SIGNIFICANT STAGES IN THE BIOCHEMICAL RESEARCH WORK OF GYÖRGY HEVESY

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It would hardly seem a novelty that György Hevesy was one of the greatest chemists of our century. He spent most of his life abroad and was on friendly terms with almost all great men of modern natural sciences. He lived from 1885 to 1966 and kept contact with his country fellowmen in Hungary during his whole career. His name was associated with radiochemistry rather than with biochemistry. Yet, if I were asked to decide which of the two was his actual field of research, I should hesitate whether not to say the first one. To put it more precisely, I should perhaps say: his research work fell on the borderline of the two. The grounds for my opinion are that most of his work was devoted to solving biochemical problems by way of the radioactive tracing which he discovered in 1913. In 1943 he was awarded the Nobel Prize for having worked out this method.

It is not by chance that there was such a long interval between the two dates. All this time was necessary to prove the wide applicability of tracing; its physiological utilization presents certainly one of the most important aspects from a scientific point of view. Processes which had been untraceable before lent themselves to studies and as a result of successful experimental work, tracing was accepted as one of the standard methods in physiology and biology. Full credit is due to György Hevesy in this field. He had made the first steps already in the beginning of the twenties, and a glance into his collected works shows the preponderance of articles in this field. This confirms my statement about his special field. Indeed it may perhaps lead us to the conclusion that the author himself might have meant the same.

Naturally enough, it is impossible here to give a detailed review of György Hevesy's works in biochemistry. The most important stages, however, can be followed, as well as the great scientist's persevering work in an emerging field of science. First I am going to survey his efforts as a young man, then the achievements following the discovery of artificial radioactivity which has brought a decisive change in the history of tracing, and, finally, I am going to say a few words about the research work to which he devoted himself in Stockholm in later years. I shall try to show how, with the ingenious applica-

tion of the method, he managed to go all the way from the examination of living organisms to the limit of the molecular level.

1. Biological application in the twenties

According to an anecdote the idea that tracing should be applied in biology came up in Rutherford's laboratory in Manchester during teatime. Staring at his cup Hevesy told Moseley, a classic of X-ray spectroscopy, pondering on the subject of the conversation: "How interesting it would be to trace the way of tea inside the human body" — he said dreamily.This happened in 1913, and the remark seemed to be utterly Utopian.

It was certainly not more than a bizarre idea. True enough, it was the year when Hevesy had discovered that lead compounds could be detected even in very small quantities if radioactive isotopes were admixed to them but this applied only to lead, resp. to materials which had active isotopes. There were not many of them at the time. The thought that water, the basic material of tea, could also contain isotopes, seemed inconceivable.

So it seemed in the beginning that tracing would be applicable in a relatively narrow sphere, and mainly for analytical purposes. Analytical application, however, implied that a number of new processes could be studied, even those where main difficulty has been the hardly detectable small quantity of material.

Several such tasks required definite knowledge about the changes in the concentration of lead. This was important because at firts the tracing method had been worked out only for lead.

The methodological significance of his discovery was clear to György Hevesy. He conducted series of various tracing examinations as early as the 1910s. Some of them were worked out with the help of co-authors, often with Hungarian colleagues. Gyula Groth, László Zechmeister, Erzsébet Róna assisted in some of his work. They measured diffusion rates, investigated phase transformations, explained the molecular rearrangements of organic materials.

It seemed obvious that experimental work should be extended also to biological problems. A great hindrance was that lead, like the other heavy metals, was unsuitable for this purpose because of its toxic effect. So the attractive possibility could merely be indicated and the first exploratory steps attempted. Hevesy did not hesitate to make them.

The breakthrough came in 1923. Hevesy made his first physiological experiments with radioactive lead isotopes. In his earliest paper he had pointed out that radioactive lead isotope absorbed by a vegetable organism could be traced inside the plant. The first step suggested the second, namely that animals should also be examined. The relevant results were published a year later by Hevesy.

The foregoing makes it clear that the limited opportunities allowed only preliminary examinations. Hevesy could certainly not go into the depths of the individual organs, still less could he study the physiological processes. His early experiments, in the 1920s, followed the way of lead in the living organism.

Further Progress depended on finding further radioactive isotopes suitable for analytical purposes.

2. Development of biological tracing after the discovery of heavy water

It is quite common in the history of science that an idea, seemingly all Utopian, turns into tangible reality. Moseley, too, meant it rather as a joke that the way of tea should be followed. But what was regarded as Utopian in the beginning of the 1910s became reality in the thirties.

Heavy hydrogen, the deuterium had been discovered by that time. The credit was due to the American chemist Clayton Urey who proved experimentally the existence of the deuterium foreseen by thermodynamic and quantum theory calculations. He won the Nobel Prize for his work in 1934.

