electrophoresis is simple. Thus, it can be understood that its development can always be carried further. The great interest in electrophoresis which immediately followed the pioneer work of TISELIUS gave an impetus to the

[1] E. WIEDEMANN, Helv. chim. Acta *30*, 639 (1947); *30*, 648 (1947).
[2] H. SVENSSON, Koll. Z. *87*, 181 (1939).
[3] H. SVENSSON, Koll. Z. *90*, 141 (1940).
[4] E. WIEDEMANN, Helv. chim. Acta *30*, 639 (1947).
[5] J. W. BURNS and L. K. HENKE, Rev. Sci. Instr. *12*, 401 (1941). – E. WIEDEMANN, Helv. chim. Acta *30*, 648 (1947).
[6] E. WIEDEMANN, loc. cit.
[7] O. LAMM, Nova Acta reg. Soc. Sci. Upsaliensis *10*, 6 (1937).

production of electrophoresis apparatus in many countries[1]; the following Fig. 5 shows a modern apparatus as designed by the author and built for the physical-chemical laboratory of Sandoz Ltd., Basle.

It has been possible to introduce numerous improvements upon the author's apparatus of 1939/40[2]. The apparatus now comprises only a two component bench, between the two rigidly connected halves of which the all-glass thermostat, and, below this, the refrigerator are placed. The optical axis is on the eye level of the seated observer. All electrical accessories and control devices are built into the observation end of the bench, so that all regulation including the adjustment of the diagonal slit (if PHILPOT-SVENSSON's method is used) can be carried out while controlling the picture. In the

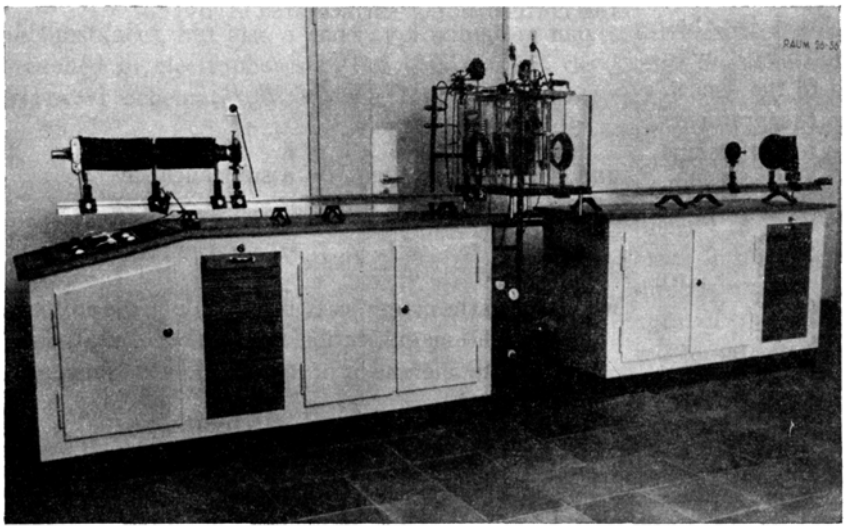

Fig. 5.

cupboard-space below the bench experimental notes, cell holders, glass cells and other auxiliary and spare parts can be systematically stored.

All parts of the optical equipment are placed on two fixed prismatic rails, one 1800 and the other 2200 mm in length. The symmetrical, two component schlieren lenses were constructed according to new calculations[3]; the chromatic aberrations of these lenses, between FRAUNHOFER's lines C and F, amount to, at the most, 0·06% of the focal distance; the spherical aberrations are reduced to quite negligible values. For the cameras, especially sharply focussing lenses of the triplet type are used, the anastigmatism and distortion of which are corrected to a high degree within the field of view. The two-component cylindrical lens system for obtaining the PHILPOT-SVENSSON diagrams is also completely free from distortion. The correction of all the aberrations of this system is at present being undertaken, and owing to the new cylindrical lens system further improvements in the sharpness of the pictures can be expected in the near future.

The two slits used in the method of direct diagram recording have been constructed anew[1] and guarantee, besides the most precise adjustments, a PHILPOT-SVENSSON picture completely free from distortions and differences in focussing, and the reduced base line of which must, of necessity, be reproduced in the right place under all conditions.

