Electrophoresis over the Seventeen Brugge Colloquia*

By

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Introduction

I am greatly honoured by the invitation to the XXth Anniversary Meeting of your Society of Electrophoresis. As a younger brother of only 17 colloquia, I wish to congratulate you, the elder boy of 20, on his anniversary. Omedeto gozaimasu. The Committee of the Brugge Colloquia hopes that this contact, which is still hampered by the large distances between Europe and Japan, will lead to fruitful collaboration between research people.

A discussion of electrophoresis based on the seventeen Colloquia on Protides of the Biological Fluids held each year since 1953 at the beginning of May in Brugge, Belgium (1–17) is the occasion to make a survey of some technical developments and of the methodological progress which occurred during the past years referring more than once to the Brugge meetings.

As our personal interest in electrophoresis was at first with technical problems regarding zone electrophoresis and later electrochromatography on paper and accompanied simultaneously by a growing interest in lipoproteins, this review will stress the problems and relate to examples and results from these particular fields. A complete summary or synthesis of the seventeen meetings was anyhow impossible whereas someone's personal engagement in a field may be more instructive even through its shortcomings.

I. From Boundary to Zone Electrophoresis

Properties of a molecule such as molecular weight, size and shape are extensively used for differentiation of biologically active molecules in the UCF (ultracentrifugation) and in chromatography. These properties are the consequence of chemical composition and structure from which also derive the electrical charges present on the surface of protein molecules in solution interacting with the solvent. The resulting net charge is used to move molecules in an electrical field during any of the electrophoretic procedures.

A general remark valid for any separation method is that each method is accompanied not only by the advantages but also by the drawbacks of the principle on which its selectivity is based. As an example and as far as differentiation is based on mobility, the same $\alpha$-lipoprotein behaves on agar as an $\alpha_1$ in fresh serum and as a $\beta$ (rapid) fraction in old serum due to a loss of fatty acids during storage (18).

Inversely two different proteins can present the same mobility under given conditions. So more than one method, and inside one method several technical variants are complemen-

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tary in order to separate and to identify a fraction completely. The purity of a fraction is
often only a temporary status maintained for as long as a new method uncovers some hidden
heterogeneity. As a result of this situation a wealth of electrophoretic procedures was
developed and all of them still have their own domain and none can be forgotten.

The decisive development in the practical application of an electrophoretic separation
procedure to protein mixtures was the free boundary method of Tiselius who overcame
thermal convection by dipping the whole cell in a cooling bath. At the Academic Lecture
of the fifth Colloquium, he said “I cannot help pointing out the great kindness of Nature,
who has provided a density optimum of water just where we need it (+4°C) to suppress
thermal convection in free electrophoresis”. In this way it became possible to study solu-
tions of considerable conductivity, such as blood serum, and to increase the potential gradient
in the electrophoresis tube considerably (19).

The interest in the anti-convectional property of paper—known since König (20)—was
revived by its success as a tool in chromatography on paper and applied to electrophoresis
(21). Great attention was devoted to the technological aspects of paper electrophoresis and
this experience was transferred later to gel and other substrates (22).

A survey of the interaction of the factors involved in migration on a supporting medium
can be summarized in a comprehensive pattern of the factors influencing migration (Fig. 1).

![Diagram of migration factors](image)

Fig. 1. Schematic representation of the factors inducing
migration during electrophoresis.

The electrical energy is the origin of two different physical factors: the first factor is the
field itself which causes migration of the buffer ions—ionophoresis—simultaneously with
migration of the macromolecules and the second factor is heat, which increases not only the
mobility of the charged particles, but also evaporation causing concentration of the buffer.
The original conductivity, pH, and ionic strength of the buffer tend to be modified through
ionophoresis. The buffered medium has anticonvectional properties and also initiates pro-
cesses, such as adsorption, which influence migration.

There is another important factor, namely the buffer flow inside the buffered medium,
which influences migration. This flow has three main sources: (1) evaporation into the
chamber favoured by heat production from electrical energy, (2) the hydraulic effect of the
level of the buffer vessels with respect to one another and to the level of the paper and lastly
(3) the electro-endosmotic effect as the interaction between the electrical field and the buf-
fered medium. This can be demonstrated nicely by adding glucose to the buffer and the
effect can be seen as a flow towards the cathode (Fig. 2) (22).

