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THE ROLE OF CHROMATOGRAPHY AND ELECTROPHORESIS IN BIOMEDICAL SCIENCES

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SUMMARY

Various facets of the history of clinical chromatography are considered, with emphasis on their application to the present state of the art. The work of Goppelsroeder on capillary analysis is reviewed, followed by a discussion of C.E. Dent's work on aminoacidurias, Lato's thin-layer chromatographic studies of sugars in urine, Ian Bush's approach to automated chromatography, as well as the extensive possibilities of high-voltage electrophoresis shown by Clotten and Clotten. It is interesting that none of these methods has been applied extensively in diagnostic work. Capillary electrophoresis, which was developed by Hjertén in 1969, seems to have gained new interest recently. At present, although it seems obvious that chromatographic methods could solve numerous diagnostic problems, surprisingly little work is being done outside the larger hospital laboratories. Some of the reasons for this situation are discussed, as are the future prospects of the *Biomedical Applications* section of the *Journal of Chromatography*.

INTRODUCTION

Having been asked to contribute a paper on the above topic, I propose to discuss here the past, present and future of biomedical applications of chromatography and electrophoresis. However, before starting on this topic, I would like to mention that 1988 is a year of anniversaries for the *Journal of Chromatography*. In chronological order: Professor Edgar Lederer is celebrating his 80th birthday, Dr. Erich Heftmann his 75th, Dr. Ivo Hais his 70th, Dr. Karel Macek his 60th and the *Journal of Chromatography* its 30th.

Incidentally the *Biomedical Applications* section is 11 years old. The first issue appeared January 1977 and the Preface stated that:

> The journal will publish papers dealing with the following aspects. developments in and applications of chromatographic and electrophoretic techniques related to clinical diagnosis (including the publication of normal values); screening and profiling procedures with special reference to metabolic disorders; results from basic medical research with direct consequences in clinical practice; combination of chromatographic and electrophoretic methods with other physico-chemical techniques such as mass spectrometry.

HISTORY

I propose to discuss selected topics which seemed of interest, and the way in which they have been accepted by medical practitioners.

Capillary analysis

Goppelsroeder, a contemporary of Tswett, developed "die Capillaranalyse", which is simply frontal analysis of liquids on filter-paper strips, ca. 50 cm long, in a closed cupboard. He observed numerous properties of his system: speed of movement of numerous liquids by ascending development, the concentration of dilute solutions by adsorption and the frontal chromatograms obtained when several solutes were present (especially with dyestuffs). Goppelsroeder published several books dealing with the analysis of body fluids.

Fig. 1 shows the title page of one of his books that appeared in 1904. It contains extensive studies of numerous pathological conditions using also colour reagents to reveal various compound classes. Fig. 2 shows the results of a typical study involving 507 samples from 178 patients and carrying out analyses at weekly intervals.

In his discussion he already voices the same hopes for his method as were expressed in the Preface of the first issue of the *Biomedical Applications* section of this journal, i.e., that his chromatograms will prove an aid to the practising physician (Fig. 3).

I was unable to establish what impact his books had on medical science. They were certainly the starting point from which the late E. Stahl was to launch himself into chromatography in a closely related field, pharmacognosy.

Paper chromatography

Paper chromatography in a two-dimensional form for the separation of all of the usual amino acids was developed in 1944 by Consden et al. [1]. It was immediately applied to medical problems and was highly successful in the study of a number of metabolic diseases. A review by one of the most active workers in the field, the late C.E. Dent, listed in 1947 results on acute yellow atrophy of the liver, the Fanconi syndrome, cystinuria, phenylketonuria and Bence-Jones proteinuria.

When I visited Professor Dent around 1950, he had a small ward of several beds attached to his chromatography laboratory. Figs. 4 and 5 show some of the results presented by him in a subsequent lecture [2].

Studien

über die Anwendung

der

CAPILLARANALYSE

I.

bei Harnuntersuchungen

II.

bei vitalen Tinktionsversuchen

von

FRIEDRICH GOPPELSROEDER.

Mit 130 lithographischen Tafeln
und 12 Lichtdrucktafeln, wovon eine nach Photographie
und 11 nach Mikrophotographie.

Verhandlungen der Naturforschenden Gesellschaft in Basel. Band XVII.

BASEL
Buchdruckerei Emil Birkhäuser
1904

Fig. 1. Title page of Goppelsroeder's book [19].

The development of paper and thin-layer methods for diagnostic purposes is still continuing today. The books by Ivor Smith containing fully worked out clinical methods have been invaluable in demonstrating the possibilities to clinical workers [3].

TAFEL 45.

Chemische Reaktionen auf die mit 507 Harnproben von 178 Kranken in 86 Krankheitsfällen erhaltenen Capillarstreifen.

EG bedeutet Eintauchgrenze. EZ. = Eintauchzone. Strich — = keine Farbenreaktion.

	Krankheiten.		Nach Eintauchen des Capillarstreifs in gemessenes Gemisch von 1 Volum concentrirter Salzsäure und 1 Volum wasser		Nach Eintauchen des Capillarstreifs in fast kochende Fehlingsche Flüssigkeit.		Nach Betupfen des Capillarstreifs mit Rohrzuckerlösung und concentrirter Schwefelsäure		Nach Betupfen des Capillarstreifs mit Salpetersäure haltiger Salpetersäure.	
			Oberer Streiftheil	Unterer Streiftheil	Oberer Streiftheil	Unterer Streiftheil	Oberer Streiftheil	Unterer Streiftheil	Oberer Streiftheil	Unterer Streiftheil
II	**Athmungsorgane**									
5	Bronchiektasia 24 Nov		dunkel fleischrot	Rosa	gelb	—	Spur fleischrot	—	zuerst violet dann rot	—
		1 Dez	Rosaschein	—	gelblich	—	—	—	gelbliche Bänder	—
		8 Dez	lebhaft fleischrot-braun	fleischrothlicher Schein	gelb	—	braungelb	—	lebhaft orangegelb	E.G lebhaft orangegelb
6	Bronchitis I 25 Nov		hell fleischrothlich	—	—	—	Spur blauviolet	—	gelbe Bänder	—
	II	1 Dez	hellrosa fleischrot	—	Spur gelblich	—	hell fleischrothlich	—	gelbliche Bänder	—
		8 Dez	lebhaft rosafleischrot	Rosaschein	gelblich	E G violetlich	—	—	gelb	—
		22 Dez	violetlich fleischrot	—	Spur gelb	—	—	—	gelbe Bänder	—
		29 Dez	hell violetlich fleischrot	—	—	E G hell violetlich	—	—	gelbliche Bänder	—
	III	28 Nov	violetlich fleischrot	—	hell gelblich	—	—	—	gelb	—
		5 Dez	Krapprosa	—	E.G. rothlicher Hochschein sonst nur gelb	—	obere Hälfte fleischrothlich	—	lebhaft gelb	—
		12 Dez	lebhaft fleischrothlich	Rosa	sehr schwach gelblich	—	violet fleischrothlich	—	gelbe Bänder	—
		19 Dez	Rosa	hell Rosa	gelblich	—	—	—	gelb	—
	IV	2 Dez.	Rosa	—	hell gelblich	—	—	—	gelbe Bänder	—
		9 Dez.	lebhaft Krapprosa	fleischrothlicher Schein	zu oberst sehr hell gelblich	—	obere Hälfte sehr hell fleischrothlich	—	obere Hälfte ziemlich lebhaft gelb	—
		23 Dez	lebhaft fleischrot	sehr hell fleischrothlich	gelblicher Hochschein	—	lebhaft fleischrot	—	hell gelbe Bänder	—
	V	5 Dez	lebhaft fleischrot	hell Rosa	gelb bis Eintauchsgrenze	fast zur	Spur bräunlich fleischrothlich	—	gelbe Bänder	—
		12 Dez	sehr lebhaft fleischrot	sehr hell fleischrothlich	Spur gelblich	—	Spur fleischrothlich	—	gelbe Bänder	hell gelbliche Bänder
	VI	5 Dez	lebhaft schmutzig rotviolet	hell fleischrothlich	Spur gelblich	—	sehr hell fleischrot	—	zuerst violet dann rothbraun Bänder	—
		12 Dez	lebhaft fleischrot	sehr leise fleischrothlich	sehr leise gelblich	—	sehr hell fleischrothlich	—	lebhaft gelbe Bänder	—

