BIOCHEMICAL CONTRIBUTIONS TO ENDOCRINOLOGY

EXPERIMENTS IN HORMONAL RESEARCH

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The Lane Medical Lectures, 1956

PREFACE

The following five papers — the Lane Medical Lectures of 1956 — were delivered in their original form last autumn at the Medical School of Stanford University, in San Francisco. They have been revised for publication, and I should like to thank the Stanford University Press for arranging for their appearance in book form.

To be invited to lecture at an American university is a compliment, and if that university be Stanford, then it is a distinction; but if Stanford University appoints one to the Lane lectureship, then that is indeed a very great honor. I must commence by expressing my very deep appreciation of having been invited to give this series of lectures, and I must also mention my awareness of the great responsibility that went with the honor.

To Professor Windsor C. Cutting I owe a special debt of gratitude for the careful preparation made by him in arranging for the lectures. It was also a pleasure to discuss, with him and with his colleagues at San Francisco and at Stanford, the subject of the lectures both before and after they were delivered.

I also wish to acknowledge the help of my colleague, Dr. E. T. Knudsen, in putting the lectures in a form suitable for publication, and I should like to thank Mrs. E. I. Barron, who has played a major part in the preparation of the manuscript.

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I INTRODUCTION TO BIOCHEMICAL RESEARCH IN ENDOCRINOLOGY

I have chosen my title of "Biochemical Contributions to Endocrinology" because it is one that covers almost completely the work of myself and my colleagues in the Courtauld Institute of Biochemistry during the last thirty-odd years. Much of this work has been published a good many years ago but it occurred to me that it would perhaps be of interest were I to say in greater detail how the work developed. No doubt a great deal can be gained by recounting the mistakes one has made and the attempts to rectify them which lead very frequently in directions quite different from those in which one originally started.

We may perhaps commence with some general observations of modern research in relation to therapeutics. Today we know that scientific research in medical and allied subjects is constantly scrutinized with a view to its application to therapy, and there is a feeling that this has always been so. It is in fact a very modern trend, and certainly this attitude developed only within the last hundred years. While the nineteenth century can definitely be described as the golden age of surgery, we have to wait until the twentieth century before we can say that modern medicine really began. Medical historians, and particularly those interested in the scientific aspect, tell us that modern medicine began with Harvey's description of the circulation in the seventeenth century. While this may be true from a historical point of view, it is certainly not true from the practical angle. If one reads Harvey's publications carefully, one can see quite clearly that his great discoveries were not in any way connected in his mind with therapy. Despite the descriptions in the famous work *Exercitatio anatomica de motu cordis et sanguinis* (24), the Galenical concept of the four humors—blood, phlegm, yellow and black bile—still

dominated the minds of physicians and was not finally disposed of until the end of the eighteenth century and the beginning of the nineteenth.

Of course, one of the great handicaps of the earlier physicians was their lack of really powerful drugs. The great pharmacopoeias prepared by the Royal College of Physicians, dating from 1618, contained what we now know to be practically worthless drugs. Apart from opium, quinine, and, later, digitalis, there were really no effective therapeutic agents whatsoever, and we can therefore see, if we study the treatment of a disease such as pneumonia throughout the sixteenth, seventeenth, eighteenth, and nineteenth centuries, that treatment altered little. A study of the treatment of pneumonia in the seventeenth, eighteenth, and nineteenth centuries reveals that the basic treatment consisted of blistering, bleeding, scarification, dry-cupping, and, in some cases, enemas or clysters. In reading the early physicians' accounts of the treatment one cannot help wondering whether people would not have been better off without any physicians at all. The difficulty, of course, in assessing this point is that usually one has no controls, but fortunately in the case of pneumonia there is a perfect series of controls for treatment of this condition in the eighteenth and nineteenth centuries. Toward the end of the eighteenth century and the beginning of the nineteenth, the strange figure of Hahnemann (1755–1843) dominated the medical therapeutic world of Europe (23). We shall not now enter into a discussion of his fantastic theory of potentiation by dilution, which was the basis of homeopathy, and we now know that practically the whole series of drugs in his pharmacopoeia were without activity even if given in much greater doses than the minute ones employed by his system. The strange fact was that his patients, in many cases, did very much better than those treated by the members of official bodies such as the College of Physicians. In fact, so successful was he that he retired to Paris in the 1830's a millionaire, having made it all out of his therapy. We now know that his success was really the result of doing nothing and that, to use an old-fashioned expression, he had "given nature a chance." One should never lose sight of the lesson provided by this study, and I should like to quote a very witty summary of the situation given in Allbutt and Rolleston's *System of Medicine* (1).