The step from deuterium to heavy water, i.e. water containing this new isotope of hydrogen, was direct and short.

This was the scientific condition for following the way of tea inside the human body. And it was only natural that Hevesy noticed it instantly.

But heavy water was very costly at that time. There was very little of it and almost inaccessible. Hevesy however obtained some because he was well-known in international scientific life and was working for institutions where genuine creative work was in progress. Eminent scientists were frequent visitors of these institutions. Almost every protagonist of modern science made a pilgrimage to Bohr's institute in Copenhagen. Urey himself was not an exception. There by mere chance, he made friends with Hevesy, and this enabled the latter to start his further biological experiments.

This friendship started in 1923 remained alive for the next ten years. Urey generously gave Hevesy a few litres of water which contained 0.6% heavy water. His further experiments in biology were helped by his assistant, Hofer who was extremely skilled in measuring the density of water and worked as Hevesy's collaborator.

The firts experiments were made with goldfish. He still was unable to penetrate into the inside of living organisms; he wanted to find out whether or not the water exchange of goldfish could be investigated with heavy water. This led him to the principle of isotopic dilution, i.e. to the discovery of a basic methodological theory.

After this research has been extended to human beings. First he measured

the amount of water content, then the average life span of water molecules in the body.

An important event of this period was a visit by the famous physician A. Krogh, winner of the Nobel Prize in 1920 at Hevesy's place of work in Copenhagen. This visit was due to his interest in Hevesy's experiments with heavy water and with the newly discovered artificial isotopes.

So in the beginning of the thirties real progress was still impossible. The lack of isotopes still hindered the extension of tracing to physiology.

3. The prevalence of tracing in the period of artificial radioactivity

While conducting research with heavy water, Hevesy experienced a strange thing. According to measurements, the change of water of the goldfish exceeded 100%. This indicated that a certain amount of the water was in interaction with the materials of the body. For proving this, the organism could no longer be considered a unit; penetration and the examination of inside materials was necessary; this, however, was a biochemical problem.

The most suitable material for the first experiments in this field was still heavy water. Hevesy investigated the exchange processes between heavy water and the hydrogen of fat. These investigations soon included the exchange between other constituents of the body and heavy water. (It should not be forgotten that the also made attempts to utilize heavy water in another field. A few years later he conducted permeability studies with heavy water. His assistant in this work was Jacobson.)

It should be noted, however, that Hevesy has never been considered the true classic of this particular method. Rudolf Schönheimer, an American biochemist of German origin had started investigations on exactly the same subject with very similar methods at the same true. Hevesy abandoned investigations with tracer hydrogen after the discovery of artificial radioactivity, consequently he did not examine the exchange of hydrogen in fat either, whereas Schönheimer used heavy water for fat syntheses and introduced the material obtained as a feeding stuff for animals. Deuterium worked its way through the adipose tissue and metabolism became an easy subject to study. The papers Schönheimer wrote after 1935 can be regarded as classical works in this branch of science.

We should not forget, however, that the indicator-method had also its opponents. S.P.L. Sörensen, for example, considered this method irrelevant and even unreliable. This was all the more unfortunate as Sörensen had a good name in biochemistry especially because of his achievements in the analysis of proteins. His judgement was probably based on the poor opinion of his close collaborator, Linderström—Lang, about the early (1923) work of

Hevesy. (By the way Linderström—Lang became Sörensen's successor in the institute.)

But it was more important that Krogh was enthusiastic. He and his collaborator Ussing made several experiments with the indicator-method and their experience was definitely positive. Later Ussing became an outstanding expert of the method. Krogh called the attention of several scientists in Copenhagen to the new method, but it was only a few years later that its use was really appreciated, after Hevesy had worked in Sörensen's institute in collaboration with Linderström—Lang.

At the time the isotopes P^{32} and N^{15} so important for research were already known. Hevesy used P^{32} in his examinations of skeleton renewal. This meant that one part of the organism, one organ became the subject of study.

He had to cope with two difficulties at the time. The first was that he had no experience in the methodology of physiological, resp. medical experiments. He had no technical personnel to help carry out his ideas. Fortunately Chiewitz the surgeon, could offer him some help, mainly by giving instruction to his technician to feed also Hevesy's animals, and to help him dissect mice. Without his generous help it would have been impossible for Hevesy to progress.

The other difficulty was that P^{32} was available only in very limited quantities. This difficulty was overcome with the help of several favourable coincidences. Bohr celebrated his 50th birthday in 1935. He received 10 000 kroner as a gift on the occasion and spent this sum on a radium-berylium source which served to produce artificial radioactive isotopes. Naturally he shared the material with his old friend.

