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SYMPOSIUM ON "ALDOSTERONE AND EPITHELIAL ACTIVE SODIUM TRANSPORT" BASEL, JULY 23–24 1971

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OPENING REMARKS

Prof. Thölen

LADIES and gentlemen,

We are very happy that it was possible for all of you to participate in our Symposium and I would like to welcome you to Basel.

It was Dr. Voûte's idea to arrange this meeting on Aldosterone and active sodium transport across epithelia, and because of the great interest which you have demonstrated I guess that he was perfectly right. Dr. Voûte has done the

whole work of organization and I would like to thank him here and now for all he has done.

Before we start our scientific session Prof. Koller, Head of our Medical Department, will show us the importance of our town of Basel in the medical sciences over the last centuries. Then Prof. Reichtein and Prof. Wettstein will give us some introductory historical remarks on steroids and especially on Aldosterone.

I wish to express my gratitude to Ciba-Geigy Inc. for their financial support. Without this support it would not have been possible to organize this Symposium.

Perhaps a last remark: The whole Symposium will be published in the Journal of Steroid Biochemistry at the beginning of 1972. Our thanks go to the Editor, Dr. Pasqualini, and to Pergamon Press for their help in this matter.

I wish you a successful meeting!

Prof. F. Koller

Prof. Thölen, ladies and gentlemen,

I should like to extend a hearty welcome to all of you on behalf of the University of Basel, its Faculty of Medicine and the Department of Internal Medicine. The latter has realized that fundamental science—represented in such a distinguished manner by the participants of this symposium—is not only the monopoly of the so called theoretical disciplines, but that it is indispensible for the development of clinical medicine as well. Dr. Voûte and his laboratory are integrated in the Department of Medicine, and I think the fact that so many prominent scientists have accepted his invitation demonstrates that he is appreciated by the specialists in the field of aldosterone physiology.

As we are meeting in Basel, you may allow me some historical remarks: The University of Basel is by far the oldest in Switzerland; it was founded in 1460, shortly after the great Council of the Church which was held in our city from 1431 to 1448. As a consequence of this illustrious meeting, the Pope Pius II, who before his election had attended the Council and had become acquainted with Basel, laid the foundation of the University. The spiritual life of Basel received a mighty impetus by this foundation and was further stimulated by the development of the printing craft by Amerbach and Frobe. In this way the ground was prepared for humanism. The greatest humanists of this age, Erasmus of Rotterdam, Thomas Platter and the famous artists Urs Graf, Holbein the younger and—some decades earlier—Konrad Witz, came to Basel and made the city one of the most important cultural centres of Europe in the beginning of the 16th century. Medicine too was influenced by these developments: Paracelsus was called to Basel and proposed an almost revolutionary change in medical education; Vesalius edited his famous work "De fabrica corporis humani" in this city. Later on, Felix Platter, Ziegler and Bauhin (well known for the Valvula Bauhini) acted here as distinguished teachers of medicine.

In the 19th Century, Friedrich Miescher, Professor of Physiology at our university, discovered the nucleoproteins in the leucocytes of wound infections. He urged the university authorities to create a chair of biochemistry, the first in Switzerland, which was offered to Gustav Bunge.

As representatives of clinical medicine, I should like to mention at the turn of the century Friedrich Müller, the great teacher, and Wilhelm His, the discoverer of the bundle of His, known to every medical student today. In 1948 and

1950 Basel got Nobel prize laureates: Dr. H. P. Müller for the discovery of the insectidical properties of DDT, Prof. T. Reichstein for his work on steroid chemistry. Allow me to add to this, that in the forties of this century the Swiss Academy of the Medical Sciences was founded in Basel by Prof. Gigon and that, at the present time, this city is making a remarkable effort for the development of fundamental sciences in biology with the establishment of the "Bio-Zentrum", which will be inaugurated in the near future and will be integrated into the university. On the other hand, the pharmaceutical industry of Basel, of worldwide reputation, realized long ago that any progress in the field of pharmaceutics can be achieved only by sponsoring scientific research in a generous way. Along this line, Hoffmann-La Roche has recently founded the Basel Institute for Immunology and Ciba-Geigy the Friedrich Miescher Institute for fundamental biomedical research where scientists work completely independent of any commercial purposes.

In concluding, I should like to express my hope that Basel will become today a centre of biological sciences in a similar way as it was a centre of humanism in the 16th Century.

