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A Self-Recording Strip Photometer for Paper Electrophoresis and Paper Chromatography

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Paper electrophoresis was first developed by Wieland and Fischer in 1948 [1] for amino acid analysis, but was quickly and almost simultaneously modified in several laboratories for the purpose of serum protein analysis [2–5]. In these first investigations the separated proteins were analysed quantitatively by retentiometry [2], or, by dying the strips, eluting the dyed zones and measuring the eluates photometrically [3–4]. Grassmann and Hannig [6] first described a quantitative method for paper electrophoresis in which use was made of a manually operated paper strip photometer. In this method pherogram strip was moved, mm by mm, over the slit of a photometer, the density values read and plotted om mm paper against distance from the starting line. The areas of the peaks thus obtained were then measured by a planimeter, these areas being directly proportional to the protein concentrations. Relative concentrations of the serum components were obtained by expressing as percentages the areas of their corresponding peaks compared with the area of all peaks on the densitogram.

The manually operated strip photometer was first developed for paper chromatography by Block [7], and Bull, Hahn and Baptist [8]. Block has used values of optical density, plotted on mm paper, and Bull *et al.* have used percentual transmission values plotted on semilogarithmic paper. Block [9] has also published another, "maximum colour density" method, where optical density is measured only at the centre of the spot. As absolute rather than relative concentrations are usually required in paper chromatography, and as the colour intensity of the reaction product of each amino acid with ninhydrin is different and dependant upon handling conditions, reference standards are usually necessary for the measurement of amino acid paper chromatograms.

We have developed a self-recording photometer for the measurement of paper electropherograms, as well as paper chromatograms. The method has been described briefly in an earlier publication [10]. The new instrument is a combination of a strip photometer of the usual type, and the self-recording galvanometer of the Heyrovsky-polarograph. The movements of the phero-

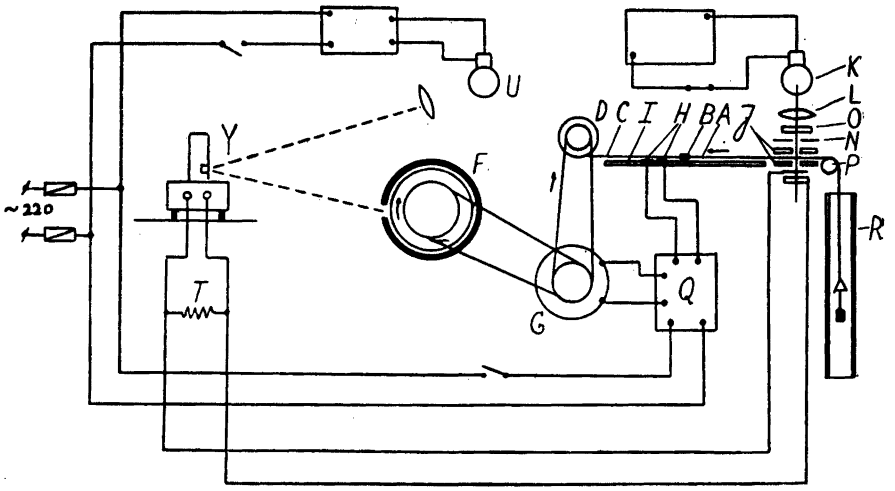

Fig. 1. Scheme of the self-recording strip photometer.

gram strip and the recording drum are synchronized by the polarograph motor. Densitograms are made upon photographic paper containing an extinction scale, divided into narrow columns, and the area of a densitogram peak is obtained as the sum of the extinction values of the columns under that peak. These areas, which can be termed the "extinction areas" of the peaks, are in direct relationship to the amount of dye in the corresponding zone, in contrast to the planimetered areas or "absorption areas" of the peaks.

Logarithmic recording of density values, required by Beer's law, is obtained by a method differing in principal from the above, using an instrument independently developed by Dr. A. Wagner *. This instrument is provided with a "logarithmic galvanometer", which gives directly the "extinction areas", which are measured by planimetring.

The present paper describes in detail our instrument and its use for the measurement of serum electropherograms and one-dimensional amino acid chromatograms.

DESCRIPTION OF THE APPARATUS.

The apparatus (Fig. 1) is a combination of a photometer and a self-recording galvanometer. A galvanometer of the Heyrovsky polarograph Model 1939 has been used in our apparatus, as it happened to be at our disposal, but other corresponding recording galvanometers can evidently be used. Parts G, F and Y originate in our arrangement from the polarograph, which is described in detail in Heyrovsky's monograph [11] and elsewhere in polarographic literature.

* Personal communication. It is a pleasure to acknowledge here a discussion with Dr. Wagner on some theoretical aspects of strip photometry.