Two cameras, a 9×12 cm (with central shutter and plate-holder with rack and pinion movement for taking, at the most, 12 pictures on the same plate) and a 24×36 mm (Leica model IIIc with focal plane shutter) can be used according to choice, the larger being preferable for scale pictures and the smaller for series of PHILPOT-SVENSSON diagrams. The degree of precision thus obtainable makes it, however, only necessary in exceptional cases to resort to the scale method.

The current necessary for working the electrical equipment is taken from the mains. The thermostat refrigerator is controlled by two differentially operating thermoregulators, so that if one regulator fails the experiment is not interrupted. The temperature of the thermostat remains constant within $\pm {}^1/_{100}$ of a degree. The stabilization of the direct current for the U-tube is so efficient that the cell potential is held constant to within 2–10 mV, even when the mains voltage fluctuates about 20 V. The switching on and off of the current to the cell also operates an electric timer which records the duration of the experiment to an accuracy of 0·1 sec. In other respects, numerous accessories ensure that the manipulation of the apparatus remains as simple as possible and that faulty operation is prevented automatically.

Two connections enable the alternative use of a tungsten band lamp or various monochromatic light sources such as sodium lamps, etc.

For the exact determination of electrophoretic mobilities $u(v)$, as well as for their comparative measurement both in the region of the descending and that of

[1] The present manufacturers of electrophoresis apparatus are: L. K. B. Produkter A. B., Stockholm (apparatus according to TISELIUS-SVENSSON for PHILPOT-SVENSSON pictures), Ad. Hilger, London (apparatus according to MCFARLANE for scale pictures), Klett Inc., New York (apparatus according to LONGSWORTH for schlieren scanning pictures), Strübin and Co., Basle (apparatus according to WIEDEMANN for PHILPOT-SVENSSON pictures, see Fig. 5).

[2] E. WIEDEMANN, Schweiz. med. Wschr. *75*, 229 (1945).

[3] Manufacturer: Kern and Co., Optical Works, Aarau. The author is indebted to Dr. LOTMAR, Aarau, for helpful advice.

[1] E. WIEDEMANN, Helv. chim. Acta *30*, 648 (1947).

the rising boundaries, significant progress in cell building has recently been achieved[1]. The corresponding volumes of analytical electrophoresis cells can now be adjusted to within 0·005 cm³ of one another, that is about 0·16%.

The specific conductivity $\varkappa$ of the solution under examination, which is normally deduced during the experiment, can be conveniently determined by the measuring cell of SHEDLOVSKY[2], the author's modified form of which can be immersed very comfortably in the thermostat.

The main-question in electrophoretic experiments is that of obtaining characteristic and comparable data regarding the test substances, and since their behaviour in almost every case depends to a very great extent on the medium, it should be the rule to dissolve them only in well-defined and suitable buffer solutions, into which they must often be introduced by dialysis. It is, however, by no means immaterial what kind of buffer system is employed, for its nature exerts a very marked influence on the sharpness and separation of the gradients, as has been shown by the work of LONGSWORTH[3], DOLE[4] and SVENSSON[5]. Slow moving buffer ions are generally more convenient than quick ones, but, nevertheless, have the disadvantage that the selection at present available is not sufficient to suit every case, and that they are especially unsuitable for the preparation of mixtures of variable pH. Experiments carried out in our laboratory, particularly with serum and plasma, have led to the preferential use of the buffer mixture proposed by MICHAELIS[6], in 1931. This contains sodium chloride, veronal-sodium, sodium acetate and hydrochloric acid, and offers, merely by alteration of the hydrochloric acid concentration, the very large pH-range of 3·5 to almost 10, at a practically constant ionic strength μ, which should be chosen at 0·1 to 0·2. As this buffer contains slow moving ions in its veronal and acetate radicals, it also separates well, and since the appearance of "β-globulin disturbance"[7] is almost completely prevented, it can be regarded as universally usable. The experiment appears promising and it is intended to improve this buffer's good properties still further by replacing all the anions and some of the cations with slower moving ones.