Friedrich Goppelsroeder

Fig. 2.

TAFEL 44. *Chemische Reaktionen auf die mit 507 Harnproben von 178 Kranken in 86 Krankheitsfällen erhaltenen Capillarstreifen.*

EG bedeutet Eintauchgrenze. EZ = Eintauchzone. Strich — = keine Farbenreaktion.

	Krankheiten.		Nach Eintauchen des Capillarstreifs in ein bestes Gemisch von 1 Volum concentrirter Salzsäure und 1 Volum Wasser		Nach Eintauchen des Capillarstreifs in fast kochende fehlingsche Flüssigkeit.		Nach Betupfen des Capillarstreifs mit Rohrzuckerlösung und concentrirter Schwefelsäure		Nach Betupfen des Capillarstreifs mit Salpetrigsäure haltiger Salpetersäure	
			Oberer Streiftheil	Unterer Streiftheil	Oberer Streiftheil	Unterer Streiftheil	Oberer Streiftheil	Unterer Streiftheil	Oberer Streiftheil	Unterer Streiftheil
Fortsetzung		22 Dez.	lebhaft fleischrot	Rosaschein	gelb	E G violettlicher Schein	fleischrötlich	—	roter Rand	—
		29 Dez.	fleischrötlich	—	Spur gelb	E G violettlich	Spur fleischrötlich	—	braunlich gelbe Bänder	—
III		24 Nov.	Rosa	—	—	E G hellviolettlich	—	—	braunlich violettliche Bänder	—
		1 Dez.	Rosarötlich	—	Spur gelblich	E G Spur violettlich	Spur fleischrötlich	—	braunlich gelbe Bänder	—
		8 Dez.	lebhaft fleischrot	—	[illegible]	—	fleischrötlicher Schein	—	lebhaft gelb	—
		22 Dez.	fleischrötlich	—	Spur gelb	E G fleischrötlich	sehr hell fleischrötlich	—	braunlich gelbe Bänder	—
		29 Dez.	schwach fleischfarbig	—	schwach gelblich	—	Spur fleischrötlich	—	braunlich violettliche Bänder	—
IV		25 Nov.	fleischfarbig	—	—	E G violett	—	—	rotviolette Bänder	—
		2 Dez.	fleischfarbig	—	—	E G violett	—	—	rotviolette Bänder	—
		9 Dez.	Spur fleischrötlich	—	Spur gelblich	E G violettliche Hochspur	Sehr hell fleischrötlich	Spur Rosa	gelbbraune Bänder	—
		22 Dez.	ziemlich lebhaft krapprosa	fleischrötlicher Hornschein	gelblich	E G blauviolettlich	lebhaft fleischrot	—	lebhaft maisgelb	E G lebhaft canariengelb
		30 Dez.	leise ziegelrötlich	—	—	—	fleischrötlich mit violettlichem Schein	—	rotvioletter Rand	—
V		27 Nov.	schmutzig fleischrot	schmutzig fleischrötlich	lebhaft gelb	Spur gelb	Hochschein fleischrötlich	—	lebhaft gelbe Bänder	hellgelbliche Bänder
		4 Dez.	lebhaft fleischrot	—	—	—	Spur fleischrötlich	—	rotbraunlich Gelb	—
		11 Dez.	fleischrötlich	—	gelblich	—	s s s hell fleischrötlich	—	—	—
VI		28 Nov.	schon fleischrötlich	Rosa	gelblich	E G violettlich	hell fleischfarbig	—	braungelbe Bänder	—
		5 Dez.	fleischrot	—	gelblich	—	Spur fleischrötlich	—	rote Bänder	—
		12 Dez.	fleischrot	—	gelb	E G violettliche Hochspur	hell fleischrot	—	braunlich gelbe Bänder	—
		19 Dez.	fleischrot	leise Rosa	Spur gelb	—	s s schwach fleischrötlich	—	gelbe Bänder	—

Friedrich Goppelsroeder

Fig. 2. Results obtained by Goppelsroeder's capillary analysis method with urine from patients suffering from bronchiektasia and bronchitis. From ref. 19.

Two-dimensional paper chromatograms had the drawback that development took up to 4 days. However, this was overcome by the use of more rapid thin-layer methods. In the case of phenylketonuria, I repeated one of the published one-

Ich habe mich nicht über den Rahmen des chemischen Gebiets hinausbegeben und das allein dem Physiologen zustehende Gebiet nicht betreten. Wird es einstmals möglich werden an der Hand des ausseren Bildes von Harncapillarstreifen und noch mehr an der Hand der mit Harncapillarstreifen erhaltenen chemischen, spektroskopischen und auch Fluorescenz-Reaktionen die verschiedenen mehr oder minder schweren verschiedenartigen Fälle von pathologischen Prozessen, welche durch Veränderungen des Harnes sich kundgeben und die Diagnose des Arztes unterstützen, zu erkennen? Wird es möglich werden mit Hilfe der Harncapillaranalyse wenigstens für eine grosse Zahl von möglichen Harnbestandteilen, rascher wie bis jetzt mit den bisherigen Methoden zu ihrer gegenseitigen Absonderung auf, ihnen eigentümlichen und ihnen speziell zugewiesenen Streitzonen und dadurch zu ihrem schnelleren Nachweis zu gelangen? Wird es möglich werden mit Hilfe der auf Capillar- und Adsorptionserscheinungen beruhenden Capillaranalyse auch solche Spuren von Harnbestandteilen, welche sonst kaum oder nur nach langwierigen chemischen Trennungsoperationen isoliert werden können u. wenn auch nur linienbreite Zonen zu bannen? Es sind dieses Fragen, welche sich dem auf diesem Gebiete Forschenden von selbst ergeben. Mögen sie später bejahend oder verneinend beantwortet werden, so behalten doch solche Arbeiten als *Studien* ihren Wert, welcher den Forscher, der ja bis an sein Lebensende *Studiosus* bleibt, für alle aufgewendete Mühe reichlich belohnt und vielleicht auch Andere zur Fortsetzung der begonnenen Arbeit anspornt.