The chromatogram- or pherogram-strip A and the recording drum F of the self-recording galvanometer Y have their movements synchronised by a constant speed motor G. A is fastened by a metal clip B to a thin string C which is wound around a reel D. To prevent sagging, A, B and C are sliding upon a glass strip I. H is a pair of thin copper foils. Its position on the glass strip I can be changed to correspond to different chromatogram lengths. When clip B slides over H thus making contact, relay Q switches current from motor G and galvanometer lamp U.

The photometer lamp K (fed from an accumulator) emits an even illumination upon opening M, having first passed through a condensor lens L, an adjustable slit N and a filter O. The glass cell J consists of two microscope slides (see Fig. 2). The chromatogram is cut to be 10.2 mm in width and the opening in J through which it slides is made 10.3 mm in width. J is covered with black paper in which an opening M, 10.0 × 2.0 mm, is cut; thus there is no possibility of extraneous light passing the strip at its edges.

To the other end of chromatogram A a small weight is fastened. This weight which keeps the chromatogram strip taut, moves up and down in a tube R to avoid acting as a pendulum. P is a roller.

The diameter of the axis of reel D is 0.95 cm. The string is wound from side to side upon it. Thus, 20 rotations are needed to draw a 60 cm long chromatogram through the apparatus. Half of this speed (*i.e.* 30 cm by 20 rotations) is obtained by fixing to the metal clip B a roller, around which the string C is drawn back to the side of the reel D, where it is fixed. (The Heyrovsky polarograph has a 1 : 20 reduction gearing which makes the drum F rotate once while the reel D rotates 20 times.) The photographic paper is selected so that its sensitivity is just high enough to give a distinct curve even when the

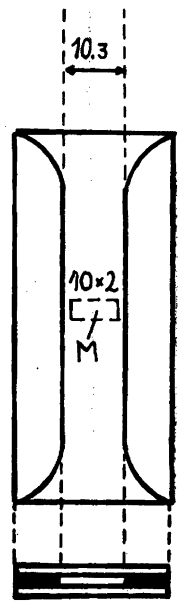

Fig. 2. Glass cell for the sliding chromatogram strip.

galvanometer deflection is rapid. The apparatus can then be used in an ordinarily illuminated work-room. Kodak "Bromars" soft paper and "Labaphot" photostat paper have been ideal for this purpose under our conditions.

We have used a barrier layer photocell, 22 × 40 mm (made by Evans Electroselenium Limited, Harlow, England). The sensitive area of this cell is 5.1 cm^2. The working characteristics of a cell improve as the ratio of sensitive area in use versus total sensitive area approaches unity, and so although this cell has given good results, a smaller size might be preferable, as our slit width has to be kept below 2 mm. However, even with this photocell complete linearity of the current output is obtained under all working conditions because of the high sensitivity and low inner resistance of the galvanometer at our disposal. It has an inner resistance of *ca.* 400 Ω and a critical resistance of *ca.* 7 000 Ω. Damping is effected by the use of a parallel shunt T of 7 000 Ω. Its high sensitivity (1 μA causes a deflection of 30 cm at 1 m distance) enables the use of low light intensities and narrow slit widths.

Slit width has varied in our conditions from 0.5 to 1.5 mm. Calibration of the slit width and control of evenness of illumination is easily made by exposing to different slit widths strips of photographic paper in the place of the chromatogram strip. The photometer part of the apparatus is seen in Fig. 3.

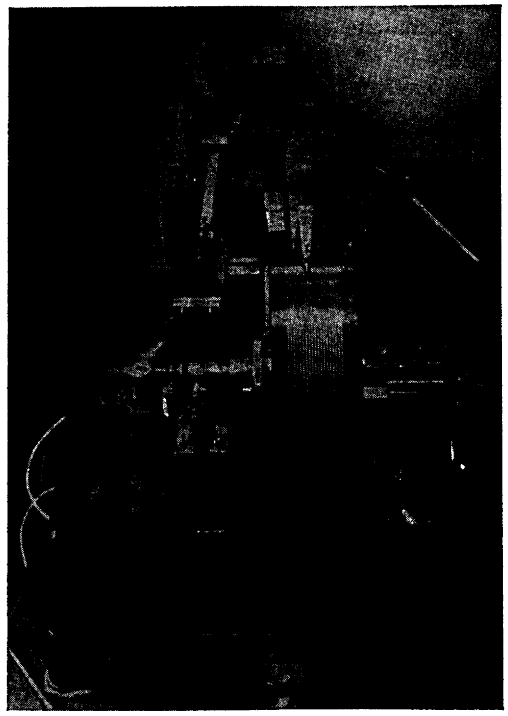

Fig. 3. Photometer part of the apparatus with a chromatogram strip in drawing position.

