Observations on Chromaffin Tissue

HERMANN K. F. BLASCHKO

Department of Pharmacology, University of Oxford, England

My friendship with Ernst Fischer and my interest in the chromaffin tissue both date from the same period. In 1923 I went to Göttingen, where Ernst and I spent our internship together in the Medical Clinic of the University, under Erich Meyer. Ernst left Göttingen to join Albrecht Bethe in Frankfurt, and there he stayed until he emigrated from Germany. I left Göttingen to start work with Otto Meyerhof, and in 1933 I too came to England, where I met Ernst again, when he worked at the Marine Biological Laboratory in Plymouth. In the nineteen twenties we had both been in England, at University College, London, in A. V. Hill's laboratory, but at different times.

I made my first experimental acquaintance with chromaffin tissue when, in February 1924, a male patient aged 36 years was transferred from the Göttingen Neurological Clinic to the ward to which I was then attached, with the diagnosis diabetes mellitus. He excreted large but variable amounts of sugar in the urine. Blood pressure readings varied from 208/128 to 225/125 mm Hg. I take these and some of the following details from a paper by Biebl and Wichels (1925). The patient died of a cerebral hemorrhage, and tumours of the suprarenal gland were found on both sides. Biebl and Wichels describe the positive chromaffin reaction given by the tumour tissue. They do not mention that they gave us, my friend Rudolph E. Siegel (M.D., later physician and historian of Medicine, first in Frankfurt and now in Buffalo, N.Y.) and me, a small amount of fresh tissue. Siegel was at the time demonstrator in the Pharmacology Department under Wolfgang Heubner; he collected the heads of frogs that had been decapitated for use in the student's class. In the evening we prepared an extract of the tumour tissue, and we were thrilled to see that the pupils dilated when we instilled the extract, just as they did when we applied a solution of adrenaline. I am afraid we omitted to determine the adrenaline:noradrenaline ratio!

Soon after I came to Cambridge in 1934 I took up the study of adrenaline. My friends and I described the action of amine oxidase on adrenaline, noradrenaline and dopamine. This was followed, in 1939, by the suggestion of the main pathway of catecholamine biosynthesis, a pathway that has since been firmly established by the work of a great number of laboratories. However, even after the discovery of noradrenaline, both in adrenergic nerves and in the chromaffin tissue, the validity of the pathway suggested remained in doubt. So we read in 1952: "It is therefore hard to regard hydroxytyramine as a serious candidate for the synthesis of noradrenaline" (v. Euler, 1952).

Here in Oxford, we decided to make a more systematic study of the specific biochemical properties of chromaffin tissue. In 1950, a visitor to our laboratory from Switzerland, Dr. H. Langemann, described the presence of a very active L-dopa decarboxylase in the bovine adrenal medulla. Since Langemann's publication (1951), this observation has been amply confirmed and extended, not only to chromaffin tissue from other species but also to adrenergic neurones and to the brain. Today it is well established that chromaffin tissue is able to catalyse the various steps in the formation of the catecholamines from L-tyrosine.

Parallel with the work on the enzyme equipment of the chromaffin tissue, studies have been made of its cytology. The work of the Oxford laboratory started from a finding, made with A. D. Welch, that it is possible to prepare cell-free homogenates of chromaffin tissue in which the catecholamines were present in a sedimentable form (Blaschko and Welch, 1953). In the sediment the amines were present in a state in which they exerted only a small fraction of their biological activity, but on adding distilled water all the activity could be released in an instant (Blaschko, Hagen, and Welch, 1955).

Further analysis of these findings led to the characterization of the chromaffin granules, and these structures have been isolated from the mitochondria (Blaschko, et al., 1956; Blaschko, Hagen and Hagen,

1957) and from the lysosomes (Smith and Winkler, 1966). All these particulate elements have not only been separated by centrifugation techniques, but they have also been seen by the electron microscopists.