Fig. 3. The final part of Goppelsroeder's book [19] expressing the hope that his chromatograms will prove an aid to the practising physician.

dimensional methods and was able to obtain results within 3–4 h of taking the blood sample. Thus planar chromatography is suitable even for direct analyses by the practitioner.

I would also like to mention the work of M. Lato and his group, summarized in a review by Ghebregzabher et al. [4]. In the correspondence relating to the review of his work for publication, he pointed out to me that although excellent high-performance liquid chromatographic (HPLC) methods for sugars were then already available, these were finally unavailable to the diagnostician who needed the results. A typical result is shown in Fig. 6.

Professor Ian Bush's approach to clinical chromatography

The late Professor Bush was not only an outstanding chemist and biochemist, but had also degrees in mathematics and medicine. His approach, which he once expounded to me during a rather good lunch (Mastrostefano, Piazza Navona, Rome), is worth recording as I do not think that he ever published it as such.

The problem that he was dealing with was to survey the steroid profiles in serum in connection with possible physiological changes during transatlantic flight. First he considered how "normal values" are obtained. For these he felt that data on at least 400 persons would be required. The usual "statistical" data

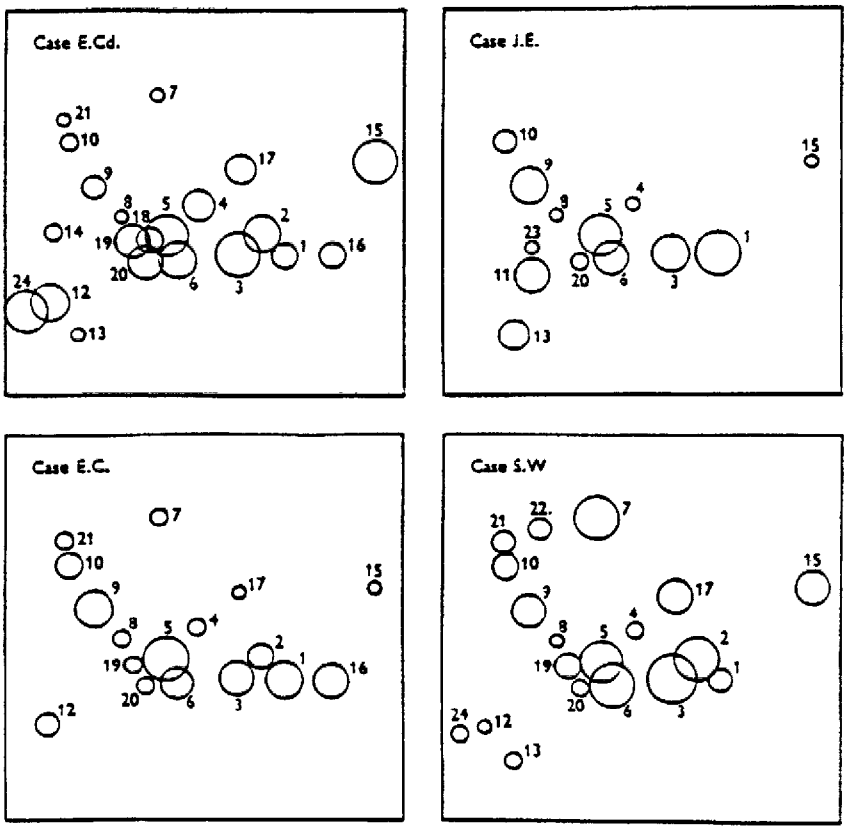

Fig. 4. Chromatograms from 25 μl of urine from four cases of Fanconi syndrome. They were specially chosen to illustrate the very different patterns that may be found in the disease. Owing to the method of representation the overlaps between neighbouring spots are grossly exaggerated here. Identification of spots: 15 = cysteic acid; 16 = aspartic acid; 17 = taurine; 18 = hydroxyproline; 19 = histidine; 20 = citrullic acid (or β-alanine); 21 = phenylalanine; 22 = tryptophan; 23 = methionine sulphoxide (probably); 24 = unidentified. From ref. 2.

on a sample of about 20 persons are generally dictated by the size of a hospital ward and the hesitation of most clinicians to work with larger numbers. In his opinion, however, these samples are not sufficiently large.

A serum profile has 20–25 steroid peaks and to obtain meaningful results at least duplicate analyses of at least ten samples over a minimum of 2 months would be required. This would involve analyses producing 200 000 results.

Professor Bush considered gas chromatography (GC) to be tactically useless for such a venture as it would require an army of laboratory technicians injecting something like ten gas chromatographs with a total of 200 samples a day. No laboratory could arrange this easily (I think that nowadays the same problem would arise if one wanted to use HPLC).

His solution of the problem consisted in the preparation of paper chromatograms with 30–50 samples per sheet. He had designed a machine for evenly spraying with reagents, which sprayed and heated the chromatogram automatically

[5]. These chromatograms were then photometrically evaluated by another apparatus designed by him, which permitted the rapid quantitative photometry of paper chromatograms [6,7].

At this point I would like to quote from the "Discussion" in his paper [6][a]:

> Although acceptable analytical performance compared with other chromatographic methods is an essential feature of direct scanning of paper chromatograms, and could not have been expected on the basis of published results up to 1954, the main advantage of the method is its speed and potential productivity. Although conventionally sized paper chromatograms are slow to run compared with gas–liquid chromatograms, large numbers can be run at the same time in cheap and relatively compact apparatus, and the running time involves no personnel working time[2,3] The overall productivity of the scanning method is thus very much greater than any other existing method of quantitative estimation by chromatography for comparable capital and running costs. The present machine produces a chart record of a 50 cm chromatogram (equivalent to approx. 2000 theoretical plates for a typically overrun strip[29]) in 50 seconds, *i.e.*, at rates of 504 chromatograms in seven hours' running
>
> In fact, the present machine has never been used to process more than about 200 chromatograms in any one day even though it has serviced simultaneously up to four separate research projects most of its working life The preparation of extracts suitable for chromatography, and the processing of the chart records are the rate-limiting steps in analytical procedures of this type. The present machine has processed approximately 25,000 chromatograms in its working life despite a considerable amount of "down time" spent on modification, development work, and transport between laboratories. In the last 12 months one research group carrying out a large scale survey of ten individual urinary steroids by a modification of the general fractionation scheme of BUSH AND WILLOUGHBY[6] has been able to process over 1500 urines (*i.e.*, approx 5000 chromatograms) using this apparatus, at the same time as it was being used for two other major research projects.

Professor Bush then also considered that the research worker faced with 200 000 items of quantitative data would of course be unable to handle these and envisaged the direct coupling of his photometer to a suitably programmed computer, which furnishes the normal values directly.

I find it still remarkable that this project was worked out around 1965, more remarkable still that in a critical examination of the then available techniques, viz., paper, thin-layer and gas chromatography, he found paper chromatography to be the most suitable and that the whole project, although published, did not find any other group ready to adopt his undoubtedly sound arguments and methods.