It was already observed, when the original TOEPLER schlieren method was introduced, that the two halves of the picture, the rising and descending boundaries, which should be mirror images of one another, always differ somewhat. This is accentuated when the mixture of test substances is brought into the medium by means of dialysis. In this case, besides the invariably observed difference between the sharpness of the corresponding curves, two additional gradients become visible, the greater in the field of the rising boundaries and the smaller in the field of the descending ones. These move very slowly and as they do not originate from the test substances, they have been termed "extragradients" by TISELIUS. It has been known for about 8 years that these extragradients are, in principle, buffer salt gradients which arise from a difference between the medium and the upper layer of the "same" buffer solution. This, in itself, is a consequence of the Donnan equilibrium set up by dialysis. This dialysis must, however, in general precede the experiment, in order to bring the test substance into the required well-defined medium. More exact consideration of these processes has fully justified this interpretation, and has further shown that the value of the extragradient δ in the field of the rising boundaries is always greater than that of the corresponding gradient ε in the field of the descending ones. In this connection, the mobilities of the rising gradients are found to be greater than those of the descending ones. Since the descending boundaries move in their own medium, the conductivity $\varkappa$ of which is taken as the basis of the calculation of the mobilities $u(v)$, and as the ε-boundary is always the smaller, the image of the descending boundaries is the one usually evaluated. For the determination of the area, the value of the ε-boundary must therefore be deducted, and this has been made possible by LONGSWORTH[1] and DOLE[2] by the use of a special buffer mixture (e.g. veronal-sodium – veronal) in which the extragradients move very slowly, or even backwards, and so are separated from the other gradients in the picture. It may be remarked that this method of determining the concentration, customary in America, requires a still further calculation.

Another procedure has been proposed by SVENSSON[3], that of increasing the ionic strength μ in the experiment to about 0·25, and at the same time reducing the protein concentration to the still permissible minimum of about 0·5%. In this way the extragradients as well as the mobility differences in the two parts of the picture become small and both picture anomalies can, in general, be ignored.

Some little while ago, experiments were initiated in our laboratory with the object of entirely eliminating the extragradients and thus keeping both parts of the picture in an electrophoresis experiment strictly equivalent with respect to the determination of the area

[1] The author is indebted to Dir. M. KREIS, Heerbrugg, for helpful advice.

[2] TH. SHEDLOVSKY, Am. Chem. Soc. *52*, 1793 (1930).

[3] L. G. LONGSWORTH, Chem. Rev. *30*, 323 (1942); J. Phys. Coll. Chem. *51*, 171 (1947).

[4] V. P. DOLE, J. Clin. Invest. *23*, 708 (1944); Am. Chem. Soc. *67*, 1119 (1945).

[5] H. SVENSSON, Ark. Kem. *22*, A, 10, 1 (1946).

[6] L. MICHAELIS, Bio. Z. *234*, 139 (1931). – Cf. E. WIEDEMANN, Schweiz. med. Wschr. *76*, 241 (1946).

[7] D. H. MOORE and J. LYNN, J. Biol. Chem. *141*, 819 (1941). – Cf. H. A. ABRAMSON, L.S. MOYER, and M. H. GORIN, Electrophoresis of Proteins, Reinhold Publ. Corp., New York 1942, p. 185.

[1] L. G. LONGSWORTH, Chem. Rev. *30*, 323 (1942).

[2] V. P. DOLE, J. Clin. Invest. *23*, 708 (1944).

[3] H. SVENSSON, Ark. Kem. *22*, A, 10, 1 (1946).

