

ning of one line is complete. A section of the steel band is seen to the left in the diagram, in front of the phototubes.

The electronic problem was to achieve a linear amplification of the DC from the phototube. This was solved in the following way. The DC generated in the phototube is modulated by an oscillator and the rectified amplitude is then proportional to the light intensity. The AC from the phototube is amplified and rectified and fed into a comparator together with a sawtooth voltage. When the sawtooth voltage has attained the value of the above-mentioned, amplified and rectified AC amplitude a synchronizing pulse is emitted. Thus, during the time needed for the sawtooth voltage to increase, a pulse train is emitted with a certain number of pulses proportional to the light intensity. This computation is a synchronised process governed by another phototube. At the moment when the  $3 \mu^2$  area of the sample is to be measured, the sawtooth voltage is started. The values of the light intensities and the synchronizing pulses are stored in the heads of the memory.

When it is convenient, these values are printed as per cent transmission point by point as they occur in the sample. One must be sure, however, that the transmission values never exceed 100%, and an initial control is therefore necessary. The apparatus has an advance control and an operation control. The advance control renders the transmittance visible on the dekatron tubes of the panel. Individual values can also be computed. The operation control, seen to the right of the panel, is a continuous register of the localization and the number of measured values that have been registered.

The printing system consists of a pulse-counting device and an IBM writer. Trigger circuits ensure that the dekatrones emit their information in the right order.

Figure 3 shows an example of values obtained from a section through a spinal ganglion which has been exposed at 8–10 Å in an X-ray microspectrograph. The radiogram was then inserted in the computer and scanned. When the transmission values have been printed on the square of paper, the image of the preparation is projected over this area in the same scale and the outline of the cells are drawn. Details of this information machine will be published elsewhere.

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#### *Zusammenfassung*

Der vorliegende Artikel beschreibt einen Zellenanalysator, der die Lichttransmission in einem mikroskopischen Präparat absucht und quantitativ berechnet. Das Präparat, das in einem wahlfreien Teil des elektromagnetischen Spektrums untersucht werden kann, teilt sich in 12000 Messpunkte ein, gewöhnlich jedem  $3 \mu^2$  im Präparat entsprechend. Die Transmissionswerte in Prozenten werden automatisch in der Ordnung, wie sie im Präparat vorkommen, ausgeschrieben, wobei sowohl die Verteilung als auch die Menge der untersuchten Substanz leicht aus dem Ziffernbild, das die Maschine gibt, berechnet werden kann. Die Berechnung der 12000 Messwerte dauert 4 min.

## PRO EXPERIMENTIS

### A Simple Paper Chromatographic Method for the Study of Serum Protein Patterns in Health and Disease<sup>1</sup>

FRANKLIN and QUASTEL<sup>2</sup> applied paper chromatography to the separation of plasma proteins and showed that the patterns exhibited in chromatograms of pathological plasma vary greatly, according to the disease, indicating distinct changes from the normal. The chromatographic patterns obtained by FRANKLIN and QUASTEL were criticised and shown to be artefacts by HALL and WEWALKE<sup>3</sup>. Recently TAUBER and PETIT<sup>4</sup> have described a two-dimensional paper chromatographic method for the separation of a portion of each fraction of albumin and  $\gamma$ -globulin from a mixture of the two. Complete separation of the proteins was not possible by this method.

A simple circular paper chromatographic method for the separation of serum proteins is described in this note in the hope that it may prove useful for clinical diagnosis and prognosis of diseases.

The procedure adopted for obtaining the chromatograms was similar to the one described by GIRI *et al.*<sup>5</sup> for the separation of amino acids in blood. 40  $\mu$ l of the serum is spotted at the centre of a Whatman No. 1 filter paper (18.5 cm diameter). Immediately after spotting, the "wick" is inserted at the centre of the paper and the chromatogram is developed with about 40% of aqueous alcohol as the solvent. The spot should not be allowed to dry on the paper before development. After the solvent front reaches a distance of about 8 cm from the centre of the paper, it is dried at 90–100°C for about 5 min. The protein fractions are delineated as channelled and circular zones by staining with the dye bromophenol blue as follows:

- (1) Dipping for about 3 min in 0.2% bromophenol blue in saturated alcoholic HgCl<sub>2</sub> solution for staining and fixing the serum proteins on the paper;
- (2) Rinsing in 0.5% acetic acid solution;
- (3) Final washing in water to remove acetic acid and drying at 60–70°C.

Figure 1 is the typical pattern of normal serum.

For better comparison of the difference in the patterns showing the variation in Albumin-globulin ratio, it is convenient to run a mixed chromatogram which may be carried out by spotting 10  $\mu$ l of normal and pathological serum on the circumference of a circle (3 cm diameter) drawn at the centre of the paper, diametrically opposite to each other and developing the chromatogram as described above. Figure 3 is the chromatogram of the patterns of normal and liver cirrhosis sera. The difference in composition of the serum protein fractions-

<sup>1</sup> Presented before the symposium on "Chromatography" held on 3rd and 4th April 1953 under the auspices of the Society of Biological Chemists, India, and published in an abstract form in the Proceedings of the Society of Biological Chemists (India) 11, 33 (1953).