In another part of this paper I shall also mention other methods which in the hands of their inventors opened up great possibilities in clinical chemistry, but were steadfastly ignored by most other workers.

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[a] Bush here considers the so-called "Bush systems" for steroids, which develop very rapidly (4–8 h) and are very susceptible to temperature variations and need completely closed development chambers. However, when developed with the necessary consideration they yield very compact spots, hence 2000 theoretical plates are usual.

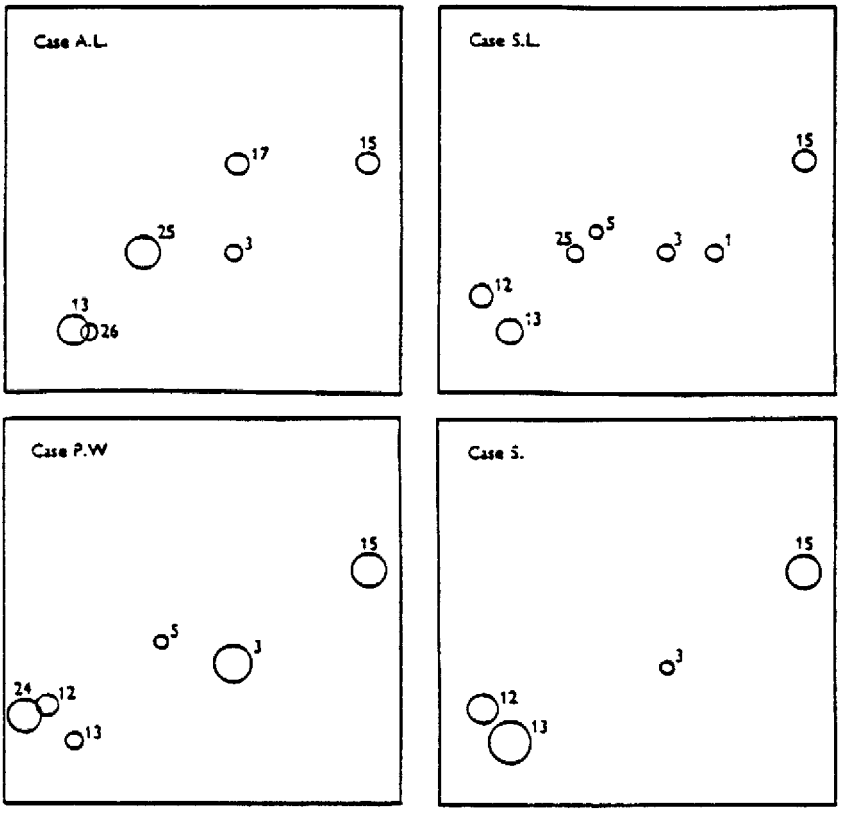

Fig. 5. Chromatograms of 25 μl of urine from four cases of cystinuria. The same conventions are used as in Fig. 4. 25 = On-citrulline, an unknown substance moving very close to the area occupied by citrulline; 26 = ornithine; this substance requires a separate chromatographic step to be performed in order to distinguish it from lysine. It has not yet been looked for in this way in the other three urines. From ref. 2.

Paper electrophoresis

The first separation of serum proteins by paper electrophoresis was published by E.L. Durrum in 1950, i.e., only a few years after the general acceptance of paper chromatography. I would like to suggest that the idea of separating substances by paper electrophoresis was only evident when chemists were already familiar with working with paper strips.

I can still remember when this new technique was very critically viewed by clinical workers and I was informed that of course playing around with paper strips can hardly be considered serious work. Fortunately, Kunkel and Tiselius [8] in 1951 pointed out clearly that in their opinion paper electrophoresis offers several advantages over the Tiselius apparatus.

There are numerous studies of pathological sera by paper electrophoresis, some fairly extensive, such as that by Wuhrmann and Wunderly [9]. For some time paper electrophoresis was used for diagnostic purposes by numerous practitioners. The technique seems to have appealed more to medical men than chromatographers, perhaps because the manipulations required are not so very different

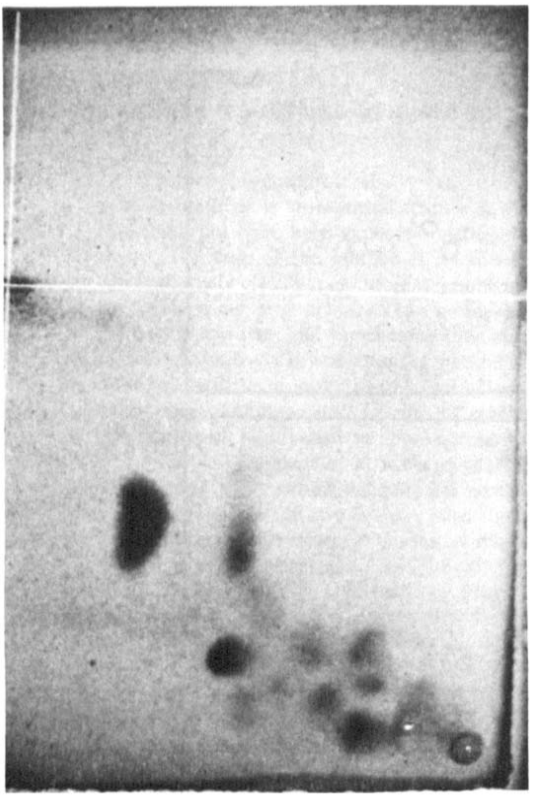

Fig. 6. Two-dimensional chromatogram of untreated urine from a subject affected by an intestinal absorption disorder. Sorbent: sodium tetraborate–sodium tungstate (0.024 *M* $Na_2B_4O_7$+0.0125 *M* Na_2WO_4)-impregnated monolayer of silica gel G-60 (Merck)-syloid 63 (W.R. Grace) (2:1, w/w). Solvent systems: ethyl acetate–2-propanol–water (2:1:1) and ethyl acetate–acetic acid–methanol–water (6:1.5:1.5:1). From ref. 4.

from those used in histology (staining, washing, etc.) and because an electrophoretic analysis was easily completed within a day.

Rather than presenting photographs of electropherograms of pathological conditions, Fig. 7 shows an electropherogram that was kindly sent to me by Dr. E.L. Durrum in 1950. In the 38 years that it has been in my possession, it was used as a bookmark in the report by Dr. Durrum and I find it remarkable that it has stood up to well to ageing. I wonder with which other clinical technique actual results could be preserved for such a long time.

High-voltage paper electrophoresis

High-voltage electrophoresis, that is, at 50–100 V/cm, was developed by Michl [10] and improved apparatus was built mainly by Gross [11] and Werner and Westphal [12]. Although protein separations are poor at high potentials, very good and fast separations of low-molecular-mass substances can be obtained.

