PONTIFICIA
ACADEMIA
SCIENTIARVM

COMMENTARII

Vol. II N. 38

G. B. MARINI BETTOLO

ARNE TISELIUS
(1902-1971)

EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

PONTIFICIA
ACADEMIA
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COMMENTARII
Vol. II - N. 38
pag. 1-24

ARNE TISELIUS
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Pontifical Academician

SVMMARIVM — Agitur de publico praeconio quod Auctor — cum Sessio Plenaria Pontificiae Academiae Scientiarum haberetur — die 14 Aprilis 1972 recitavit.

The premature disappearance of Professor Arne Tiselius, is not only a great loss for the Pontifical Academy of Sciences, who had the honor to have him as one of the most distinguished members, but also for world Science.

Many scientists have the privilege to bring a contribution, sometimes very important, to the progress of Science, but only a few have had the chance to impress on Science itself a new direction, opening with their work new avenues: Arne Tiselius belonged to the latter.

In effect we owe to Tiselius our fundamental knowledge of the chemistry and physico-chemistry of proteins, which he could obtain in a considerable interdisciplinary effort applying

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Paper presented on April 14th, 1972 during the Plenary Session of the Pontifical Academy of Sciences.

a physico-chemical approach to the solution of biological and biochemical problems.

His discoveries have had a great impact not only on pure Science but also on the development of modern medicine and in particular of immunology, enzymology, pathology and therapeutics, as well on many other fields of practical application, as clinical chemistry.

* * *

Arne Tiselius was horn in Stockholm, on August 10th 1902. From his father, a well known mathematician he had herited the enthusiasm for science. He followed the courses at the University of Uppsala, where in 1924 was graduated Fil. magister in Chemistry, Physics and Mathematics.

He began his scientific carrier as assistant to a famous scientist, The Svedberg, who had dedicated the greatest part of his activity to the study of ultracentrifugation as a mean for the separation of proteins.

During the twenties and the thirties the more important problem both for chemist and biologist was the knowledge of protein structure.

The main cause which prevented any progress in the field was the impossibility, with the current separation techniques to obtain pure chemical substances.

In effect all the attempts to characterize the various proteins came up against difficulties, due to the lack of a convenient method for the separation of the complex mixture of substances of high molecular weight.

Svedberg's ultracentrifugation of proteins constitutes the first contribution in solving this problem on the basis of physico-chemical methods.

This solution requires a high level theoretical approach and an advanced experimental technique.

The young Tiselius devoted his first years of research

work to the developing of these methods, thus adquiring a complete knowledge of the techniques, and moreover of the importance of the problem of the separation of the proteins.

The results of Svedberg's researches had shown that protein may sediment at the pure state, whereas up to that time all the evidences were against this conclusion.

Tiselius on the basis of these facts, instead of using for the separation of proteins the sedimentation rate, tried to utilize their property, as other colloids, to migrate in an electric field. He applied the basic knowledge of electrophoresis of colloids to the proteins in solution, using, to localize the migration, the method of moving boundaries: the latter were determined by a delicate interpherometric system (Schlieren Methoden).

In 1930 he presented his thesis on this argument, where he demonstrated, using an apparatus which he had planned and constructed for this purpose, that through the examination of the sharp boundaries obtained during the electrophoresis, it was possible to characterize the unitary homogeneous proteins.

At the present time this result seems to be obvious: but we must here remember that this was the first demonstration that the protein macromolecules were unitary and homogeneous.

Only five years later he took over again his researches in this very promising field on the basis of the first results which had aroused considerable interest among all the biologists and biochemists, who were asking for a new experimental approach to the study of proteins.

The improvement he brought during this second period of research on protein separations, in the optical system, in order to obtain better determination of the moving boundaries, in the cells to avoid the negative thermical effects, gave rise to a new type of electrophoresis apparatus.

With this apparatus Tiselius was able to make the first quantitative separations of blood serum and to detect and determine four main protein components which were named by him albumin, α-globulin, β-globulin and γ-globulin.

Of these four new proteins he could give the electrophoretic properties and establish their homogeneity and constance.

We must now remember that all applications of these basic findings in the field of immunology, pathology and therapy (who does not know the use and the role of gamma globulin), and their implications and applications to the field of public health were made possible by the work of a young physical chemist, who used perhaps for the first time in history of science, a purely mathematical and theoretical approach of the study of one of the most difficult and fascinating problems of biology and of the knowledge of life.