Prof. Reichstein

Mr. Chairman, ladies and gentlemen,

I accepted this kind invitation with reluctance only because I was away for two weeks on a botanical excursion and had to get up this morning at four o'clock and drive very many hours in order to arrive here on time. So I had no time to prepare anything to give you a really historical review. What I can do is to produce a few personal remarks as far as I remember them, my memory being not very good. The foundation for the whole chemical work on the adrenal hormones was laid by Thomas Addison in 1855, i.e. nearly 120 years ago. He then described a fatal syndrome for the human being, today called Addison's disease, and stated that the only cause he could detect was the destruction of the adrenal glands, a really remarkable discovery for that time. It took rather long until similar things could be proved in animal experiments. C. E. Brown Sequard claimed about a year after Addison's paper that he could produce similar symptoms in animals after adrenalectomy but as far as I know these experiments are not accepted as reliable because the methods were not adequate at this time. It was only about 80 years later (around 1930) that different investigators, especially in America, e.g. Rogoff and Stewart (1928–1929), Hartman and Brownell and particularly W. W. Swingle, J. J. Pfiffner and their co-workers (1930–1932) showed with reliable methods that the adrenal glands are essential to life in animals and that their main function is to produce a hormone or a mixture of hormones which is necessary for normal life. That was the base for chemical work and this chemical work can perhaps be classified in two periods: the one period was around 1930–1935 when three groups of workers, that was Oscar Wintersteiner and his associates at Columbia University, E. C. Kendall and co-workers in the Mayo Clinic at Rochester and my collegues and myself first in Zurich and then in Basel took up the chemical work on the adrenal extracts. At this time it was not yet possible to follow isolation work quantitatively by biological methods. The available test methods were too crude and swallowed too much material. As soon as it was recognised that the main biological activity resided in fractions rich in highly hydroxylated steroids, such fractions were separated as far as possible and

the individual crystalline steroids tested in animals. In this work nearly 50 different compounds were isolated from adrenal extracts and some of them were shown to have activities connected with the function of the adrenal gland. The availability of pure compounds enabled physiologists to follow more closely the biological effects of these compounds. The two compounds from this period which turned out to be of particular interest were corticosterone and cortisol (hydrocortisone). It was known from earlier work (Loeb *et al.*, 1933 and others) that one of the main functions of the adrenal glands was their action on water and electrolyte metabolism. But these two compounds had very little activity in this respect; on the other hand, they showed very remarkable effects on carbohydrate and protein metabolism. And when Hench, Kendall and their associates (1949) in the Mayo Clinic demonstrated the powerful anti-inflammatory properties of cortisone and cortisol particularly in artritic conditions, this had such important consequences both for theoretical and practical medicine and for the pharmaceutical industries that many people forgot, at this time at least, that in the adrenal still another problem was hidden and that was just the compound responsible for the control of the water and mineral metabolism. This compound, later to be known as aldosterone, is present in the gland in much smaller amounts than cortisol and is much more difficult to crystallize; therefore, the methods were not at first available to isolate this compound in pure form. But all three groups which I just mentioned stated that something else is present in the gland which is much more active, for instance in Swingle and Pfiffner's dog test, than any of the pure compounds isolated so far. Kendall referred to such material as "the amorphous fraction", of course still a complicated mixture. It was about, if I remember correctly, in 1953 when I was in London that Prof. E. C. Dods introduced me to two of his co-workers, Dr. S. A. Simpson and Dr. J. F. Tait, who had just developed a very sensitive method in mice in which they could detect this compound as well as a paperchromatographic system which could trace this compound in paperchromatograms. The ground was thus laid for other chemical work. Prof. Dods suggested that we cooperate together and we were fortunate that Dr. A. Wettstein and Dr. R. Neher of CIBA-Aktiengesellschaft also joined us in the effort to isolate this compound. We had the help of the Organon people in Holland who sent us an adequate amount of crude adrenal extracts, and we had already accumulated sufficient knowledge of how to work for separating such extracts particularly with partition chromatography and other sensitive methods. So, when these extracts arrived it was only the work of a few weeks before aldosterone could be isolated in pure crystalline form and its structure elucidated. Aldosterone was also isolated in crystals, only a few weeks later, by Mattox *et al.* in the Mayo Clinic and by a group of chemists under the guidance of L. H. Sarett et Merck *et al.* in the U.S. who also performed degradation work (1955), confirming the structure we had published. Chemical work culminated in the first total synthesis of racemic Aldosterone by a group of workers at Ciba-Aktiengesellschaft Basel under the leadership of A. Wettstein (1955).

That was in a few words a personal view of the history of aldosterone.

Thank you!

Prof. Wettstein

Mr. Chairman, ladies and gentlemen:

I cannot add very much to Prof. Reichstein's explanations on the early history

From left to right: Prof. Reichstein, Sylvia Simpson, Dr. Tait, Prof. Wettstein, Dr. Neher and Joseph von Euw.

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of aldosterone, but I feel I should at least try to correct one or two of his understatements concerning his contribution to the field.