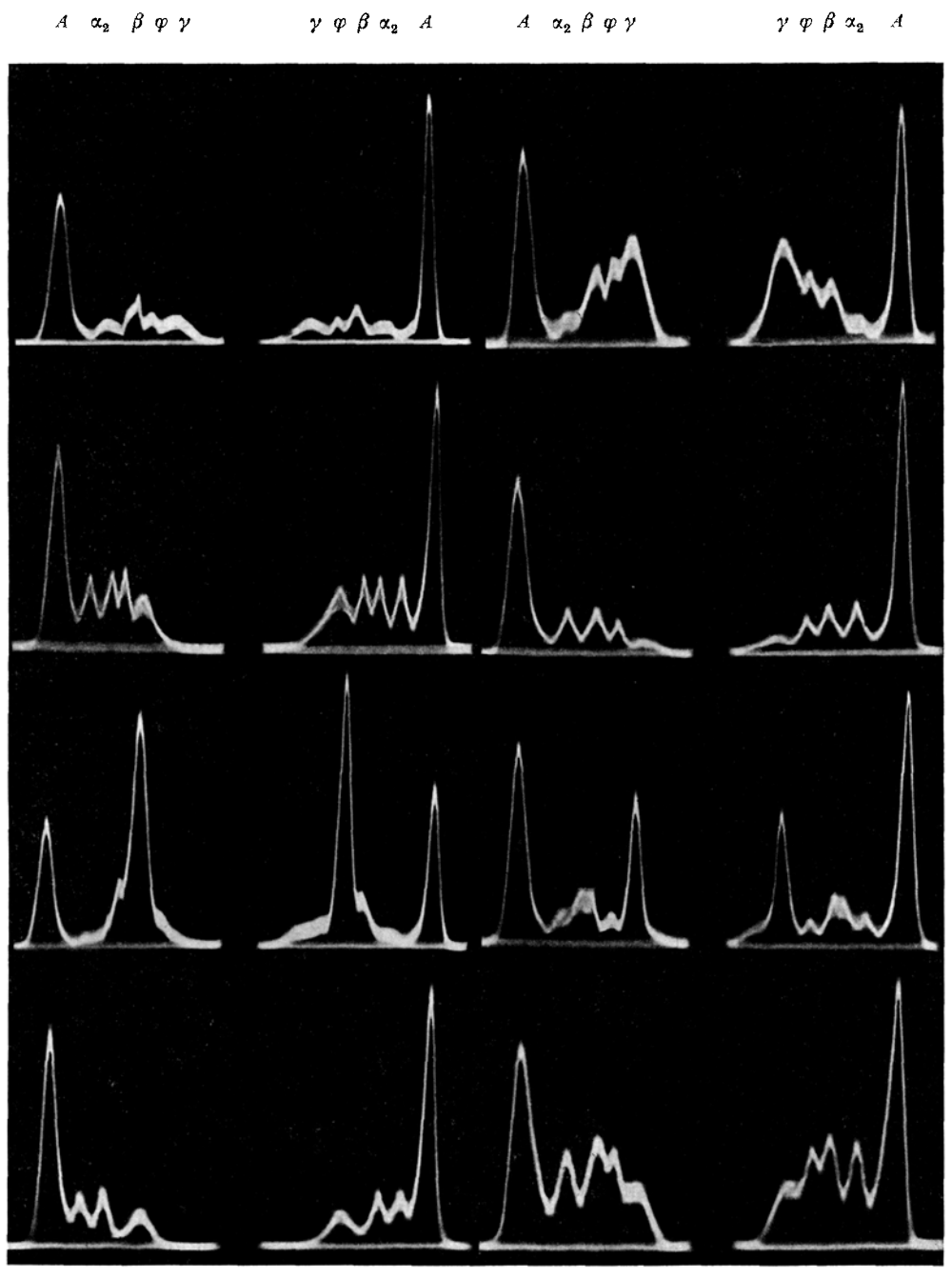

Fig. 6a. PHILPOT-SVENSSON diagrams using the cell with centre partition. Extragradients eliminated. Leica photographs.

and the concentration values, and approximating closely to the same value for the mobilities $u(v)$. Pursuing further the experiments of LONGSWORTH and MCINNES[1] and of LAGERCRANTZ[2], it could be shown that it was, in principle, only necessary that the salt concentration and conductivity of the upper layer of buffer solution should be the same as those of the protein medium in order to achieve complete elimination of the extragradients and at the same time to make the mobilities of the rising boundaries agree with those of the descending ones[1]. This method of working permits not only the use of the buffer desired and the choice of medium ion strength and protein concen-

[1] L. G. LONGSWORTH and D. A. MCINNES, Am. Chem. Soc. *62*, 705 (1940).

[2] C. LAGERCRANTZ, Ark. Kem. *19*, A, 7, 1 (1945).