Power supplies have been the subject of big concern. Once the ill effects of Joule’s heat
due to a residual alternative ripple had been eliminated by good electronics, there was still
the choice between constant voltage and constant current. Under constant voltage and in open strips, gels or curtains, evaporation—with concomittant concentration of buffer solvent and conductivity—increases with increasing voltage and easily ends up in a catastrophic buffer concentration on the supporting medium (Fig. 3). With constant current, however, the amount of energy admitted to the substrate does not vary with increasing conductivity. The voltage drops and there occurs no harm to the equipment. The influence of room temperature, which is considerable at constant voltage, is neutralized under constant current conditions acting as an outstanding feedback regulation.

II. Electrophoresis on a Supporting Medium

The success of paper which enabled zone separation, resulted in the search for analogous types of supporting media out of which even non-electrophoretic methods such as gel filtration eventually developed (Porath 23). But in spite of the introduction of many new supporting media, PAPER is still extensively used. During electrophoresis on paper, however, chromatographic effects such as absorption, ion exchange and partition chromatography affect the separation. In this respect the saturation of the paper with an albumin containing buffer, as introduced by Lees and Hatch (24) lowers the absorption to the paper and favours the separation of lipoproteins into the Fredrickson patterns (25) (Fig. 4).

The introduction of IMMUNOPHORESIS IN AGAR by Grabar and his coworkers at the second Colloquium (26, 27, 28, 29) is one of the major events in our Colloquium history.
It is utterly impossible to review the achievements of this method and to describe its impact on our knowledge of proteins.

However, in spite of its tremendous achievements, immunology does not have a simple answer for all the problems of molecular variation and some critical remarks about the dogma of immunological differentiation may be useful. One cannot expect to find fractions other than those to which the host was sensitized. If under pathological conditions an antigenic structure is slightly modified, covered up by some prosthetic groups or uncovered by the lack of them, this might well mean that pathological body fluids may contain a given protein but in a slightly different and immunologically unrecognizable form. Due to a different setup of the immunological sites, these may not react with the antiserum prepared with the normal protein.

Let us take an example in the field of lipoproteins. Recently Gotto demonstrated the existence of a partially degraded or incomplete apoprotein in plasma of ABL (A-β-lipoproteinemia) (30). Such a situation of circulating lipid free apoprotein is comparable to heavy chain or light chain diseases in the immunoglobulin system. In this context it seems that analogies between protein systems should be looked for and this might lead to a simplification of our protein concept which is now still in the process of becoming more complex every day. The study of protein fragments shows building blocks common to proteins with very different functions. Of course immunology will be one of the tools in this process of simplification of the complexity it created itself.

With the aid of radio tracers Lang demonstrated how precipitation in agar may be due to interference of non-immunological factors (31). Non-immunological precipitation lines with starch and other reagents were obtained by Gillert (32) and interpreted on a precipitation line of the amylopectin in the starch with given lipoproteins. This COMBINATION-ELECTROPHORESIS, as it was called, is the sister of immunoelectrophoresis. Some reagents used in that method could be applied to specific gel filtration which now use antigens or enzymes attached to the dextran network, the so called unsolubilized enzymes. Immunological interference and association with accompanying proteins can lead to false conclusions not only on antigen-antibody relationship but also on presence or absence of antibodies in a given antiserum.

Out of this critical study of precipitation inhibition and coprecipitation the overtaking electrophoresis also called CROSSED-OVER ELECTROPHORESIS, was developed by Lang (33) and used as a quantitative immunological method. The idea itself of taking advantage of the crossing over of fractions had been maintained by ourselves (22) and used extensively by Nakamura (34). Culliford used this technique, which is very economical with the antigen, in the identification of blood stains for forensic purposes (Fig. 5) (35).

The irregular and complex composition of agar was overcome by the introduction of agarose. Araki had shown how agar consists of two components: a neutral main com-

Fig. 5. Schematic representation of crossing-over electrophoresis.
ponent or AGAROSE, which is a linear polysaccharide free of ionizable groups, and agarpectin (36). The isolation of agarose from agar was difficult and solved by the precipitation method of Hjertén (37). Uriel demonstrated that agarose is an advantageous replacement of agar as a substrate for electrophoretic separations and immunochemical analysis and developed a series of ingenious detection methods for enzymes and other active groups (38).

Although lipoproteins could not be separated on agar because of the precipitating effect of the sulphate groups, clear separation of all lipoprotein groups can be obtained on agarose.