The cytologists have in recent years obtained evidence of the separate storage of adrenaline and noradrenaline. It has been possible to show that noradrenaline and adrenaline are differently distributed in a sucrose density gradient. The first indication of this was obtained by Eade (1956), who used the bovine adrenal medulla. Even better resolution of particle fractions containing adrenaline and noradrenaline respectively was obtained by Schümann (1957) who used homogenates of chicken adrenal gland. It might be mentioned here that Coupland and Hopwood (1966) have recently described an electron microscopic method that distinguishes between adrenaline- and noradrenaline-storing granules.

A biochemical study of the chromaffin granules began with the discovery by Hillarp, Högberg, and Nilson (1956) of large amounts of adenosine triphosphate (ATP) in the adrenal medulla. We were able to show that the bulk of the ATP was present in the chromaffin granules (Blaschko et al., 1957).

Lysis of the chromaffin granules releases not only the low-molecular-weight constituents, but also a considerable fraction of the granule protein (Blaschko et al., 1956). This protein has recently been studied in our laboratory. A preliminary purification of the soluble protein fraction was achieved by Mrs. Karen Helle (Helle, 1966a and b: *see also* Blaschko and Helle, 1963). This work has been continued by Smith and Winkler (1965) who described a method of obtaining the main soluble protein fraction of the bovine chromaffin granules in a pure form. Using this method, Mrs. Helle has been able to show in Bergen that this protein has antigenic properties (Helle, 1966). With the help of this specific antibody, Banks and Helle (1965) have recently shown that upon stimulation of the perfused adrenal gland by carbachol, the medulla releases into the perfusate not only catecholamines, but also the soluble granule protein. This finding has added a new fact to our knowledge of the physiology of the chromaffin cell. It is of particular interest in view of the recent finding by Douglas and Poisner (1966) that the ATP is released intact from the chromaffin tissue when the amines are released. It seems that catecholamines, ATP and soluble protein are released together when the chromaffin cell is stimulated.

Another constituent of the chromaffin granules is phospholipid. It has recently been found that the phospholipids of the chromaffin granules exhibit one distinctive feature: they are relatively rich in lysolecithin, a hydrophilic phospholipid usually present in the tissues in very small amounts (Blaschko et al., 1966). It is of interest that the presence of lysolecithin in the adrenal medulla has been known for some time (Hajdu, Weiss and Titus, 1957). It is interesting that another amine-storing cell, the mast cell, is rich in lysolecithin (Keller, 1962).

In 1924, the diagnosis phaeochromocytoma was not arrived at during the patient's lifetime. Today many of these tumours are removed. Forty-one years later, in 1965, we were able through the kind cooperation of Sir George Pickering, Regius Professor of Medicine at Oxford University, to obtain such a tumour that had just been removed by operation. The cytology of the tumour will be described elsewhere in collaboration with Dr. A. H. T. Robb-Smith. The tumour tissue contained 59% of adrenaline and 41% of noradrenaline. A homogenate of the tissue in isotonic sucrose was prepared, and on centrifugation about two thirds of the total catecholamines were found in the sediment; this is a figure very similar to that found in normal chromaffin tissue. Also the distribution of the catecholamines upon ultracentrifugation over a sucrose density gradient was normal, and the molar ratio, catecholamine:ATP of 5.1 was similar to that normally found in the fraction in which the amine-storing granules were principally recovered. The main difference between tumour tissue and normal chromaffin tissue was in the very high catecholamine content per unit of weight. In the electron micrographs, chromaffin granules appeared to be very numerous.

It is to be hoped that more of these tumours will be studied by these techniques, as this might help to lead to a correlation between clinical symptoms and amine storage.

An attempt has been made to give in this article a review of some of our activities since I first met Ernst Fischer in Göttingen many years ago. I have not related the work of our laboratory to that of others. Also, I have only described observations made on chromaffin tissue. The catecholamines have acquired a much wider importance with their discovery in adrenergic neurones and, more recently, in the central nervous system. However, the study of a more homogeneous tissue has the advantage of greater simplicity. Some of the findings made on the adrenal medulla, e.g., the enzyme studies, are of immediate relevance to nervous tissue. To what extent the new findings on amine storage and release have a counterpart in neurones, remains to be elucidated in the future.

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