Later Hevesy obtained also stronger specimens. By a fortunate accident on his journey to Japan he met one of the inventors of the accelerator, E.O. Lawrence, who showed him a small-size sample of a proton accelerator still in its initial, experimental stage. As a token of their friendly relations Lawrence also sent utilizable isotopes to Hevesy. Martin Kamen produced P^{32} himself and his isotopes had a higher degree of activity than the other preparations available in trade.

Hevesy work benefited considerably from the help of A. Krogh. Partly Lundsguard himself, and partly his staff helped Hevesy to lean the methods of medical and physiological research.

A symposium in 1937 was an important landmark in the application of tracing in biochemistry. The subject was the applicability of the P^{32} isotope and many specialists participated in it whose opinion was by no means indifferent for the cause. Meyerhof, winner of the Nobel Prize for Medicine in 1922, who had gained distinction for investigating the enzymes in carbohydrate metabolism, was also present. However, his opinion of the application of the P^{32} as a tracer was not favourable. According to him a major disadvantage

was that the exchange equilibrium between the labile ATP phosphates and the inorganic P^{32} might develop too fast.

Another famous participant of the congress (apart from Joliot-Curie) was J. K. Parnas, another successful researcher of carbon hydrate metabolism. His opinion was not so negative as Meyerhof's. He understood the potential advantages of applying the P^{32}. Agreement led to cooperation. Soon after he started joint experiments with Hevesy; they examined certain reactions of the phosphates of gylcerine.

These experiments led also to a more general approach of phosphatide metabolism, based on previous experiments on the formation of phosphatide in chicken embryos. On this he worked not with Parnas but with Ladislaus Hahn.

He tried to extend investigations with P^{32} as far as possible. Therefore he soon switched from phosphorus metabolism to new directions. He described in an essay how the P^{32} incorporates into the red corcpuscles.

Aten was his collaborator in this and in further works. Together they worked out the methods which led to the marking of erythrocytes with P^{32}. This method was widely applied later.

Soon he started biochemical experiments with the K^{42} isotope which had become accessible in the meantime. But he found himself confronted with a basic methodological problem. Not only that the amount of this material was very limited in the mid-thirties but the activity of the hardly obtainable preparations was also inadequate. Law-activity isotopes make the tracing examinations extremely complicated in biochemistry. This was the reason for the frequent measurement defects with which he tried to examine the problems of potassium metabolism.

This should not lead us to the conclusion that these experiments were a total failure. On the contrary, they helped him to discover important facts. He demonstrated that K^{42} could penetrate into the erythrocytes. This was a significant achievement at this early stage of research. Hevesy never forgot the surprise shown by the physician Rehberg, later Krogh's successor, upon hearing the news. Earlier Rehberg had strong doubts whether potassium would diffuse into the erythrocytes and he was very much surprised at the positive results. This was not the only achievement of permanent value with the K^{42}. He proved with measurements that the proportion of the incorporation of potassium into the tissues was strongly increased by muscle work.

Experimenting with radioactive potassium isotope did not mean his giving up research with the P^{32}. He investigated the renewal of brain phosphatides together with Hahn, and the incorporation of phosphorus into the enamel of teeth with W. Armstrong and W. Arnold.

It was clear that the artificially active isotopes did really increase the possibilities of tracing. In that period Hevesy searched new and new ways of application with the aid of many colleagues.

4. The application of tracing in nucleic acid research

Hevesy worked extensively to explore the biochemical role of nucleic acid (DNS). Although the great importance of DNS is due to its genetic influence, at the time György Hevesy did not concern himself with this aspect; his attempts were much more general and exploratory.

The aim of his research carried out with Ottensen in 1939 was to examine the circulation of nucleic acid. Soon they found that nucleic acid incorporated P^{32} surprisingly slowly. It was surprising because the renewal of the other phosphorus compounds in the liver was very quick. Their experiences required some kind of explanation. They thought that it was a case of methodological difficulty; the diffusion of P^{32} in nucleic acid does probably not measure the renewal of the latter but the formation of new cells.

This conclusion inspired a new direction of research. Hevesy presumed that cell-growth could be studied with this method. And what was the typical sphere of cell-growth? The formation of tumours. So he began to wonder about the formation of nucleic acid in tumours. He studied specially the influence of X-ray radiation on the process of tumour formation.