Thousands and thousands of serum protein analyses performed all over the world, confirmed the early findings of Tiselius and moreover have shown the implication of this method in the knowledge of the behaviour of these biopolymers: the chemical interpretation of immunological response, the detection of particular diseases through the study of the protein composition of blood serum, the characterization of the properties and the use in medicine of γ-globulin.

The new method was not only confined to the study of human proteins, but it has made possible the study of proteins in different fields of natural sciences both in the animal and in plants, and to contribute to the understanding of hybrid molecules and even a new approach to the chemistry of nucleic acids.

The success of this new avenue open to scientific research culminated in 1948, ten years later, with the Noble prize for Chemistry to Arne Tiselius, was in certain way surpassed by the simple method, he named « zone electrophoresis » published for the first time in 1950.

Although well aware of the importance of the method of the « free electrophoresis », he certainly realized that the technique was extremely difficult and sophisticated, and thus could not have so widespread among all laboratories, where protein analysis is performed and especially not in hospitals where the diagnosis through the protein pattern had proved to be of fundamental importance.

Moreover the moving boundaries method was purely analytical and could not be used, for preparative purposes.

Since 1927 he had begun new experiences in order to separate proteins on a support of gelatin using two coloured proteins of algae: the phycoerythrine, red, and the phycocyanine, blue.

Although successful his first results appear only 23 year later in a number a papers dealing with the technique of *zone electrophoresis* i.e. of an electrophoresis of proteins where the substances and the electrolyte are both supported on an inert material.

In the many aspects of application this method appears as « paper electrophoresis », « starch electrophoresis », electrophoresis on agar or on polyacrilamide which now permit in a few hours sharp and quantitative separations of complex protein mixtures.

With a very simple technical means, which can be also manifactured by the research worker in his laboratory, electrophoresis becomes a tool now easily avalaible for all.

At the present time proteinogram has become one of the most common but important clinical analysis and this is due to the pioneering and developing work of Tiselius.

Tiselius himself was very proud of this simple system which he preferred to the free electrophoresis apparatus, mainly for its advantages due to the simplicity and flexibility of the system. We may now argue that without the previous fundamental work on the electrophoretical properties and behaviour of protein in solution, this new method would not have been conceived.

The zone electrophoresis has disclosed even more avenues to research and its applications. The high sensitivity of the method can be even multiplied by the use of immunoreactives thus disclosing with Grabar the innumerous applications of immunoelectrophoresis, one of the most important tools in modern biochemistry.

Discontinuous electrophoresis, the so called disc-electrophoresis, is another important application of the basic techniques.

We may today consider that in the very field of clinical medicine the knowledge of the protein components of blood and serum and their relative rates were invaluable for the development of this discipline.

Another field where Tiselius devoted most of his research activity, and which under certain points of view is closely related to the former, is the separation of two and more substances on special adsorption supports. Now we call the system adsorbtion chromatography which has become a Science through Tiselius work.

At the time he entered the field very little was known except a great deal of empirical data, about the principles and the limits of this technique already largely used for the separation of organic substances.

We may perhaps find the motive of these researches in the desire and preparative to approach, through different pathways, the problem of the separation of pure substances from very complicate mixtures. At the same time he was in effect engaged in the work of the electrophoresis of proteins.

In this case he used the previously acquired experience formed during his stay (1933-35) at the Frick Chemical Laboratory in Princeton where he had studied the diffusion of water into the crystals of zeolites.

For this purpose Tiselius applied the physico-chemical and mathematical approach to analyse the phenomena which take place in a chromatographic column.

To this purpose he chose, to separate known mixtures of sugars amino-acids and peptides, a column of active carbon as absorbent.

From these researches absorbtion chromatography finds its theoretical basis. Even the nomenclature of this technique was introduced by Tiselius, and until now commonly used like: *frontal analysis, displacement analysis* and *elution analysis*.

As the carbon was too active for its extremely large surface and retained or denatured all the proteins in the column, he studied a new adsorbent less active and convenient for this kind of separation.