<sup>2</sup> A. E. FRANKLIN and J. H. QUASTEL, Science 110, 447 (1949); Proc. Soc. Exp. Biol. Med. 74, 803 (1950). – A. E. FRANKLIN, J. H. QUASTEL, and S. F. VAN STRATEN, Proc. Soc. Exp. Biol. Med. 77, 783 (1951).

<sup>3</sup> D. A. HALL and F. WEWALKE, Nature, 168, 685 (1951).

<sup>4</sup> H. TAUBER and E. L. PETIT, Proc. Soc. Exp. Biol. Med. 80, 143 (1952).

<sup>5</sup> K. V. GIRI, K. KRISHNAMURTHY, and T. A. VENKITASUBRAMANIAM, Lancet, Sept. 20, 562 (1952).

albumin and globulin of the two samples of serum is clearly shown by the reduction in the length of channels relating to albumin zone (the outer zone) and enhancement of the intensity of the colour of the globulin zones (inner zones) in the case of liver cirrhosis (Fig. 2) and in

albumin and is characteristic of serum containing low concentration of this fraction, while the increase in the intensity of the colour of the globulin zones (inner zones) indicates the increase in globulin fractions as in the case of liver cirrhosis.

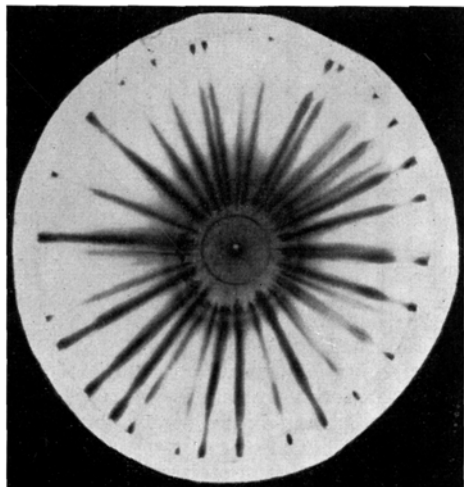


Fig. 1.—Circular paper chromatographic pattern of normal serum.

the case of normal serum the increase in the length of the channels, touching the solvent boundary showing thereby the increase in albumin fraction, compared to the pattern of the liver cirrhosis serum. The shortening of the channels of the albumin zone without reaching the solvent boundary line indicates low concentration of

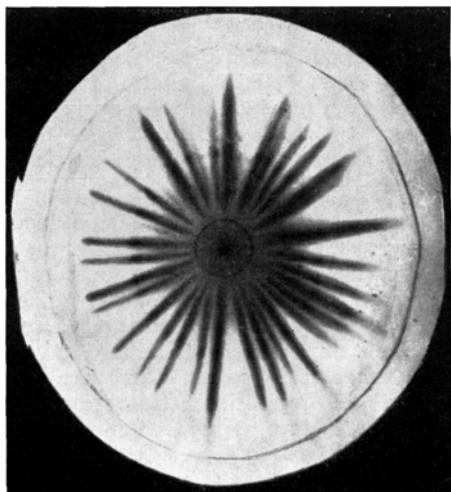


Fig. 2.—Circular paper chromatographic pattern of the serum of a patient with cirrhosis of liver.

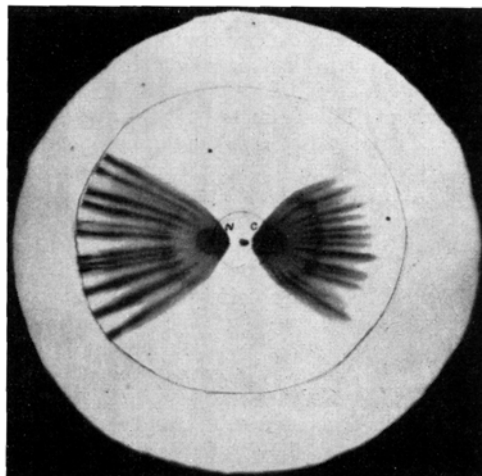


Fig. 3.—Circular paper chromatographic patterns of normal serum, and the serum of a patient with cirrhosis of liver.

Normal serum and the serum of liver cirrhosis patients show characteristic patterns in a number of cases investigated. The chromatographic experiments were always supplemented by the simultaneous determination of the electrophoretic pattern. The visual chromatographic patterns showing the variations in the albumin-globulin fraction is in agreement with the electrophoretic patterns obtained<sup>1</sup>.

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#### *Zusammenfassung*

Es wird eine einfache Methode zur Trennung und Differenzierung der Serumproteine mit Hilfe der Papierchromatographie (Rundfilter-Papierchromatographie) beschrieben. Die Chromatogramme zeigen charakteristische Unterschiede der Albumin- und Globulinfractionen bei normalen Seren und bei Seren von Patienten mit Leberzirrhose.

<sup>1</sup> By using the Swiss Micro-electrophoretic apparatus of the firm Messrs. Kern & Co.