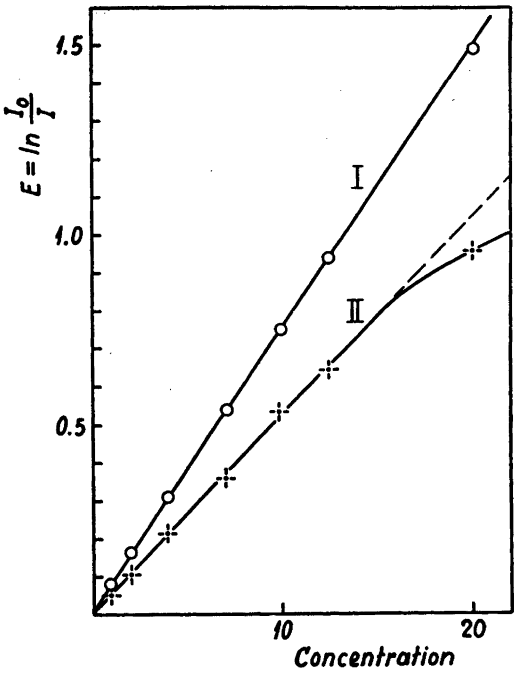

Fig. 4. I. A series of dilutions of amidoblack-10B, measured by Beckman Quartz Spectrophotometer at 620 mμ. II. The same by the strip photometer using Ilford filter N:o 626.

METHOD

Calibration of the apparatus

Deflection of the Heyrovsky galvanometer is directly proportional to the electric current. As current output from the photocell is in linear relationship to illumination as mentioned above, it depends solely on the filter, whether linearity between extinction values and concentration is obtained with substances which follow Beer's law. Linearity of the strip photometer was controlled by measuring a dilution series of amidoblack-10 B in 1 cm cells with the instrument. For this purpose glass cell J was removed, an adequate boring for Beckman cells made and the photometer turned through 90°. The same solutions were measured by the Beckman spectrophotometer. Of the filters at our disposal best results were obtained with the Ilford filter No. 626, No. 607 being inferior. As can be seen from Fig. 4, curve II, complete linearity is obtained with this filter over the finely divided part of the extinction scale, up to $E = 0.8$. The transmittance of this filter and the absorbance of amidoblack-10B can be seen from Fig. 5. Amidoblack-10B follows Beer's law in solution (Fig. 4, curve I).

Strict linearity between density values and dye concentration is necessary in those cases where no reference standards are used, as in the case of paper electropherograms where different peaks, low and high, are directly compared with each other. When standard curve technique is used, as in most cases in paper chromatography, complete linearity is not so essential although the form of the standard curves improves with linearity. For convenient measurement of peak areas in extinction units, areas which are directly

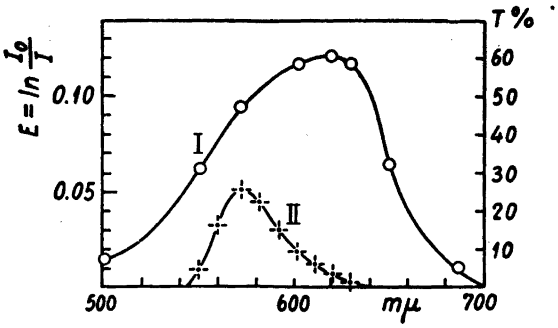

Fig. 5. I. Optical density of amidoblack-10B (left scale). II. Transmittance of Ilford filter No. 626 (right scale).

proportional to the amount of dye in the corresponding zones, the scale given in Figs. 7—9 was designed. It was calculated according to the equation $L = A\,(1-e^{-kc})$, drawn in large size and reduced photographically. This scale is exposed on the recording papers prior to their use by the help of a 10 × 20 cm negative (also commercially available).

Measurement procedure

The measurement procedure is similar for paper electropherograms and paper chromatograms, except when the standard curves for the latter are constructed by using merely heights of the peaks (Block's "maximum intensity"). In this case it is not necessary to expose the scale on the registering paper and the higher drawing speed can be used (Fig. 6 B).

When "extinction areas" are to be measured, the scale, given in Fig. 7—9 is first contact-copied on to a 10.5 × 24 cm piece of suitable photographic paper with the aid of a 10 × 20 cm negative. One edge of the paper is placed in red light exactly along the ∞-line of the negative. This edge is marked with pencil after exposure and, when placing the paper on the recording drum, this edge is placed below the "10 cm" mark.

A 10.2 cm broad strip is cut from the most suitable place of the pherogram or chromatogram with the aid of a straight-edged glass rule and a heavy razor blade. The strip is rendered transparent by keeping it for 10—15 mins. in a trough filled with Grassmann and Hannig's transparancy-oil, and the surplus of the oil is absorbed by drying the strip 2—3 times between sheets of filter paper.