The clinical and diagnostic applications of this technique were exploited mainly in the institute of Professor L. Heilmeyer. A book describing their results was

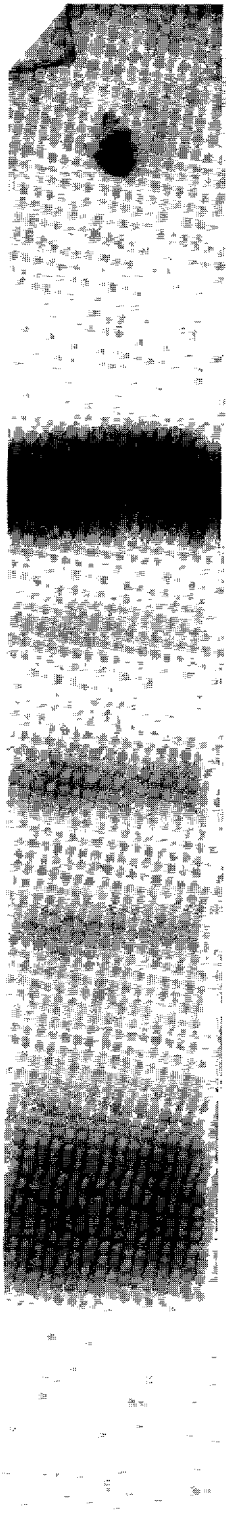

Fig. 7. Serum protein electropherogram made by Professor Durrum in 1950 – it has lasted 38 years with no visible deterioration.

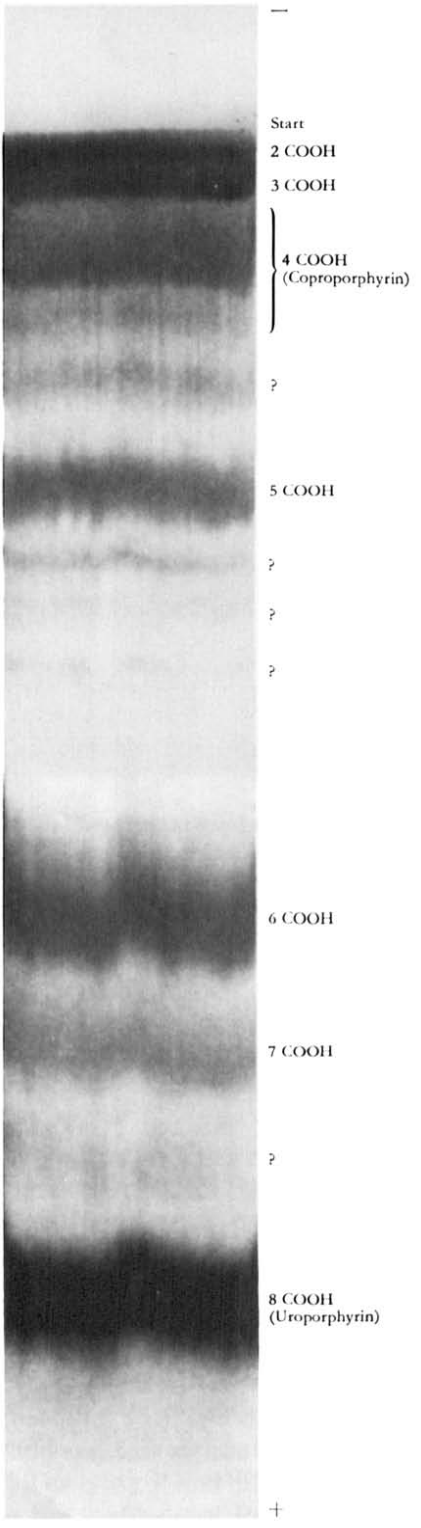

Fig. 8.

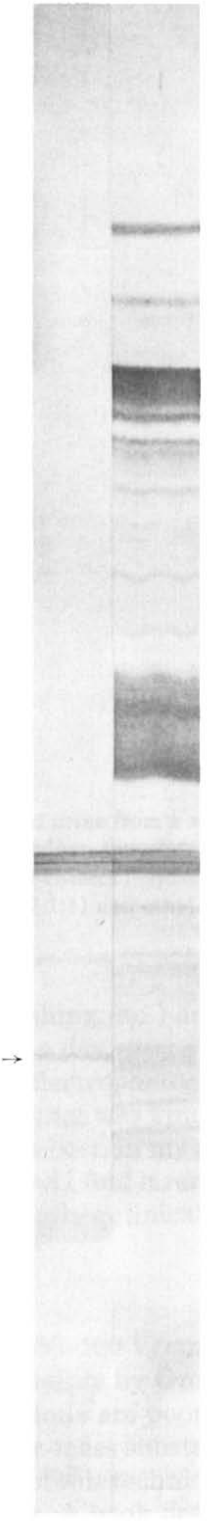

Fig. 9.

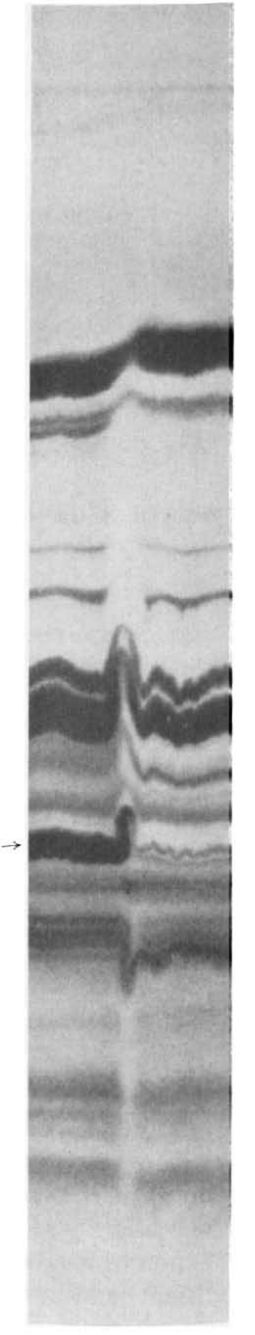

Fig. 10.

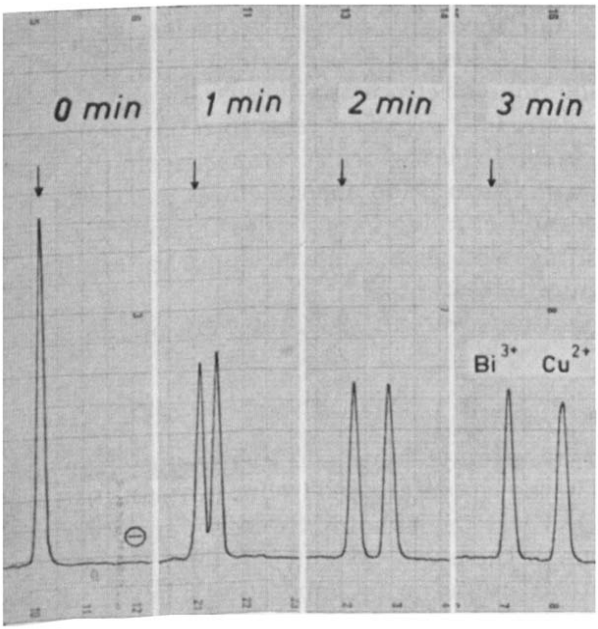

Fig. 11. Separation of inorganic ions (Cu^{2+} and Bi^{3+}) by free zone electrophoresis. Medium, 0.1 *M* lactic acid. Sample: 10 μg of $Cu(NO_3)_2 \cdot 3H_2O$ and 7 μg of $Bi(NO_3)_3$ in 8 μl of 0.1 *M* lactic acid. Inner diameter of electrophoresis tube, 3 mm; current, 8 mA; voltage, 2540 V; temperature of cooling water, 10°C. The arrow indicates the position of the starting zone. The scans were made at the times indicated. From ref. 14.

published by Clotten and Clotten [13] in 1962. The numerous illustrations (e.g., Figs. 8–10) show that "plate numbers" of the order of 160 000 can be obtained in 120 min, separating, for example, 28 ninhydrin-positive zones from a deproteinized serum.