He found very suitable calcium phosphate and successively hydroxylapatite, a stable form of the same calcium phosphate. This adsorbents allowed separations not only of small molecules but also of proteins.

I rembember that in 1954 he showed me with pride in his laboratory the experience he was running in order to separate by chromatography the proteins. Also in this case, in order to follow better the separation, he used the two chromoproteins he had already tried in 1927 the electrophoresis experiments, phycoeryhrine and phycocyanine.

Later, coupling the adsorption and ion exchange techniques on resins, also developed by him, he obtained excellent separations of proteins in a pure state.

In the daily exciting research work with his collaborators, advancing in different fields of science from molecular biology to theoretical chemistry he found the way to develop many new ideas from the experimental observations.

From one of these he found the basis to develop a new important method, today fundamental for the study of biopolymers: the use of molecular sieves.

He had observed that during electrophoresis certain supports like homogeneous gels, i.e. starch, do act as molecular sieves.

He applied the same principle to the column chromatography using in this case granular beds of the same gel.

The following developments of this technique, with his collaborators, Porath and Flodin, led to the dextran gels and later to those of polyacrilamide and agarose.

Those who have familiarity with the work in the field of biopolymers know well how many possibilities this finding has open to biochemical research. In effect, *gel filtration,* as now the method is named, enables us to substitute for proteins the more troublesome dialysis by a simple passage in a column of the solution, and also to separate proteins according their molecular weigth as well as to understand some difficult problem of association of macromolecules and interaction between proteins and other molecules.

At present we may consider that a great part of the progress of the biochemical methods of purification of biopolymers is connected with the use of the gel filtration.

We have reported three of the main lines of research of Arne Tiselius, but we could here quote many more. His interests were extremely broad and as above reported in his Institute at the University of Uppsala he followed with his coworkers many different important fields of research.

We may here report as further examples the solvent-solvent extraction developed with Albertson for the separation of macromolecules, using two water rich immiscible phases; the electrophoresis in capillaries, studied with Hijerten and which may have further developments in the microdeterminations of proteins, and the density gradient centrifugation investigated with Svensson.

We have here tried to give a short account of the work developed by Tiselius and his collaborators in the Institute of Biochemistry at the University of Uppsala, in the long years of intense work in an interdisciplinary field where physicochemistry and biology come together for a better knowledge of the problems of life.

Tiselius for his high scientific merit received during his life many honors and awards in every part of the world: first of all the Nobel prize for Chemistry in 1948.

He was also called to important scientific positions both in Sweden and in International Organisations: he was for many years Vice President of the Nobel Foundation, scientific adviser of the Swedish Government and Vice President of the international Union for pure and applied Chemistry.

He had accepted these positions not as honours but as a new task and work for the progress of Science towards new objectives useful for mankind.

The great problems faced today by scientific research both in the national and international field, i.e. the choice of methods and of priorities and also of the utilization of the results, found Arne Tiselius in the front line.

First of all his constant preoccupation was to avoid the misuse of science, as has happened in the last years more than once, with terrible consequences also for the future of mankind.

The second point was the position of the scientist, with respect to the choices and priorities in scientific research, both on national and personal level for the implication that this decision constitutes both for the planner and the single scientist.

The scientist must solve the antinomy between the necessity for research to be rewarding directly or indirectly in the social or the economic field and the imperative of the freedom of the research worker.

This problem unknown in the past, when research was considered a mere curiosity for laymen, has now been developing since the world spends billion dollars every year and research represents a small but already sensible percentage of the investements of the nations, and on the other hand the small number of research workers has enormously grown.

Of these new problems, which are now the problems of every scientist who desire to insert science in our modern world as a real factor of progress, Arne Tiselius has given us a very acute and expressive assay in this very Academy of Sciences in april 1970, during the last session he was present with us.

We may consider his words as his last message to us and to all the peoples who believe in Science as a true factor of progress for mankind.

« The dilemma is above all, that "free research" can no longer be as free as it used to be, although most responsible people would admit that freedom in basic research is essential for human progress in all fields.

The limitations thus put on our freedom are chiefly a consequence of the enormously increasing demands placed on research, both fundamental and applied, which cannot be met by a corresponding increase of funds and personnel. There is, however, another equally important factor: the research worker himself in making his own priorities is often aware of his duties to help a suffering world and he may therefore feel inclined to prefer a short-range project of obvious practical importance to a long-range plan which scientifically may seem more significant, and where he may have a greater chance of doing his utmost in his specific capacity.