[1] E. WIEDEMANN, Helv. chim. Acta *30*, 168 (1947).

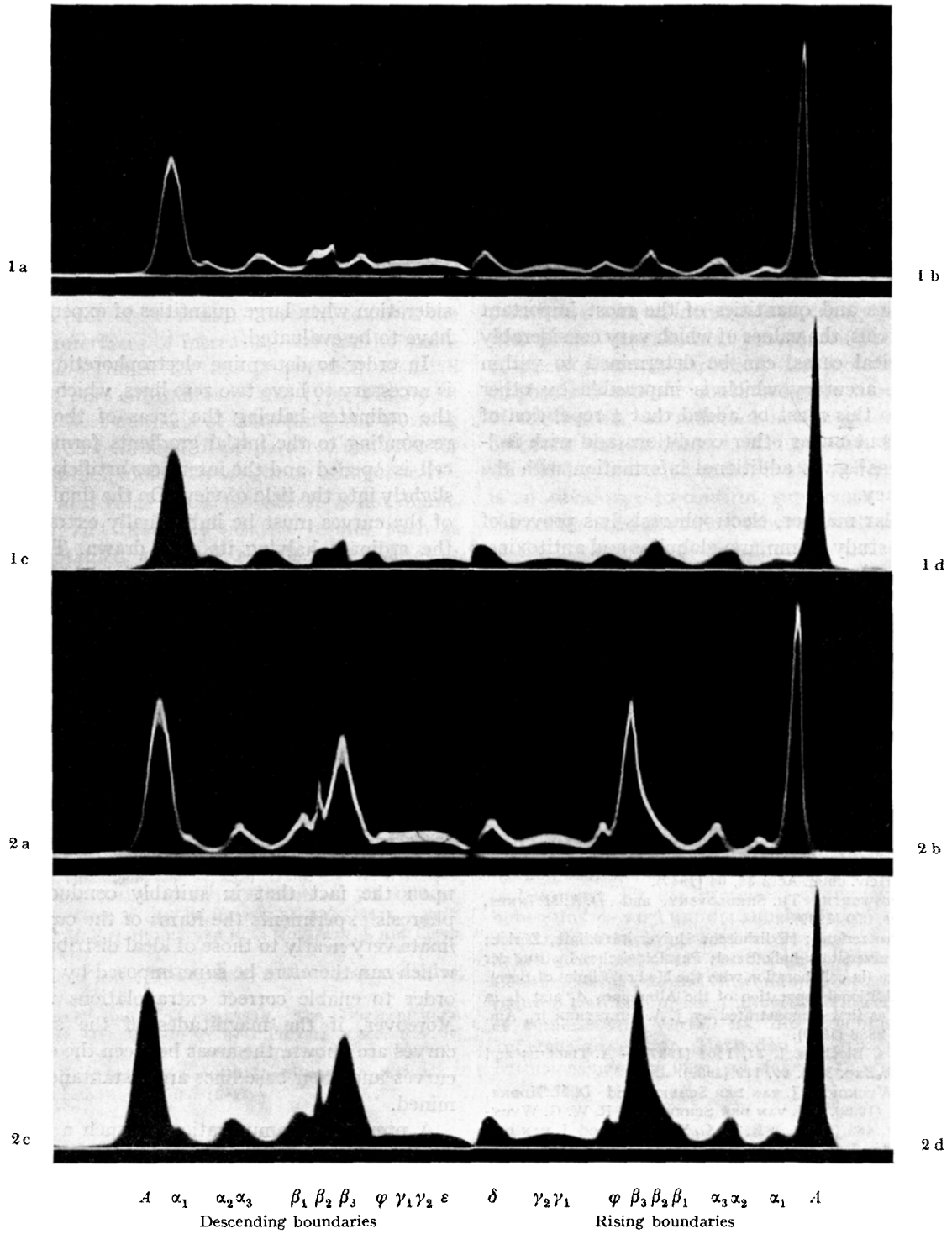

Fig. 6*b*. Similar diagrams to Fig. 6*a*, using a cell of double height. 1: Normal human plasma. 2: β-Myeloma plasma. a–b: PHILPOT-SVENSSON technique. c–d: PHILPOT technique (similar in appearance to schlieren scanning photographs). Leica photographs.

trations, but also makes it possible in every case to check the evaluation of the area of the one part of the picture against that of the other. Thus the results gain in certainty and the calculation of the concentrations is simplified as corrections are unnecessary. The values desired can also be deduced from the usually sharper picture of the rising boundaries, which, in the case of plasma proteins, shows no β-globulin disturbance.