However, this again is a field which for some reason did not receive the attention it merits. The book, written in German, is almost unknown. On a rainy day in Sils-Maria I showed it to Professor J. Porath, who was amazed at the work it contains and that he had not seen it previously.

Capillary electrophoresis

The first extensive study and construction of a high-performance instrument was described in a paper by Professor Hjertén [14]. He separated mixtures of

Fig. 8. Electropherogram of urine from a patient with porphyria cutanea tarda. Zones marked with a questionmark show an intense red fluorescence, but cannot be identified as any of the known porphyrins (EDTA-buffer, pH 8.6, 60 V/cm, 150 min). From ref. 13.

Fig. 9. Electropherogram of urine for the determination of xanthurenic acid (→) (pyridine–acetate buffer, pH 3.6, 60 V/cm, 120 min). From ref. 13.

Fig. 10. Comparative high-voltage electropherogram of normal urine (left) and urine from a patient with intestinal carcinoma. The arrow indicates the zone of oxyindoleacetic acid appearing as a brown-violet spot after diazotation. There is only a very small amount of this compound in normal urine. From ref. 13.

inorganic ions (Bi–Cu in 3 min), nucleobases, nucleosides, nucleotides (3–12 min), serum proteins (36 min), nucleic acids, viruses and cells.

Until Everaerts produced similar zone electrophoretic separations, 12 years later, this technique lay practically dormant [15]. More recently it has been taken up by various groups, using very fine capillaries, which of course present the same problems with electro-osmotic flow and adsorption phenomena as paper electrophoresis, which is after all only a bundle of random capillaries of about the same dimensions and has the great advantage that phenomena all along the "capillary" can be readily observed by spraying with reagents.

To show just how good the separations were in 1969, the work of Hjertén is illustrated in Figs. 11–14.

Instrumental chromatography

Apparatus for the inferometric detection of zones and thermostatic chromatographic columns was developed in the laboratory of Tiselius in 1940–1946 and the instruments were marketed by LKB (Stockholm, Sweden). However, the instruments were not popular. One of them was bought, just after the war, by Professor A. Liberti (and later stood around in my laboratory), but it proved impossible to persuade students to do their thesis work on it. The background and attitudes of chemists at that time were not adjusted to instrumental research.

Soon after there were a host of papers describing refractometers, pH meters and conductivity detectors in relation to either adsorption or ion-exchange columns. It took the approach of Moore and Stein [16] in 1954, that is, about 10 years later, to automatic amino acid analysis to produce the breakthrough that made instrumental chromatography acceptable.

The early machines took about 24 h for a complete separation of amino acids; by 1969 Mondino [17] was able to modify the technique to such an extent that a total analysis took only 220 min. Without resorting to HPLC instrumentation, the speed of this latter technique was almost matched.

To sum up this sketchy historical survey, one can say that there are numerous methods that have been worked out for general and specific diagnostic problems. It is not surprising that some were employed only by a single research group, as the choice of alternative methods available for solving a given group is large. In my introductory course on chromatography destined for students of "sciences naturelles", I discuss about 40 different concepts (and these are only the more important), of which most are possible alternatives, if suitably developed. On the other hand, we have no real guidelines as to which of these will or should give better results.

THE PRESENT

I would like to start here with a story that the late Professor G. Sartori told me. During the Second World War, one female student at the Istituto di Chimica, Rome, was given the task of helping the war effort, namely to investigate whether banana skins could be used as cattle feed. On analysing banana skins for cellulose,