Much of the "research about research" done, deals too much with a comparison in scientific productivity under different conditions or in different countries, measuring the output in number of pages or titles of communications or by other means where quality does not come into the picture.

I am not sure that a penetrating study of the optimal conditions for scientific creativity would lead to practical recommendations to governments, research councils or foundations how they should spend their money. But it might lead to an awareness in the back of their minds which could influence their decisions. And to others it would be fascinating to know more about the act of creation of great science.

Now to return to the dilemma in the selection of priorities in the world as it is to-day. An increasing number of scientists are becoming aware of the sufferings of the world to the extent that they suffer themselves. And they know — perhaps better than most people — which enormous risks we are running as our capacity to make use of our discoveries has outgrown our ability to prevent the misuse. I can very well imagine that in this situation a scientist would be willing to sacrifice his long-range plans for some more immediate goal, if this appears particularly desirable in the present situation. And I can also imagine that now the optimal conditions for creativity — as I have just discussed, would apply to such cases even to people who earlier would never have thought of sacrificing some of their so-called freedom.

To decide about priorities in research is, as we all know extremely difficult. It requires an almost prophetic vision. The contribution of leading scientist to the immediate needs of mankind may also take the form of suggesting or urging priorities — they should have the vision, more than anybody else, what the future perspectives and possibilities are ».

He was a very simple, likable and cordial man, although under a certain delicate reserve.

As an orator he was sober and concise, but extremly sharp and convincing.

Those who have had the privilige to contact and approach him realized immediately that he always tried to ensure that his interlocutor would not be overawed by his great personality and wisdom.

He was able to dedicate, without any sign of impatience, his precious time to any young associate and even to any researcher from all the Countries of the world, who wished to have from him on this or that problem.

He was in effect in his discussions a real promoter of initiatives and ideas. He was a great scientist but not a lone scientist: he did like to work together with a team of collaborators, who were orientated by him in different fields of biochemistry and biophysics, trying to harmonize the various aspects of the research, which was going on on many directions in his outstanding unit, the Biochemical Institute of the University of Uppsala, which he had created and formed in many years of teaching and research.

He was a Master, a real Magister, in the best acception of the word: he dedicated himself mainly to the teaching in classes and seminars and even in the laboratories, because he believed that it was his moral obligation to transmit to his disciples his ideas and ideals for Science.

This was the way he wished to hand on the torch of wisdom to the younger generations anxious to overcome the past.

We may now hear from its own words how he considered the relations between the professor and the disciple, in that, we may define a constructive antithesis, which must perpetuate progress in the future through different ways and means.

His words, the expression of an experience not only of a Master but also of an exceptional Disciple, should be meditated in this very historical moment when contestation is widespread in the Universities and research laboratories throughout the world.

« The privilege of having worked under a great master, to receive inspiration from him and to witness how scientific work is done at its best. This does not involve simply imitation, but sometimes opposition. You know, as well as I do, what a great inspiration a young apprentice may feel when he finds that the professor is wrong. Much of the influence takes the form of criticism — that is positive criticism — but also if on some occasions the senior scientist can explain to his collaborators not only what engage his mind just now, but also what he hopes to do, how he feels about the situation in a certain field, also perhaps his disappointments and sufferings, facing failures or contradictions. This is to "prepare the minds".

The prepared mind is no doubt an essential background but it is not enough. *A deep personal involvement is necessary,* *one has to live with one's problem and one even may have to suffer with it.* It is interesting that the deeper one penetrates this subject, the more obvious is the conclusion that creativity is basically the same in science as it is in the arts and in music. This may seem strange to some laymen who tend to believe that science is only cold logics and thus a rather inhuman occupation ».

These thoughts are the expression of a man who has always had for Science the enthusiasm which was manifestated by a life of dedication to teaching and research.

They are also the index of deep feelings which guided him in the scientific research towards the happiness of the creative action.

"When a new thought is born, or when one of the deep secrets of Nature yields to searching scientist — in this very act of creation — the is a pure and primitive happiness, deeper than anything of this kind wich can never be granted a human beeing to experience" and above all the expression of a great and inspired spirit.

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