An important aspect of gel electrophoresis is the molecular sieving effect which is particularly pronounced in POLYACRYLAMIDE and STARCH GEL. The electrophoretic mobility of a protein molecule is determined by a balance of the electromotive force applied to it and the frictional retardation which it undergoes during its movement through the buffer solution. In buffers employed without support or stabilized by carriers as paper or agar, frictional retardation is so small compared to the net charge that proteins of different molecular size but equal electrophoretic mobilities migrate together. When the stabilizing medium consists of gels with well defined pore size, separation is the resultant of mobility and frictional retardation. Such gels acting as molecular sieves permit very detailed resolution of complex protein mixtures. The most widely used medium of this sort is the gel of partially hydrolysed starch proposed by Smithies (39) and a gel of still finer pore size obtained by polymerizing acrylamide as described by Weintraub and Raymond (40), the resolving power of which outranks that of starch gels. In so called disc electrophoresis on polyacrylamide columns perfectly sharp patterns are obtained. An example is the check on the separation of native albumin into monomers and dimers as shown in Fig. 6.

An important advance has been the introduction of CELLULOSE ACETATE as a supporting matrix by Kohn in 1957 at the 5th Brugge Colloquium (41). The new supporting

Fig. 6. Separation of BSA in its monomeric and aggregated components on polyacrylamide gel.

Fig. 7. Transparency of cellulose acetate when dipped in white oil.
medium was more uniform than the various grades of filter paper and is completely transparent after immersion in white oil (Fig. 7). In the clinical routine laboratory, agar—although introduced earlier—did not develop into a competitor for paper because the manipulations such as the pouring, handling, staining and drying of weak gels are unpractical. With cellulose acetate these disadvantages do not exist and the technique was rapidly miniaturised to the semi micro and later to the microscale, allowing the handling of more samples on a small bench space in a shorter time (42). The method was also adapted to immunophoresis (43).

Regarding quantitation, the protein fractions can be scanned after staining with Ponceau S. For lipoproteins, however, the cellulose acetate technique failed as the proposed staining tricks result in a questionable increase in the number of lipid-positive fractions.

III. Free Zone Electrophoresis

Any stabilizing medium—either paper or powder or even a density gradient—can interfere not only with migration and diffusion but also with the material under separation. To avoid such interference stabilization of free zone against convection by slowly rotating a horizontal tube was introduced by HJERTÉN (44) (Fig. 8). If a volume element contains protein and

![Apparatus for free zone electrophoresis according to Hjertén.](image)


has for this reason a higher density than the surrounding solution, this volume has a tendency to fall from the ceiling of the rotating tube wall. Half a revolution later it still falls but is now pressed against the bottom of the tube. Half a period later it hangs onto the ceiling again. In this way the gravity field eliminates the disturbing influence of convection. This idea is one of the wittiest combinations of simple physical laws in the field of electrophoresis. By scanning the tube in the U. V. during the run, accurate mobility determinations were made.

Another type of stabilization was introduced by Kolin (45). Its principle is magnetic
rotation. Relative to the gravitational field, magnetic rotation periodically inverts the vortices generated by thermal convection (Fig. 9, 10). Two bar magnets $M_1$ and $M_2$ are coaxially aligned so that like poles $N$ and $N$ are facing each other and are separated by a soft iron cylinder $m$. The vicinity of the curved surface of the cylinder $m$ becomes the site of a substantial radial magnetic field whose field lines are perpendicular to the cylinder.

Fig. 9. Suppression of thermal convection between two horizontal concentric cylinders. The temperature of the inner cylinder IC is higher than that of the outer cylinder OC. (a) Vortices in a stationary annular column. (b) The vortex on the right (solid line) is retarded when transferred to the left (dashed line) by a 180° rotation of the annular liquid column. (c) B: magnetic field vectors of a radial magnetic field. The electric current flow through the annular buffer cylinder bounded by cylinders IC and OC at right angles to the plane of the paper. Electromagnetic forces rotate the liquid counterclockwise. (I) injector through which mixtures of particles to be separated are injected.

Fig. 10. Simple non-collecting cell for magnetically stabilized electrophoresis. E: graphite rods serving as electrodes; A and C: electrode compartments; B: cooling bath compartment; $M_1$ and $M_2$: bar magnets; N and S: magnetic poles; m: soft-iron cylinder; R: annular space between outer and inner lucite tubes OT and IT; H: opening in OT through which injector I is inserted; T: tubing supplying mixture to be separated to the injector. Only the negative binding post is shown. The connections to the electrodes E⁺ are not shown.
surface. Around this central core and between two concentric lucite tubes an annular buffer zone is connected to the electrodes. Particles injected into the annular compartment are moved by a component of two forces: their horizontal electrophoretic mobility and the rotating flow induced in the buffer column by the magnetic field acting on the electrical current. As a result the component moves along a spiral path. Rapidity of separation is astonishing because the thread of the screwlike motion doubles at each turn. Thus the distance between two components doubles at each turn and a collecting device can be adapted. This ingenious technique is efficient for separating particles.