One end of the strip is pointed and pushed through the glass cell J. The light beam of the galvanometer is adjusted with the galvanometer's adjustment screw exactly upon the 10 cm mark of the drum (below which is the ∞-line of the scale) with closed slit. The slit is then opened just enough to move the light beam upon the O-line of the scale when the most transparent place on the strip is below the slit. The clip B and the weight are then fastened to the ends of the strip, the motor started and the drum lock opened. When the contact H is adjusted for electropherograms and "half speed" is used, the current is automatically cut off after 5 minutes, when 15 cm of the pherogram has been drawn through. The recording drum has then turned half way around. A second pherogram can still be recorded upon the same paper, after which the paper is changed in the photographic darkroom and the numbers of the strips just measured marked with pencil behind the recording paper.

'Half speed' is always recommended when the area of the peaks is to be measured, as with pherograms, as it gives peaks of twice the breadth (10 cm densitogram length corresponding to 15 cm strip length). Ordinary speed is recommended when only "maximum intensity" is measured. The relationship densitogram : strip length is then 1 : 3.

After development of the densitogram and scale and drying of the paper, the areas or heights of the peaks are measured. For the measurement of areas the densitogram is

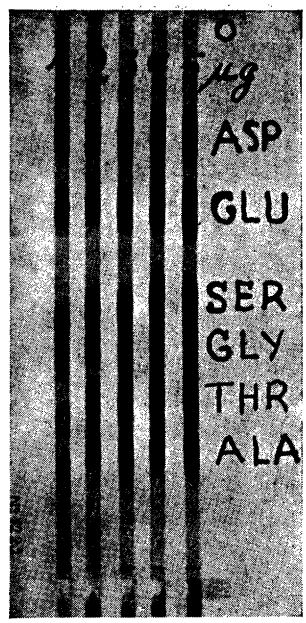

Fig. 6A. Strips for standard curves with 1—5 µg spots. Solvent: water-saturated phenol in NH_3-atmosphere. Paper: Whatman No. 1. 40 hrs, 20° C. Strips cut after ninhydrin reaction.

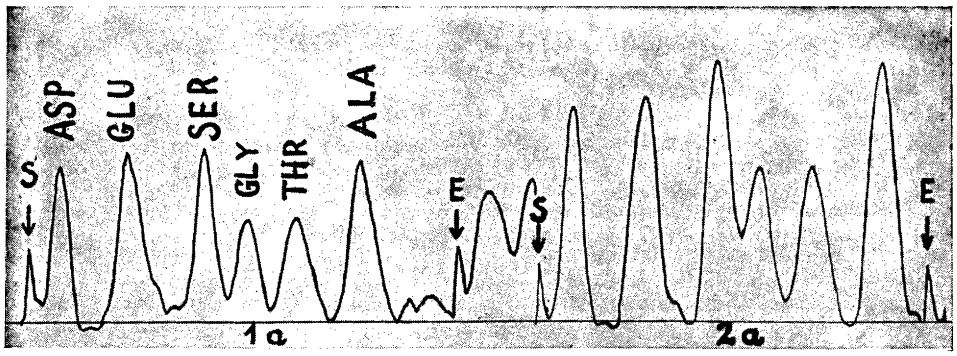

Fig. 6B. Two first strips of Fig. 6A (1 and 2 µg spots) drawn with ordinary speed for measurement by peak heights. S and E = pencil marks showing start and end.

first divided into peaks, after which the density values of the 1 mm broad columns under each peak are read. This is conveniently done with the help of a sharp needle, which is kept in the right hand. To avoid double readings the columns can be marked with needle pricks as they are read. The sum of the columns under each peak, the so-called "extinction area" of the peak, is calculated either from memory or by a computer operated by the left hand. The measurement of one densitogram in this way takes 3—4 minutes. The "extinction area" of a peak is directly proportional to the amount of dye in the corresponding zone, provided that the peak is not higher than $E = 0.8$. If any peak is

Fig. 7A. Two paper electropherograms of normal human serum. Two 10.2 mm wide strips have been cut for measurement from the middle of both pherograms. Pherogram 26 made on "Elphor"-paper, 27 on Whatman No 1 paper.