He worked together with Professor H. v. Euler in Stockholm. Euler was an excellent biochemist, a Nobel Prize-laureate in chemistry in 1929. He himself studied malignant tumours at the time. He had rats who had developed Jensen sarcoma, and proved to be remarkable experimental material. They investigated the blocking effect of X-ray radiation on the formation of nucleic acid in the first place, and their results were a valuable contribution to experimental research in this field. Lucie Ahleström, a highly skilled assistant of Euler's layed a great part in this work. Her task was to recover nucleic acid and various other phosphorus compounds from the radiated tissues. Measurements of radioactivity were made in Copenhagen. So research was complex not only from a methodological point of view but, in addition, the work was carried out in different places. This certainly did not make the situation any easier.

It was quite evident that Hevesy chose Stockholm as his place of refuge when he had to flee from Nazi persecution from Copenhagen in 1943. Krogh and Rehberg, also went there, so there was nothing to prevent them keeping up the professional consultations and friendly talks they had got used to in Copenhagen. The working conditions also improved.

His joint work with Euler and Ahlström progressed. The change of scene accelerated Hevesy is work. As a rule he planned the experiments and Ahlström carried them out with great inventiveness and enthusiasm.

His most important results were the following: he demonstrated a 50% decrease in the formation of nucleic acid in rats exposed to radiation. The method leading to this conclusion was again tracing the incorporation of P^{32}

into nucleic acid. They made the important observation that left-side irradiation influenced not only the left-side tumour but also the sarcome on the right side. They also proved that the effect was independent of age. The effects on new-born rats were exactly the same as on fully matured specimen.

Hevesy's research on nucleic acid was made easier now with the availability of radioactive carbon isotope.

The $CH_3C^{14}OOH$ produced from it could be built into the purins of the nucleic acid. This allowed Hevesy to study how the C^{14} got incorporated into the nucleic acid of radiated mice. The main achievement was the discovery of the extremely short-lived fractions of sebacic acid in the liver.

With the end of the war Hevesy had to decide whether to go back to Copenhagen or to stay on in Stockholm. Conditions of work and family interests made him decide for Stockholm. At that time he had already a laboratory of his own at the Research Institute of Organic Chemistry and Biochemistry. He managed to build up successful cooperation with Professor Häggquist, and again took up research work with heavy water this time as a joint work. They studied the toxic effect of heavy water on mice. Hevesy maintained close contacts also with the institute of Theorell, the Nobel Prize-laureate for Medicine in 1955; this was all the more natural because both were interested in the renewal of myoglobin at the time. Their relationship was close, fruitful and friendly.

Despite political perturbations and frequent moves György Hevesy was a pioneer in his field. He maintained contacts and cooperation with many scientists, including a number of Nobel Prize-laureates. His interest was concentrated on a most promising field biochemistry and nucleic acid research came to the fore of scientific development a few decades later with the emergence of genetic research.

5. Later achievements

Hevesy's extremely successful work in biochemistry had extended to several branches. His ability to adapt himself to new aspects was obvious even as an old man he had the strength to start on a new line of research work. He took up haematology in 1956, in the department of dr Kottmeier at Radiumhemmet. His first collaborator was Del Santo, later he worked with Lockner.

They continued the examination of tumours. First they induced the Ehrlich carcinoma in mice and followed its development, then they examined the spontaneous formation of tumours on mice.

Their most important work in haematology was the study of haemoglobins. They published papers on the physiological damage of the red corpuscles in rabbits. The comparison of various generations brought interesting results.

They tried to compare haemophysis in the doe-rabbit and the embryo. They did not limit themselves to mere observation but tried to influence the disease. The animals were irradiated with CO^{60} and studied the effect of irradiation on haemophysis.

The Ca^{45} opened up further fields. All the processes connected with lime circulation in the living organism could be studied now.

They experimented again with mice. The main point of the study was what skeleton fractions the mouse preserved during its lifetime. Various generations were compared again with the result that if the tracer isotope was administered to a pregnant animal the same tracer will be present in the progeny.

Similar techniques could be applied to other fields. They examined how mice preserved iron with the Fe^{59} isotope.

Together with Forsberg, experiments were carried out to describe the stability of nucleic acid in the organism of mice. They explored also to what degree the RaD in the skeleton was released during the growth of mice.

György Hevesy remained a first-rank scientist throughout his life. He was an old man when he still looked for new ways to apply tracing. He retired in 1961. This did not mean of course isolation from scientific life; as a matter of fact in his last year, in 1966, he attended a congress in Rome at the Papal Academy of Sciences, although his health was already impaired. He had received careful treatment in the hospital of Freiburg to enable him to travel to Rome. His nurse accompanied him and kept the oxygen respirator in reserve all the time. The congress had been in April, Hevesy died on the 5th of July.

Summary

Author gives a historical survey on the biochemical activity of the Noble Prize-laureate György Hevesy, who discovered the isotope tracing method and applied in biochemical examinations, as well. They form a relevant part of biochemistry regardless of the means with which they have been achieved.

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