From the large number of electrophoresis photographs taken in our laboratory since 1940, first ac-

cording to TOEPLER's schlieren method[1], then by the scale method of LAMM and, after successive improvements in technique, by the direct diagram recording method of PHILPOT-SVENSSON[2], a few examples of such PHILPOT-SVENSSON diagrams are reproduced in Fig. 6. They are photographs of pathological human plasma, before which a picture of normal plasma is placed. This electrophoresis test introduced by LONGSWORTH[3] has since developed into an indispensable determination in the laboratories of larger medical clinics[4].

The reason for this is that in a single experiment, for which about 3 to 5 cm^3 of serum or plasma are required, the mobilities and quantities of the most important plasma proteins, the values of which vary considerably in pathological cases, can be determined to within $\pm 0{\cdot}3\%$, an accuracy which is impossible by other methods. To this must be added that a repetition of the experiment under other conditions and with isolated fractions[5] gives additional information with the same accuracy.

In a similar manner, electrophoresis has proved of value in the study of immuno-globulins and antitoxins. This application was introduced by TISELIUS and KABAT[6] and elaborated by VAN DER SCHEER, WYCKOFF and co-workers[7]. Investigations on lacto- and ovoproteins[8], on casein[9] and on muscular proteins[10] have also been described. In the extensive field of plant protein research as well, work has been greatly facilitated by use of electrophoresis[11]. Numerous examples of the value of electrophoresis in the study of complicated proteids and enzymes abound in the literature. It would lead much too far to mention here, even approximately, all the work the results of which depend upon electrophoresis, whose possibilities it is only intended to indicate[1].

It remains to be noted, that the evaluation of electrophoresis diagrams, whether obtained by the schlieren scanning method, by PHILPOT's method or by the method of direct diagram recording (cf. Fig. 6), is now possible in a very much simpler, quicker and more certain fashion than previously, an important consideration when large quantities of experimental data have to be evaluated.

In order to determine electrophoretic mobilities it is necessary to have two zero lines, which are given by the ordinates halving the areas of the curves corresponding to the initial gradients formed when the cell is opened and the interfaces artificially displaced slightly into the field of view. On the final picture each of the curves must be individually extrapolated and the ordinate halving its area drawn. The distances between these ordinates and the initial zero ordinates give the different values for s, from which the mobilities $u(v)$ may be calculated according to the formula (5). The relative quantities of the components present may be determined by planimetric measurement of the areas included between the corresponding extrapolated curves and the base line.

The disadvantages of this well-known method are the difficulty of correctly extrapolating the curves and the loss of time in the planimetric measurement of their areas. These disadvantages have been overcome by the introduction of semi-automatic methods based upon the fact that in suitably conducted electrophoresis experiments the forms of the curves approximate very nearly to those of ideal distribution curves, which can therefore be superimposed by projection in order to enable correct extrapolations to be made. Moreover, if the magnitudes of the superimposed curves are known, the areas between the experimental curves and their base lines are instantaneously determined.

A previous communication on such a method was published recently[2]. A more suitable and more precise method for general use has been worked out in our laboratory[3]. A series of normal distribution curves of equal height but of differing widths is superimposed by central projection upon an enlargement (about 10 times) of the Leica picture of the gradients. The curves are matched in form with those of the picture, and are made to coincide by varying the magnification, and in this way the extrapolation is made and the areas of the

[1] A. STOLL, E. WIEDEMANN, and A. RÜEGGER, Verh. Schweiz. Naturf. Ges. 1941, S. 125. – L. EMDÉN, Helv. chim. Acta *30*, 15 (1947).

[2] E. WIEDEMANN, Schweiz. med. Wschr. *74*, 566 (1944); *75*, 229 (1945); *76*, 241 (1946). – K. H. MEYER, ED. H. FISCHER and P. BERNFELD, Helv. chim. Acta *30*, 64 (1947).