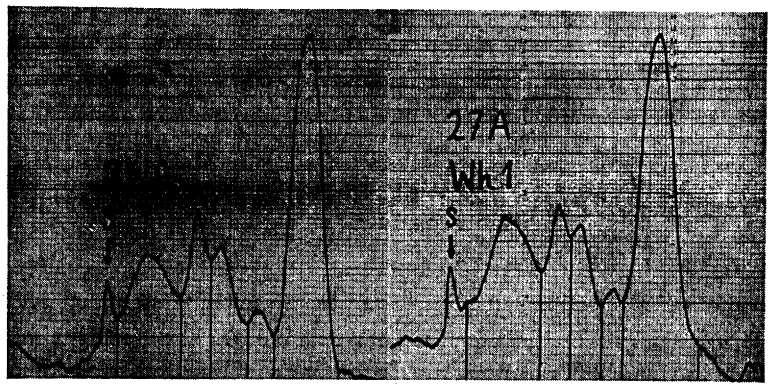

Fig. 7B. Densitograms corresponding to strips 26A and 27A in Fig. 7A, recorded on "Labaphot" photostat paper.

higher than that, it is best to make a new pherogram with a smaller sample. However, if only a few columns exceed $E = 0.8$, their values can be corrected with the help of curve II in Fig. 4, the corrected values being obtained from the dotted continuation.

The accuracy of this method of calculation was determined by allowing several persons to calculate two peaks of medium size without making needle pricks. Standard deviation was found to be 0.2 %. Although the column heights were estimated only by the accuracy of whole scale intervals, it is possible to reduce the error to a minimum by taking the overlap from one column in consideration when estimating the next column.

In the case of serum electropherograms the relative concentration of each component is calculated as a percentage of the peak area from the sum area of all peaks of that densitogram.

In the case of amino acid chromatograms either the area or the height of the peaks is measured. The absolute concentration of an amino acid is arrived at by comparing the value obtained to a standard curve made in exactly the same way with solutions of known concentrations of that amino acid.

Preparation of the amino acid chromatograms

Techniques for preparation of one-dimensional amino acid chromatograms have been published by Block [7,9,12], Bull [8] and others [13-16]. As we have in general followed the same lines as the earlier workers only some aspects of this question are treated here.

Precut strips. To make the chromatograms on narrow strips, already cut to final width [8], is theoretically simplest, since the spreading of the spots out from the strip is

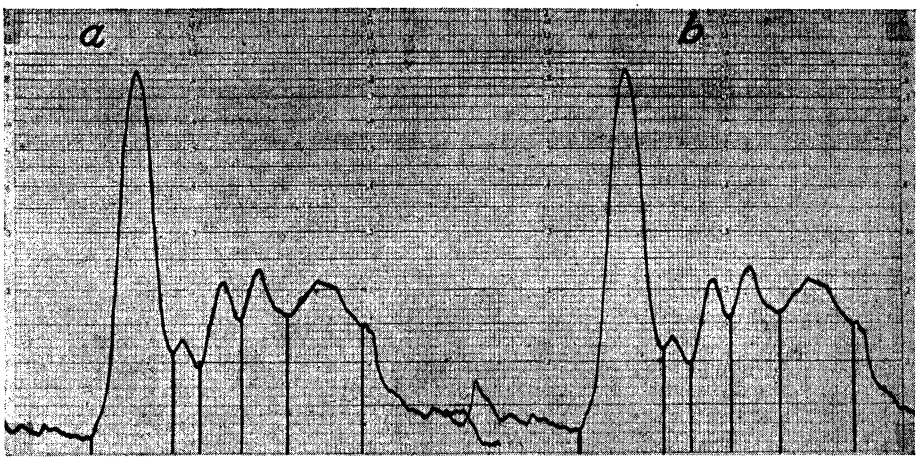

Fig. 8. Reproducibility of the recording. The same strip recorded twice on Kodak "Bromars" paper. Normal human serum.

then impossible and the total colour can thus be measured. In this case the small error caused by variations in paper thickness can also be eliminated by measuring the strips before and after ninhydrin reaction [17]. We have found that the optical density of the paper strips does not change in the ninhydrin treatment except in the region of the amino acid spots. In precut strips, however, *edge effects* are very marked. The spot length is often irregular and the highest colour density is found at the edges. This always reduces accuracy, as it makes a great difference, whether the same amount of dye is spread evenly over the strip width, or concentrated at the edges. In addition, 2 % of the strip width remains unmeasured in our apparatus. With precut strips best results are obtained by using "extinction areas", as fluctuations in spot length have no effect upon these values. On "absorption areas" they have a great effect, since these are not in direct relationship

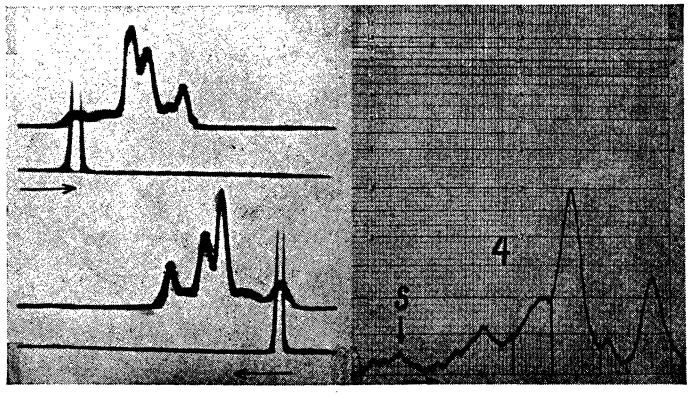

Fig. 9. A nephrosis serum analysed by Tiselius-electrophoresis (A) and paper electrophoresis (B). For quantitative results see Table 1.

to concentration but are a complicated function of diffusion (spot length) and absorption. "Maximum intensity" is of course also sensitive to all irregularities.