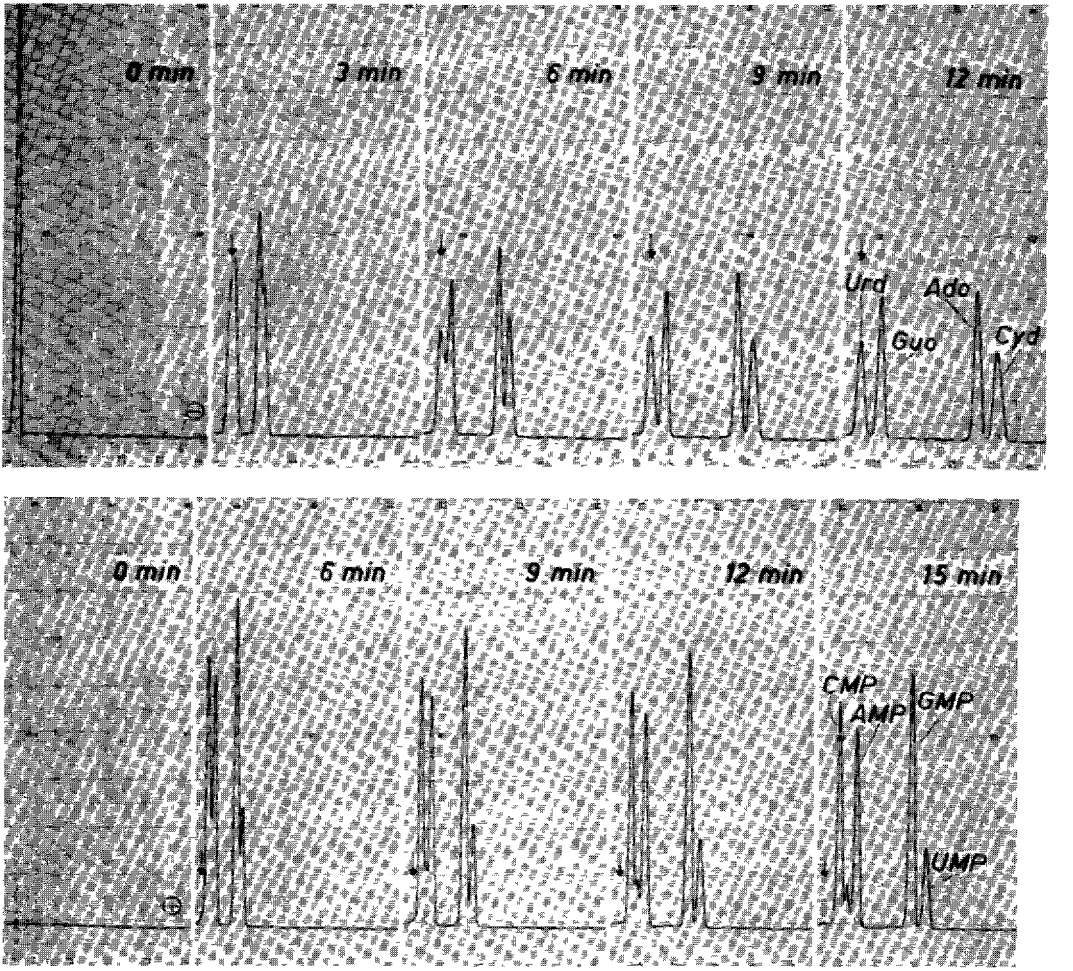

Fig. 12. (A) Separation of the nucleosides cytidine (Cyd), adenosine (Ado), guanosine (Guo) and uridine (Urd). Buffer, 0.05 *M* ammonium formate, pH 2.85. Sample, 4 μg in 15 μl of buffer. Inner diameter of electrophoresis tube, 3 mm; current, 9 mA; voltage, 3520 V; temperature of cooling water, 20°C The arrow indicates the position of starting zone. The scans were made at the times indicated. (B) Separation of the nucleotides uridine-5′-phosphate (UMP), guanosine-5′-phosphate (GMP), adenosine-5′-phosphate (AMP) and cytidine-5′-phosphate (CMP). Buffer, 0.017 *M* ammonium formate, pH 3.01. Sample, 4 μg in 15 μl of buffer. Inner diameter of electrophoresis tube, 3 mm; current, 3 mA; voltage, 2660 V; temperature of cooling water, 20°C. The arrow indicates the position of the starting zone. The scans were made at times indicated. From ref. 14.

hemicellulose, trace elements, sodium, potassium, etc., her conclusion was that banana skins would make excellent cow fodder ... only the cows would not eat it! At present we have available a splendid array of thin-layer chromatographic (TLC), GC and HPLC methods, which could greatly help in diagnostic and drug monitoring in medical practice ... only the doctors do not use them!

On the other hand there are, of course, numerous larger medical research institutes, medical faculties and large hospital laboratories where these techniques

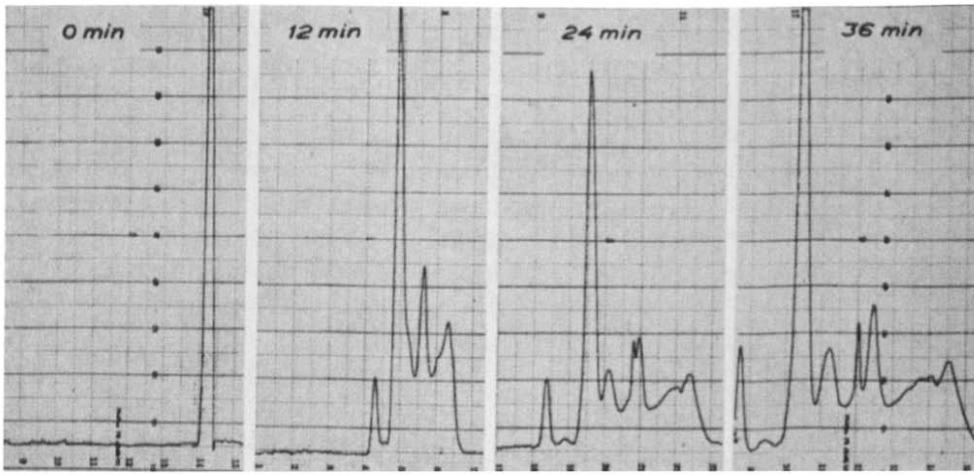

Fig. 13. Free zone electrophoresis of normal human serum. Buffer, 0.1 *M* Tris–HCl, pH 8.7. Sample, 10 μl of a 2.2% solution. Inner diameter of electrophoresis tube, 3 mm; current, 5 mA; voltage, 1320 V; temperature of cooling water, 26°C. The scans were made at the times indicated. From ref. 14.

are extensively used, but in terms of patients treated these are only a small minority. This situation is already evident from the subscriber statistics for the *Biomedical Applications* section of the *Journal of Chromatography*, which shows that the majority of subscribers are indeed those larger institutes and laboratories mentioned above. This indicates that only a small proportion of medical practitioners can have the journal on their desks. Let us examine some of the factors that may contribute to this situation.

Special skills

Most medical men hesitate to embark on chemical instrumental analysis. One of my colleagues told me that he was often approached by medical men who, when told how HPLC or GC is performed, felt that their laboratory skills were inadequate. I do not know of any course for medical undergraduates, or for chemistry students, where the student is allowed to handle independently HPLC apparatus, let alone play around with it. It is, of course, important in undergraduate work that each student has his own microscope and that he is familiar with white cell counts, differential staining, etc. This, however, seems to require fewer "skills" and less scientific background knowledge than modern instrumental methods. So, until familiarity with HPLC and GC methods become part of an undergraduate course, I fear that no headway will be made.

Economics

The capital investment necessary for HPLC and GC, especially when we look at modern "chromatography stations", is high, involving five-figure numbers (in U.S. dollars). If we consider the general price explosion and sophistication of most modern diagnostic apparatus, such as X-ray machines, it would seem that such an outlay could only be justified if the medical practice specializes in a field in which the outlay can be recovered within a short time. If the apparatus is to