[3] L. G. LONGSWORTH, TH. SHEDLOVSKY, and D. A. MCINNES, J. Exp. Med. *70*, 399 (1939).

[4] E.g. in Switzerland: Medizinische Universitätsklinik, Zürich; Medizinische Universitätsklinik Basel; Physiologisches Institut der Universität Bern (in collaboration with the Medical Clinics of Bern).

[5] Cf. the additional separation of the Albumines A_1 and A_2 in human plasma as first demonstrated by J. A. LUETSCHER jr., Am. Chem. Soc. *61*, 2888 (1939).

[6] A. TISELIUS, Biochem. J. *31*, 1464 (1937). – A. TISELIUS and A. E. KABAT, J. Exp. Med. *69*, 119 (1939).

[7] R. W. G. WYCKOFF, J. VAN DER SCHEER, and D. H. MOORE, Science *90*, 357 (1939). – J. VAN DER SCHEER and R. W. G. WYCKOFF, Science *91*, 485 (1940). – R. W. G. WYCKOFF and J. VAN DER SCHEER, Proc. Soc. Exp. Biol. and Med. *43*, 427 (1940). – J. VAN DER SCHEER, R. W. G. WYCKOFF, and F. H. CLARKE, J. Immunol. *39*, 65 (1940); *40*, 173 (1941).

[8] Cf. R. K. CANNAN, A. H. PALMER, and A. KIBNICK, J. Biol. Chem. *142*, 803 (1942). – L. G. LONGSWORTH, Ann. Acad. Sci., New York, *41*, 267 (1941).

[9] O. MELLANDER, Bio. Z. *300*, 240 (1939); Nature *155*, 604 (1945). – R. C. WARNER, Am. Chem. Soc. *66*, 1725 (1944). – HS. NITSCHMANN and W. LEHMANN, Exper. *3*, 153 (1947).

[10] E. C. BATE-SMITH, Biochem. J. *34*, 1122 (1940). – M. DUBUISSON and J. JACOB, Exper. *1*, 273 (1945). – J. JACOB, Exper. *2*, 110 (1946).

[11] A. STOLL, E. WIEDEMANN, and A. RÜEGGER, Verh. Schweiz. Naturf. Ges. 1941, S. 125. – M. FISHMAN and L. S. MOYER, Science *95*, 128 (1942). – A. M. SOOKNE and M. HARRIS, J. Research Nat. Bur. Standards *23*, 471 (1939). – O. QUENSEL, Untersuchungen über die Gerstenglobuline, Diss., Almqvist and Wiksells, Uppsala 1942, S. 59.

[1] A more complete bibliography will be published shortly in "Chimia".

[2] H. LABHART, Exper. *3*, 36 (1947).

[3] E. WIEDEMANN, Helv. chim. Acta *30*, 892 (1947).

curves are determined. This area is given by the formula $F s^2$, where F is the area of the superimposed curve for a given scale and s the linear magnification with respect to the same scale. This picture evaluation, realizable with simple accessories, makes it possibly to carry out the required area determination rapidly and surely with an error of only $\pm 0{\cdot}3\%$, and the experimental work is considerably reduced.

The method of electrophoresis is, according to its conception and development, an analytical one, the value of which is chiefly evident in investigations on natural substances of high molecular weight, especially proteins and proteids. It is also, however, so far as the stability of interfaces of increasing size can be maintained, of importance for the preparation of small quantities of pure substances of this type. Finally, it is one of the best methods of controlling the working out and carrying through of preparative methods for substances of high molecular weight on a larger scale[1].

The analytical value of electrophoresis is not diminished by the fact that only positive results, such as cannot be obtained, as a rule, except from a series of experiments, can prove the purity and homogeneity of a material examined, and that it seems to be important in every case to confirm these results by taking into consideration other suitable methods of investigation[2]. The ultracentrifuge[3], and diffusion measurements[4], may be considered as the most important of such methods. The methods and results of the ultracentrifuge are widely known through the brilliant work of SVEDBERG, so that it is hardly necessary to go into them; it may not be so well known that the diffusion measurements introduced by LAMM are of high value for controlling the purity of proteins, as only pure substances give a diffusion curve which corresponds exactly to that of the ideal distribution curve of Gauß.