Strips cut afterwards. The other possibility is to make chromatograms on wide sheets [12]. Because of partition and diffusion effects spots then spread in such a way that their surface is directly proportionate to the logarithm of concentration (provided the initial area and chromatographing length remain the same) as has been shown by Fischer *et al.* [18,19]. On wide strips the spot form remains much more regular than on narrow strips. After ninhydrin reaction strips are cut to contain the central parts of the spots. Paper correction cannot be done, as it is not possible to cut the strips in the right way before ninhydrin reaction. In comparing measurements the peak heights (Block's "maximum intensities") have given practically the same accuracy as the peak areas. We have therefore mostly used these values for the wide strips as measurement with them is rapid.

None of the techniques for preparation of chromatograms so far used, have been finally approved, as the accuracy of all chromatogram series made has been much inferior to that of the self-recording apparatus. When known solutions of pure amino acids have been analysed using wide strips and peak heights, the error for most amino acids has been 5—10 %, and for alanine, serine and aspartic acid 2—5 %. Two spots of each amino acid at the optimum concentration region, 1—10 mM/l (Block [12]), and a standard curve with 5 points (1, 2, 3, 4 and 5 μg/spot) has been used (Fig. 6A and B). Ninhydrin reaction has been carried out according to Block [12].

Preparation of the electropherograms.

The electropherograms have been prepared essentially according to Grassman and Hannig [6] in a self-constructed apparatus [10], later in the commercial "Elphor"-apparatus (Bender & Hobein, München). Filter paper obtained from the last-mentioned firm and Whatman papers N:o 1, 7 and 11 have given rather similar results.

We have followed Grassmann and Hannig's technique except in two points, these being the application of the sample and decolorization of the strip.

Application is made as follows. The strip is drawn through the buffer, put on some sheets of filter paper, and the place of starting line is heavily dried [22] by pressing it with a narrow "drier", made by winding filter paper two or three times around a glass strip. The pherogram strip is immediately fixed to the moist frame and 10—20 μl of appropriately diluted serum (1 : 2 or 1 : 3) rapidly applied to the middle of the approx. 5 mm wide dried zone. Buffer, siphoning from both sides, diminishes the starting zone, also wetting it and preventing any denaturation and irreversible absorption on dry fibres.

Decolorization procedure has been modified as we have not been able to achieve complete decolorization by dry methanol, containing 10 % acetic acid, and any surplus colour in the strip causes too low albumin and too high γ-globulin values (mean values: alb. = 53, α_1 = 5, α_2 = 10, β = 10, γ = 22 instead of those in Table 1 [10]). Of solvents tried (methanol, ethanol, butanol, acetone and dioxane, containing different amounts of acetic acid and water) "Partridge-mixture"[20], well known from paper chromatography, made according to Campbell *et al* [21]. (630 ml n-butanol + 270 ml water + 100 ml acetic acid; 48 h standing; upper phase used) has given the best results. Corresponding methanol and ethanol mixtures have been too effective causing also decolorization in protein zones. Accurate time control is also necessary with butanol-mixture.

The decolorization procedure finally approved contains 7 5—20 min. washings (5, 10, 15 and 4 × 20 min.) in methanol containing 10 % acetic acid and 5 5 min. washings in the Partridge-mixture. After decolorization the strips have to be completely white except in the protein zones.

Decolorization is the most critical phase in preparation of the electropherograms and comparable results are obtained only if it is always carried out in exactly the same way.

RESULTS

Serum analysis. For comparison, determinations were made from the same samples by Tiselius-electrophoresis and the new method. The determinations

Table 1. Protein components of normal human serum and a pathological serum, determined by optical electrophoresis and paper electrophoresis.

	Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin	Total
By optical electrophoresis:	%	%	%	%	%	%
Normal serum, mean values	59.1	3.9	8.3	14.3	14.4	100
Nephrosis serum	15	6	27	41	11	100
By paper electrophoresis:	Σ* %	Σ %	Σ %	Σ %	Σ %	Σ %
Normal serum, strip 23A	645 54.1	39 3.27	118 9.89	137 11.5	254 21.3	1 193 100.06
» » » 23B	1 014 58.1	49 2.81	149 8.54	237 13.3	300 17.2	1 744 99.95
» » » $24A_1$	816 57.5	59 4.15	116 8.17	192 13.5	237 16.7	1 420 100.02
» » » $24A_2$**	830 58.0	58 4.05	117 8.18	191 13.3	235 16.4	1 431 99.93
» » » 24B	828 57.4	47 3.26	118 8.18	181 12.6	268 18.6	1 442 100.04
» » » 25A	696 55.9	46 3.70	112 9.00	137 11.0	253 20.3	1 244 99.90
» » » 25B	1 038 58.7	71 4.02	148 8.38	211 11.9	299 16.9	1 767 99.90
» » » 26A	836 60.0	51 3.66	132 9.48	146 10.5	228 16.4	1 393 100.04
» » » 26B	708 58.2	42 3.45	118 9.70	149 12.2	200 16.4	1 217 99.95
» » mean values ($\overline{X}$)	57.5	3.54	8.92	12.1	18.0	100.0
» » standard deviation ***	1.7	0.41	0.65	0.99	1.8	
Nephrosis serum, mean values	18.8	4.4	41.9	16.2	18.8	100.1

* Σ = Sum of the columns under the peak in extinction units × 100..

** Duplicate determination to $24A_1$, not included in mean values and standard deviation.

*** Calculated acc. to $\sigma = \sqrt{\frac{\Sigma(X_\nu - \overline{X})^2}{N}}$ (X_ν = individual determinations).

by Tiselius-electrophoresis were kindly made for us by Mr. A. Louhivuori at the State Serum Institute, Helsinki.

Results with two samples of human serum, normal serum (a mixture of 10 sera), and a pathological one from a nephrosis patient are presented in Table 1 (see also Fig. 7—9). A number of determinations were made from both sera by paper electrophoresis and one strip was recorded twice by the photometer to give a picture of the reproducibility of the measurements ($24A_1$ and $24A_2$). All pherograms and densitograms were made in a routine manner, without any special precautions, and give a true picture of variations. In addition to 8 individual determinations by paper electrophoresis, mean values and standard deviations calculated from them are given for normal serum. As can be seen from Table 1, the mean values for paper electrophoresis correspond rather well to those of Tiselius-electrophoresis in the case of normal serum, except that γ-globulins give slightly higher and albumin and β-globulins slightly lower values. This may be due to differences in affinities of these components to the dye used.

In the case of the pathological serum reported in Table 1 (see also Fig. 9) the values of other components also correspond fairly well to each other except those of α_2- and β-globulins. The β-component is a lipoprotein which gives a very high value in Tiselius-electrophoresis, but only slightly higher than normal in the paper electrophoresis. On the other hand, α_2-component gives very high values in paper electrophoresis.

DISCUSSION

The accuracy of paper electrophoresis, using the method described above, is of the same degree as that of the classical Tiselius-electrophoresis and both methods give rather similar results with normal serum. Several authors [22–24] have also reported good conformity between values of Tiselius-electrophoresis and paper electrophoresis in the case of pathological sera. Although this may be true in most cases, it is certainly not always true as is shown by the one case reported here and has also been pointed out by Köiw *et al.*[25]. This is quite understandable as both methods are based on different qualities of protein components: one upon refractivity, the other upon affinity to amidoblack-10 B. Pathological sera may therefore give rather different results by the two methods especially in cases when lipoproteins are present. The methods therefore supplement rather than replace each other. The great advantage of paper electrophoresis is its simplicity and rapidity. If the analyses are made in series and the technique well standardised, one laboratory technician can easily make 5—10 complete determinations in a working day of 7 hours. The method may therefore become clinically valuable.

The error of the measurement of peak areas as sum of the columns is approx. 0.3 % and the accuracy of the apparatus is of the same degree. The accuracy of the method as a whole depends therefore entirely upon the quality of the pherograms and chromatograms. The present accuracy of *ca.* 5—10 % for both electropherograms and amino acid paper chromatograms can evidently be increased. Irregularities of the filter papers available are one of the main sources of error. A more even and thin paper quality would have two advantages: more even baseline for the densitograms and more regular zone and spot form.

For amino acid analysis the regularity of spots obtained with pure samples does not yet, of course, determine the practical value of the method. Tissue extracts and other natural samples usually contain disturbing impurities which have to be removed first. Mixtures of amino acids also have to be rather simple to be analyzable by one-dimensional technique only. Block [12] has developed a technique for measurement of two-dimensional chromatograms, where maximum colour density, an internal standard and a large number of replicate determinations are used. This method is not practical with a self-recording apparatus of this type. Strips, containing central parts of the spots can be measured, but because of the great irregularity of spots in the two-dimensional chromatograms accuracy becomes too low to make the rather laborious procedure practical.