In this context it is interesting to mention **ELECTROMAGNETOPHORESIS** which takes advantage from the force exerted upon a particle suspended in a fluid when it is submitted to a direct electrical current and to a magnetic field perpendicular to this current. The same effect can be produced by an alternating magnetical field and is then based on differences in dielectric constants. Suspended particles, such as erythrocytes, different from the surrounding fluid in the value of their electrical conductivity, dielectric constant or magnetic permeability can thus be separated. However, the available force is limited by

![Diagram](image)

**Fig. 11.** Comparison of the migration forces during centrifugation and electromagnetophoresis (E. M. P.).

The gravitational force $981 \text{ g cm/sec}^2$ is taken as a force unit.

- $g$: the acceleration of gravity
- $J$: the current density

the magnetical induction and by the current intensity. Under optimal conditions with permanent magnets of 20 kGauss and a current of 0.25 A/cm² an acceleration of about 65 g can be reached against an acceleration of 500 g commonly obtained by a conventional centrifuge (Fig. 11). However, with the development of stronger magnetic fields this principle would become operational.
IV. Electrofocusing

Electrofocusing in natural pH gradients is the latest development in electrophoretic separation and a technique of growing importance for the fractionation and characterization of proteins. Electrofocusing or isoelectric focusing, isoelectric fractionation, isoelectric separation, stationary electrolysis, isoelectric condensation and isoelectric analysis are some of the names given to the phenomena occurring to ampholytes in a pH gradient influenced by an electrical field. We selected the shortest single word: electrofocusing (EF) and derive from this immuno EF, gel EF and so on.

The idea was described in theoretical papers long before its practical use for the purpose of separating substances was talked of (46); the main obstacles to its development being of a technical nature concerning convection in the electrolyte system and the stability of the required gradient. Svensson and Vesterberg introduced a natural pH gradient composed of a family of synthetic ampholytes covering a pH range at regular narrow pI intervals and stabilized by a sucrose gradient. These ampholytes—so-called ampholines—are aliphatic polyamino polycarboxylic acids of somewhat mysterious composition (Fig. 12) (47, 48).

\[
-\text{CH}_2-N-(\text{CH}_2)_x-N-(\text{CH}_2)_x-NR_2
\]
\[
(\text{CH}_2)_x \quad R
\]
\[
NR_2
\]

\[x = 2 \text{ or } 3\]
\[R = \text{CH}_2-\text{CH}_2-\text{COOH} \text{ or H}\]

Fig. 12. General formula for the ampholine carrier ampholites.

![Graphs showing separation of BSA monomers and dimers by electrofocusing](image)

Fig. 13. Separation of BSA monomers (BSA), BSA dimers (BSA), desalted BSA monomers and desalted and defatted BSA monomers by electrofocusing in a 3–10 pH gradient.
Because the mobility of the ampholines is zero at their isoelectric point and if convection is reduced towards zero by the sucrose gradient, the pH gradient—once established—forms a continuous and stable background for the migrating proteins and peptides which must in turn assemble in the pH zone corresponding to their own isoelectric point. At the isoelectric level of a fraction in the column an active equilibrium is formed between the focusing (electric) and the spreading (diffusion) forces. As a result the focused zone remains at constant sharpness for an unlimited period of time. Sharp resolution is a resultant of a high angle of mobility over the pI point and of low diffusion, as it happens to work with proteins but not with small ions such as amino acids. Voltage acts with the square root of its value and the shallower the pH gradient the better. A mathematical treatment of resolving power was given by Svensson and is applicable to any separation method (49).

As an example of electrofocusing results, runs of monomers and dimers of albumin and of desalted and defatted albumin are shown in parallel in Fig. 13. Monomers and dimers give each two identical peaks with the same pI. The desalted monomers yield three peaks which are also found after defatting but with a conspicuous decrease of the most acidic fraction as a result of the loss of free fatty acid and a compensating rise in the more basic fraction. Such differences are not observed with other techniques and prove the sensitivity and the selectivity of the electrofocusing method. The microheterogeneity of immunoglobulins—the significance of which has still to be checked—was also demonstrated (Fig. 14).
At the XVIth Colloquium we have shown the subunits of low and high density apoproteins with this method (50).