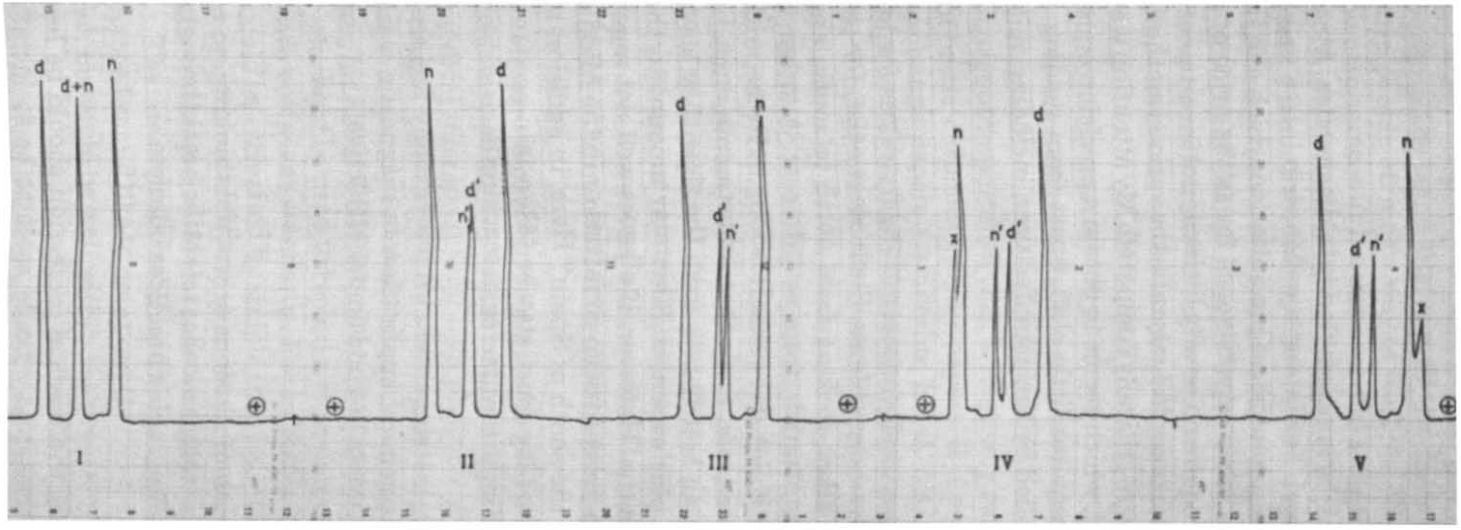

Fig. 14. Three samples run simultaneously: d = denatured T_2DNA; n = native T_2DNA; d + n = a mixture of the two. The samples (10 μl of a 2.2% solution) were applied as three separate zones. Buffer, 0.1 *M* Tris, pH 8.7. Inner diameter of electrophoresis tube, 3 mm; current 5 mA; voltage, 1320 V; temperature of cooling water, 26°C. The scans were made at intervals of 6 min. In scans I, III and V the direction of carriage movement was from right to left; scans II and IV involved movement of the carriage from left to right. Consequently, consecutive patterns are reversed. The distance between the zones d and d' remained constant during the run, as did the distance between the zones n and n', thus indicating that n' consists of native and d' of denatured T_2DNA. Note the sudden appearance of peak N. From ref. 14.

serve only two or three times a month it will certainly be outside the reach of the general practitioner.

Competitive methods

Numerous pharmaceutical companies have diversified into diagnostics. Most of these are of bewitching simplicity, such as dipping a paper strip into a urine sample and reading off a colour or an immunological precipitation which can be observed after several hours. Many are fairly expensive, as far as the price of a single test is concerned. However, they require no or very little capital investment.

In a recent thesis at the Faculty of Pharmacy in Lausanne the various methods for determining theophylline in blood were critically compared. My attention was drawn to the "Miles Seralyzer" method, which seems to obtain a precision equal to that of most HPLC methods and requires little skill, apart from reading a photometer. Also, the whole kit is supplied by the manufacturer, including suitable standards.

Maintenance

In certain areas of the world where a critical concentration of services for instrumentation exists, maintenance poses no problems. Such areas exist, for example, between North Germany and Milan, looking from north to south, and from the Paris region to approximately Munich, from west to east. Another is situated in the U.K. and another in the U.S.A. However, working in Rome, with instruments from well known companies, one finds that adjustments must be made by their technicians, who charge an hourly rate from the time they leave their base in Milan until they return, plus expenses. This may amount to the salary of a research worker for 3–6 months. However, if all goes well not much time is lost. But what is the situation in most African countries or in the Middle East, or closer to home, in Palermo or the south of Spain? Here, in addition to the cost, the inevitable considerable time lag must also be taken into account, making the servicing of instruments an important factor in reaching decisions on alternative methods.

To summarize the situation: modern instrumental chromatography has features which definitely speak against its general application in diagnostic work and at present make the option of alternative methods more attractive.

THE FUTURE

The future, by definition, is a projection of illusions or wishful thinking, so to consider it we must also assume that no new technical or scientific breakthroughs will occur that will make present chromatographic practices obsolete.

High-performance liquid chromatography

It seems imperative that in the future the medical profession should be less hostile to instrumental analysis. The easiest way would seem to be to include HPLC and GC in the undergraduate curriculum, but in such a manner that the student "gets the feel of it". The modern method of arranging chemicals and

apparatus for one afternoon in front of the student and then to let the whole class perform the same analysis, in the form of a macabre ballet, seems intended to turn people off a new way of working. We feel that each student should have his own apparatus at his disposal and have time to "play around with it".

This is definitely against the present trend and would also involve considerable costs. Hence I would say that in the near future there is little hope that students will become enthusiastic over instrumental chromatographic methods. The position could change if manufacturers of apparatus considered the importance to them of such training and would collaborate, also financially, in such a project. This, however, would involve a "perestroika" of the present attitude.

Flat-bed chromatography

I tend to agree with the ideas of the late Professor Ian Bush that paper chromatography offers the best possibilities of application in clinical analysis. One aspect of importance that is relatively easy to achieve would be the development of better chromatographic papers. It is evident from some of the commercial ion-exchange papers (Amberlite SB-2, for example) that it is possible to prepare papers with as many theoretical plates per centimetre as we have with the present HPTLC plates. In the literature only some very preliminary laboratory-scale experiments by Dr. K. Macek can be found. When long ago I suggested this project to the then most important paper manufacturer, I was told that "I do not know anything about paper making". Perhaps in the future another generation of paper manufacturers will be more broad-minded and turn out the required high-performance papers.

A. Kuhn, of my laboratory, has been rediscovering the possibilities of using aqueous solvents as eluents. Development on cellulose thin-layer plates takes about 10–20 min and the type of separations that can be obtained is shown in Fig. 2 in ref. 18.

Hence we could propose to the medical practitioner a development technique which requires no capital outlay and is as fast as HPLC. I would not encourage medical men to do their own photometric evaluation of the chromatograms. Instead, I would propose a viewing cabinet with two normal and two UV lamps and a permanently focused Polaroid camera. Such a cabinet could be put on the market for about US$ 150, of which the cost of the camera would be about US$ 100.

It could be used to record a chromatogram of several "unknowns", side by side with sufficient standards. For immediate use the spot intensity and/or spot size could be estimated by simple inspection with an accuracy of $\pm 20\%$, and the photographic record could then be evaluated in a central laboratory at a later date, if the investigation of the case warrants it.

The ideas proposed here are a topic in which I am personally interested and hence I would like to point out that they are only some of a whole range of variants which would be well within the present abilities and inclinations of medical men.

The future of the Biomedical Applications section of the Journal of Chromatography

When this section was founded it was also with the hope that chromatographic methods would become a generally applied technique in medical practice. For

various reasons, some of which have been outlined above, it remained in the research laboratory and in larger clinical laboratories and could not compete with alternative methods for the majority of diagnostic tests.

Thus, in my opinion, the *Biomedical Applications* section could continue, as at present, as a specialist journal, serving a relatively small group of laboratory scientists or it could alter its appeal by following some possible steps:

(1) The Editors could take active steps to encourage the general acceptance of suitable chromatographic methods. This could be done by organising symposia, inviting suitable reviews and inviting specific papers. Such steps are considered unusual today, the general picture of a scientific Editor being that of a "rompiscatole" (it is impossible to do this term justice in translation) who suggests shorter introductions or fewer figures, but who refrains from making editorial policy. This picture would have to undergo revision.

(2) The scope of the journal could be enlarged to cover modern diagnostic methods in general. This would offer the advantage that the journal could deal with a very important field, namely the critical comparison and evaluation of different analytical methods for a given problem.

This may seem platitudinous to an analytical chemist, but in my editorial correspondence there have been several cases of eminent medical scientists who have never heard of the percentage of false positives and the percentage of false negatives in doping analysis. In diagnostic analysis the situation is just as bad but more difficult to assess, as it leads to fewer court cases.

Such a journal, if correctly handled, could have an impact as great as that of other outstanding medical journals, such as the *British Medical Journal* and *The Lancet*.

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