The best way to obtain paper chromatograms from natural amino acid samples for analysis by a self-recording instrument of the present type would probably be through simultaneous purification and quantitative prefractionation of amino acid mixtures into groups by a suitable ion-exchange technique. The groups obtained would then be easier to fractionate by one-dimensional runs.

The high reproducibility of measurements by our method proves that it is not necessary to clamp the paper strip, rendered transparent by oil-immersion, between two glassplates as in the corresponding manual instruments. The

simple filter photometer used has also proved completely satisfactory, even though a filter with higher transmittancy would be desirable. Replacement of the filter with a monochromator would of course greatly enhance the versatility of the apparatus. Strip adapters for Beckman Spectrophotometer [26] and Cary Recording Spectrophotometer [27] have been published. As has been pointed out by Parke and Davis this makes possible the measurement of compounds, having a selective absorption in the ultraviolet, without any colour reaction.

On the practical side of the method development in the quality of chromatograms is of much higher importance, however, than development of the apparatus.

SUMMARY

A self-recording strip photometer has been constructed by combining a filter photometer with the self-recording galvanometer of the Heyrovsky polarograph. An extinction scale, divided into narrow columns, has been designed, this scale being copied photographically upon the recording paper of the galvanometer prior to its use. The scale and curve are developed simultaneously. Peak area is obtained as the sum of the column heights under that peak. For paper electropherograms the relative concentrations of protein components are calculated as percentages of the peak area as compared with the total area under the densitogram. For amino acid chromatograms either peak areas or peak heights and standard curve technique are used.

Replicate determinations of the same strip differ by approx. 0.5 %. Determinations of replicate chromatograms or electropherograms from the same sample differ 5—10 %. Normal serum gives with this method approx. similar results as with Tiselius-electrophoresis, but pathological sera may give quantitatively rather different results, possibly due to different affinity of the components to the dye used, amidoblack-10B.

The practical value and development possibilities of paper strip photometry are discussed.

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REFERENCES

1. Wieland, Th., and Fischer, E. *Naturwiss.* **35** (1948) 29.
2. Wieland, Th., and Wirth, L. *Angew. Chem.* **62** (1950) 473.
3. Turba, F., and Enenkel, H. J. *Naturwiss.* **37** (1950) 93.
4. Cramer, H. J., and Tiselius, A. *Biochem. Z.* **320** (1950) 273.
5. Durrum, E. L. *J. Am. Chem. Soc.* **72** (1950) 2943.
6. Grassman, W., and Hannig, K. *Naturwiss.* **37** (1950) 496; Grassmann, W., Hannig, K., and Knedel, K. *Deut. med. Wochschr.* **76** (1951) 333.
7. Block, R. J. *Science* **108** (1948) 608.
8. Bull, H. B., Hahn, J. W., and Baptist, W. H. *J. Am. Chem. Soc.* **71** (1949) 550.

9. Block, R. J. *Anal. Chem.* **22** (1950) 1327.
10. Miettinen, J. K. *Suomen Kemistilehti* **26 A** (1953) 49.
11. Heyrovsky, J. "*Polarographie*", Springer-Verlag, Wien, 1941, p. 219.
12. Block, R. J., Le Strange, R., and Zweig, G. "*Paper Chromatography*", Academic Press, New York, 1952.
13. Rockland, L. B., and Dunn, M. S. *J. Am. Chem. Soc.* **71** (1949) 4121.
14. Patton, A. R., and Chism, P. *Anal. Chem.* **23** (1951) 1683.
15. Redfield, R. R., and Gruzman Barron, E. S. *Arch. Biochem. and Biophys.* **35** (1952) 443.
16. McFarren, E. F., and Mills, J. A. *Anal. Chem.* **24** (1952) 650.
17. Van Os, G. A. J. *Biochim. et Biophys. Acta* **9** (1952) 11.
18. Fischer, R. B., Parsons, D. S., and Morrison, G. A. *Nature* **161** (1948) 764.
19. Fischer, R. B., Parsons, D. S., and Holmes, R. *Nature* **164** (1949) 183.
20. Partridge, S. M. *Biochem. J. (London)* **42** (1948) 238.
21. Campbell, P. N., Work, T. S., and Mellanby, E. *Biochem. J. (London)* **48** (1951) 106.
22. Körver, G. *Klin. Wochschr.* **28** (1950) 693.
23. Plückthun, H., and Götting, H. *Klin. Wochschr.* **29** (1951) 415.
24. Schneider, G. *Acta Chem. Scand.* **5** (1951) 1020.
25. Köiw, E., Wallenius, G., and Grönwall, A. *Scand. J. Clin. & Lab. Invest.* **4** (1952) 47.
26. Tennent, D. M., Whitla, J. B., and Florey, K. *Anal Chem.* **23** (1951) 1748.
27. Parke, T. V., and Davis, W. W. *Anal. Chem.* **24** (1952) 2019.

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