Recently the anti-convectional sucrose gradient was replaced by polyacrylamide (PAA), under the form of small columns by Riley (51), who showed the polymorphism of LDH activity in human liver and heart extracts with this method. A PAA thin layer lying face down on the electrodes (Fig. 15) was introduced by Awdeh (52) and used in the separation of various antibodies.

This new method raises its own problems such as the absorbance of the ampholines in the U. V. which can be avoided by scanning at 280 nm (Fig. 16). A more complex problem is the interaction between the ampholine and the polyelectrolyte under examination. This problem requires careful check of the separated fractions by electrophoresis, immunophoresis, enzymophoresis and other methods keeping in mind that some ampholines might be bound to the active sites of the protein in spite of prolonged dialysis. These problems were extensively discussed in a Round Table and Workshop at this year’s XVIIth Meeting (17).

V. Electrochromatography

The last point in this survey is a description of electrochromatography. A combination of electrophoresis with chromatography is applied in a technique where a horizontal electrical field acts on a vertical buffer flow supported by a substrate such as cellulose powder (53) or paper (54). Each particle migrates according to the resultant vector of the horizontal electrophoretic velocity and the vertical velocity of the buffer (Fig. 17). The method can be
used in two ways. If the sample is applied in a continuous way, the separated fractions can be collected in tubes at the bottom of the curtain (Fig. 18) in which case the method is called "continuous" electrophoresis. If only a spot is applied a two dimensional pattern will develop and we called this application "star" electrophoresis (Fig. 18) (55).

This same method was described as free two dimensional continuous electrophoresis by Barollier (56) and also by Grassman and Hannig (57). A buffer flow is injected between two horizontal glass plates and submitted to a transversal electrical field. A sample injected into the buffer stream is separated continuously and collected. Very sharp fractions are thus obtained but the yield of this preparative method is rather low.

As mentioned for zone electrophoresis on paper the mobility of a large ion is partly counteracted by absorption, and for this reason, in two dimensional setups, two fractions with identical electrophoretic mobility can proceed at a different speed along the same pathway, thus giving rise to a better separation than in zone electrophoresis on paper (Fig. 19).
Contact prints of the curtain onto substrate containing gel can be made to study enzyme activity (58).

However, the interweaving of electrical field and buffer flow required by this method had its own technical problems. It is a first requirement to obtain a perfect electrical field. This was realized by designing cascade electrodes where the electrode wire is washed continuously and moreover where no buffer spills from the electrode onto the curtain (59) (Fig. 20).

At the same time, there was a problem with the electrical shunt between the top of the two electrodes across the chromatographic buffer feed. This was in turn avoided by using a trickle feeder so that the connection between the two electrode wires offers the same resistance at any height of the electrode (60) (Fig. 21). Proof of the correctness of the
chromatographic buffer flow and of the cascade electrodes under these circumstances is
given by the parallel eosine spots under alternative current (Fig. 22). Under normal working
conditions the linearity of the fractions shows the validity of these two improvements.

Applications of electrochromatography are numerous and a few examples are the separa-
tion a. of micro ions, b. of isotopes, and c. of biological fluids such as serum where the method
acts as a desalter and leaves the fractions contaminated with only the buffer ions (Fig. 23, 24,
25).

Fig. 24. Separation of Na^{24} and I^{131} in serum.

Fig. 25. Schematic representation of the separa-
tion of human bile and gastric juice
taken from color originals.

Fig. 26. Comparison between ECG, UCF and
zone electrophoresis patterns.

Fig. 27. ECG pattern of human serum lipopro-
teins in a case of obstructive icterus (A)
and in a Fredrickson type III. (B)
Schematic representations taken from
color originals.

A comparison between electrochromatographic, ultracentrifugal and zone electrophoretic fractions of lipoproteins is given in Fig. 26. Patterns analogous to the Fredrickson patterns and often more detailed ones are obtained with pathological sera. An example of Type III and of the peculiar pattern proper to obstructive jaundice with its characteristic monolipoprotein are shown in Fig. 27.