The survey which has been given above on the subject of electrophoresis traces its development by LONGSWORTH, SVENSSON and other research workers from the initial pioneer experiments of TISELIUS. New progress is described in technique and methods which has been achieved in America, Sweden and Switzerland permitting both an increase in accuracy and a facilitation of the experimental procedure. The importance of electrophoresis, especially for protein research is emphasized and the continued expansion in its field of application, as reflected in the great increase in literature on this subject, is illustrated. The extent of this field is indicated and it is also pointed out that it is an advantage to confirm, supplement and amplify the results with the aid of other suitable methods such as the ultracentrifuge and diffusion measurements.

The author wishes to express his thanks to Prof. A. STOLL for his support throughout the course of his work.

Zusammenfassung

Es wird ein Überblick über die Elektrophorese gegeben, wie sie aus den grundlegenden Arbeiten von A. TISELIUS hervorgegangen und von L. G. LONGSWORTH, H. SVENSSON und anderen Forschern ausgebaut worden ist. Neuere Fortschritte der Technik und Methodik, die in Amerika, Schweden und der Schweiz erzielt werden konnten und sowohl eine Erleichterung der Versuche wie eine Steigerung der Meßgenauigkeit ermöglichen, werden besprochen. Die Bedeutung der Elektrophorese, insbesondere für die Proteinforschung, wird hervorgehoben und es wird auf die zunehmende Verbreitung der Elektrophorese hingewiesen, die sich in einem erheblichen Anwachsen der einschlägigen Literatur spiegelt. Leistungsfähigkeit und Anwendungsbereich der Elektrophorese werden umrissen mit dem Hinweis darauf, daß es zumeist von Vorteil ist, die Ergebnisse mit Hilfe anderer geeigneter Methoden (Ultrazentrifugierung, Diffusionsmessung usw.) zu sichern, zu ergänzen und zu erweitern.

[1] E. J. COHN, T. L. MCMEEKIN, J. L. ONCLEY, J. M. NEWELL, and W. L. HUGHES, J. Am. Chem. Soc. *62*, 3386 (1940). – E. J. COHN, J. A. LUETSCHER jr., J. L. ONCLEY, S. H. ARMSTRONG jr., and D. B. DAVIS, J. Am. Chem. Soc. *62*, 3396 (1940). – J. W. WILLIAMS, M. L. PETERMANN, G. C. COLOVOS, M. B. GOODLOE, J. L. ONCLEY, and S. H. ARMSTRONG jr., J. Clin. Invest. *23*, 433 (1944). – S. H. ARMSTRONG, jr., M. E. J. BUDKA, and K. C. MORRISON, J. Am. Chem. Soc. *69*, 416 (1947).

[2] E.g. A. STOLL and E. WIEDEMANN, Schweiz. med. Wschr. *77*, 664 (1947).

[3] TH. SVEDBERG and K. O. PEDERSEN, The Ultracentrifuge. Clarendon Press, Oxford 1940. – E. G. PICKELS, Chem. Rev. *30*, 341 (1942).

[4] O. LAMM, Nova Acta reg. Soc. Sci. Upsaliensis *10*, 6 (1937). – H. NEURATH, Chem. Rev. *30*, 357 (1942).

Hormonal and Nervous Factors in the Regulation of Body Temperature[1]

By G. MANSFELD[2], Budapest

The fact that VAN 'T HOFF'S rule is valid for living matter throws adequate light on the importance of the regulation of temperature, perhaps the most perfect regulatory mechanism evolved in the course of phylogenesis. Differences of a few tenths of a degree Centigrade may indicate disease, and neither the heat of the tropics nor the cold of the Arctic changes our body temperature. Inquiry about the mechanism of this most precise regulation is usually answered by comparing it to the thermostat. A more adequate metaphor is supplied by a gas-heated room in which the height of the flames and the rate of ventilation are strictly coordinated; an increase of the height of the flames is associated with a precisely adequate rise of the rate of

[1] A lecture.

[2] Institute of Physiology, University of Budapest.