Let us start just at the moment when Prof. Reichstein together with Kendall and Hench got the Nobel Prize in Physiology and Medicine. By the way, another man, Oscar Wintersteiner, had also made a major contribution to the field. Now, at that moment the hormonal spectrum of the adrenal looked quite complete: we had at hand all the genuine glucocorticoids culminating in cortisol and we had even an excellent mineralocorticoid to explain that part of the activity of the extracts, cortexone, which Prof. Reichstein had synthesized long before it was found in the adrenals.

But soon after 1949 a new area began, when John Luetscher at Stanford pointed to the existence of a strongly sodium retaining factor in certain urines. Even more conclusive were then the papers of Sylvia Simpson and James Tait from the Middlesex Hospital. They were able to prove beyond any doubt the existence of an additional substance in animal adrenals and, what is more important to the biochemist, of larger quantities in the adrenal venous blood showing that the substance was indeed produced by the adrenal. They called this substance, which could not be identical with any of the known adrenocortical hormones, electrocortine because of its biological effects on the electrolyte balance. As said before, at that time Prof. Reichstein already had a long standing in steroid chemistry and our CIBA company had also specialized for about 20 years in steroid hormones. So we both realized the immediate interest of such a new hormone and decided to work together again on the special item and to invite Drs. Simpson and Tait in London to join our group.

Everybody went immediately to hard work. Research meetings had to take place occasionally towards midnight between two trains at a bar near the railroad-station. My slide shows a photo taken at such a meeting. (Fig.) You see at your left Prof. Reichstein and Sylvia Simpson, in the middle Dr. Tait, myself and Dr. Neher who has been already mentioned, and at the far right Joseph von Euw. He was a lab-assistant of Prof. Reichstein for many many years and devoted together with him his entire life to research. What were the results of this cooperation? One happy result was the later marriage of Dr. Simpson and Dr. Tait. On the research side the group acted quicker: it could announce, as Prof. Reichstein already said, in early 1953 the isolation of the new hormone from the so called "amorphous fraction" of adrenal extracts which had been obtained long ago by separation of the great many other related constituents and also investigated by other less fortunate scientists. The last genuine adrenal hormone having resisted crystallization for nearly 20 years was thus isolated in pure form; its extremely high mineralocorticoid properties were exactly as expected. A year later the constitution of the hormone was established again in Prof. Reichstein's lab. For this purpose the 22 mg of available substance, which was about all one had in the entire world, were degraded and transformed to a compound which proved identical to a steroid already known. That the hormone is itself a steroid was not self-evident before that time, but more surprising was the fact that it is quite a strange steroid containing instead of the usual angular 18-methyl group an aldehyde group. Therefore, it is now called aldosterone by everybody as Prof. Reichstein suggested.

The synthesis of such a compound from preformed steroids presented at first sight insurmountable problems. For example, in a review from Sir Robert

Robinson's Institute it was stated that the prospects for a synthesis were very poor. Chemical reactions needed for transformations in the inert 18-position simply did not exist. Therefore, we tackled first the total synthesis, that is according to the vocabulary of the chemist a synthesis from smallest chemical compounds, in our case from propionic acid using for the first reactions some steps which Luis Sarett had described. As there were, on paper, many different theoretical pathways to continue we called for help again on other groups, besides the group of Prof. Reichstein on chemists from Prof. Prelog's Institute in Zürich and from Organon Inc. in Holland. The more promising approaches were alloted equally to the groups. With some luck our group at CIBA reached the goal first so that I could report at the International Chemical Congress in Zürich 1955, exactly one hundred years after Addison's "Essay on Disease of the Suprarenal Capsules", the complete synthesis of the racemic hormone in about thirty steps. The other groups were to follow with their modifications shortly afterwards. It was also possible then, according to Reichstein by chemical means, in our case by the use of microbiological methods, to obtain the natural enantiomer, the optically active d-aldosterone.

After this result we started investigating partial syntheses from readily available steroidal starting materials. They lead, without the necessity of separating racemic modifications, directly to the optically active hormone. The working group in Zürich with Profs. Arigoni and Jeger had elaborated in the meantime novel chemical methods for the functionalization of the angular methyl groups, methods which proved also extremely suitable, later on, for the preparation of the clinically important 19-nor-steroid hormones. In a common venture with our group they were used for the first partial synthesis of aldosterone published in 1960. This was followed by modifications which made aldosterone accessible even on an industrial scale. Among the many collaborators joining their forces, special credit should be given to Dr. Schmidlin, who is present also today.

Gentlemen, this is, in a nutshell, the story of aldosterone seen by a chemist emphasizing the cooperative contributions made in Switzerland and especially here in Basel. After what has been said you may understand how proud we feel to have this Symposium in our town. I hope that the *genius loci* which helped us a lot will assist also your deliberations for which I wish you every success.