In the study of lipoproteins, electrochromatography was used to separate the lipoprotein fractions in view of the subsequent analysis of their lipid content. The way of procedure
is aimed at the establishment of the fatty acid patterns from the different lipoproteins and
from their lipid classes within the same serum (61) (Fig. 28). A spot corresponding to 0.45 ml
prestained serum previously concentrated on the ultrafilter is applied to the curtain. After
3 hours of migration the \( \alpha \)- and \( \beta \)-lipoprotein zones are cut out of the wet curtain, eluted
with chloroform/methanol and treated in the same way as whole serum. To establish the
fatty acid pattern of cholesterol esters, triglycerides or phospholipids, serum as well as the
cut out \( \alpha \)- and \( \beta \)-lipoproteins are extracted, analysed by TLC and subsequent gas chrom-
atography. In order to obtain sufficient $\alpha$- and $\beta$-lipoprotein-lipid material, several curtains are cut out and the extracts combined.

![Diagram of lipid and lipoprotein analysis](image)

Fig. 26. Integrated lipid and lipoprotein analysis.

<table>
<thead>
<tr>
<th></th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
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<tr>
<td>Serum</td>
<td>0.39</td>
<td>0.26</td>
<td></td>
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<tr>
<td>Prestained serum</td>
<td>0.39</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>After separation with electrophotography</td>
<td>0.38</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>After separation under $N_2$ atm.</td>
<td>0.38</td>
<td>0.25</td>
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Fig. 29. Control of fatty acid ratios during manipulation.

The validity of this procedure in as far as the fatty acid analysis is concerned depends on the preservation of unsaturated fatty acids during the procedure as proved by the unchanged $18:0/18:1$ and $18:0/18:2$ ratios (62) (Fig. 29). Results obtained on the human male and also on baboon males fed with a control and an atherogenic diet are given to demonstrate the type of information that can be obtained. In the human (Fig. 30) unsaturation is most pronounced in the $\beta$-lipoproteins but is less influenced by age than in the $\alpha$-lipoprotein. This is conspicuous in the $\Delta:0/\Delta:1\Delta:2$ and still more so in the $18:0/18:2$ ratio. In the baboon on the contrary (Fig. 30), the ratios are not only very different from the human value, but desaturation occurs under the influence of an atherogenic diet.

Thus the two lipoproteins were characterized as different entities having a personal fatty
Acid acid pattern reacting on their own and in a different way under the influence of diet or drugs. It is evident that this type of information stressing the individual fatty acid patterns inside individual lipoproteins has an informational potential which is lost in the analysis of whole serum (63, 64, 65).

## Conclusion

As a general conclusion it can be stated that electrophoresis is at the origin of two lines of work, one based on the theoretical implements of the method and a second one of biological and clinical interest.

A study of mobility in the electrical field is complicated by the interaction with the anticonvensional medium but in reaction to this problem some useful developments were developed such as the introduction of rotational gravity as an anticonvensional method and the combination of flow with a perpendicular electrical field on the curtain as in electrophotography and later in the free two-dimensional methods. The introduction of gels has been a major advance in spite of or perhaps thanks to the fact that it combines molecular sieving with separation due to electrical charge.

The presence and the function of carrier ions responsible for conductance but also for maintaining pH stability within a reasonable range during the run has recently been modified by the introduction of newly synthesized ampholytes through which a nearly continuous pH gradient can be obtained between the electrodes. As a result of this new situation isoelectric fractionation becomes a reality. As a whole it may be stated that the theoretical situation on which electrophoresis is based has been used to the utmost.

From the biological side—which was only touched during this survey—the patterns have been studied for all kinds of biological fluids in many physio-pathological circumstances and for many species. The introduction of immuno-precipitation after the run was an achievement in the establishment of pathological data and is also a means of control of the purity of isolated proteins. Specific antisera paved the way in the study of molecular variation and of submolecular units. Work on the immunological filiation of a given protein has grown into a useful tool in the phylogeny of life.

These facts and methods were presented over the years at the Brugge Meetings on

<table>
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<th>FA ratio</th>
<th>Human α₁-LP</th>
<th>Human β-LP</th>
<th>Baboon α₁-LP</th>
<th>Baboon β-LP</th>
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<tr>
<td></td>
<td>Av. 25 y</td>
<td>Av. 65 y</td>
<td>Av. 25 y</td>
<td>Av. 65 y</td>
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<tr>
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<td>0.90</td>
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Fig. 30. Fatty acid pattern from alpha and beta lipoproteins in human and baboon males.
"Protides of the Biological Fluids" and this account hopes to stimulate active connection with the Japanese Society of Electrophoresis.

**Bibliography**