It is a humiliating but instructive fact that the possibility of recovery from acute disease without active treatment was established by the assumed success of a demonstrably futile system of therapeutics, the last, we may hope, of attempts to answer the absurd question, "On what universal principle should disease be treated?" When it could not be denied that persons suffering from pneumonia and other acute disorders did recover when treated with infinitesimal doses of useless drugs, it could not be long doubted that some acute diseases might get well of themselves.

The report of some cases of pneumonia which recovered in the Homeopathic Hospital at Vienna awakened thought on this subject, and an article by Sir John Forbes, which appeared in the British and Foreign Medico-Chirurgical Review [22], pressed the lesson home. Skoda [32] had given fair trial to other methods of treatment, and found that under the so-called expectant treatment the mortality of his patients from acute pneumonia was much less than when treated by bleeding, blisters and antimony. These facts were made known in England by George Balfour [2] who had followed Skoda's practice in Vienna. John Hughes Bennett of Edinburgh also published a series of cases of pneumonia treated without bleeding, antimony or mercury with unusually small mortality [3], and he gave an interesting account of the arguments of Alison, Watson, Christison and Markham. Discussion followed, but it was less prolonged than might have been supposed; as so often happens general opinion had been gradually altering, and was ready to turn at the first summons. Moreover, the advocates of antiphlogistic treatment threw away their case by the assertion that they were right in bleeding before, and right in doing nothing afterwards—not because their opinions but the nature of the disease had changed; and a presumed "sthenic type" of fevers and inflammations, with a successful heroic treatment corresponding thereto, was dwelt upon with the same satisfaction that an old man contrasts the hard frosts and heroic exploits of his youth with the mild winters and feeble powers of his contemporaries.

Pasteur's demonstration of the microbial origin of disease (30), together with the development of synthetic organic chemistry, toward the latter half of the nineteenth century changed the whole picture and we had the introduction of the first chemotherapeutic agent by Ehrlich in 1910 (21). From this period onward the eyes of therapeutists were firmly fixed on the laboratory and we find the sulphonamides in the 1930's very quickly establishing their position while the antibiotics were translated straight into medicine from the moment of their production. From the work that I am about to describe we shall be able to see that substances produced in our laboratory, such as the synthetic estrogens, very quickly found a place in therapy and the discovery of new sub-

stances secreted by the body, such as aldosterone, very quickly began to modify and explain a number of conceptions of disease.

Substances affecting oxidation in the human body

Cutting, Mehrtens, and Tainter (6) described in detail the remarkable effects on metabolism of administering 2:4-dinitrophenol. It was shown that by the oral administration of daily doses of 3 mg. per kg. body weight of 2:4-dinitrophenol a marked stimulation in metabolism could be obtained, and this was followed, provided the diet remained the same, by a rapid loss of weight. The observation that 2:4-dinitrophenol produces a stimulation of metabolism goes back a considerable way, as it was first observed in workers involved in the preparation of this substance for explosive purposes. Cutting and his collaborators pointed out that 2:4-dinitrophenol was a toxic substance quite apart from its power to raise the basal metabolic rate and that, therefore, its clinical use had to be very carefully guarded. Sir William Pope and I were engaged in the study of the biological effects of substituted phenols at this time, and we were very interested in comparing the activity of dinitro-ortho-cresol with that of 2:4-dinitrophenol. The acute toxicity of both these substances was about the same, but we were able to show by animal experiments that the dinitro-ortho-cresol was considerably more powerful than the 2:4-dinitrophenol, although, of course, the mechanism of stimulation of oxidation was undoubtedly the same (13). We now know that all substances of this type are too dangerous to be used clinically for a number of reasons. Above all, by their very powerful nature they are themselves extremely dangerous, for unless administration is continued by very frequent estimations of basal metabolic rate severe hyperthermia and death may result. It is interesting to note that it is possible to increase the basal metabolic rate by nearly 100 per cent without any corresponding alteration in the circulation rate. The only method, apart from the determination of basal metabolic rate, of following the change is to determine the oxygen saturation of the venous blood. This would be found to be very much diminished.

The appearance of bilateral cataracts in a number of patients who had used this type of drug for reducing purposes at once put an end to the therapy, and we are, therefore, only interested in

this group from a purely theoretical point of view. J. D. Robertson and I were, however, able to use dinitro-ortho-cresol to settle the real question as to whether myxedema was due solely to a reduction in the basal metabolic rate (14). Figure 1 shows a myxedematous man whose basal metabolic rate has been raised to plus 20 per cent by dinitro-ortho-cresol. The second picture shows the same patient with a basal metabolic rate of plus 10 per cent produced by the administration of thyroid extract. In the latter case, it can be seen that the signs of myxedema have disappeared, while in the former, despite the fact that the basal metabolic rate is higher, the stigmata of the disease are very clearly present. This adequately confirms the conception that thyroid hormone has a very specific action quite apart from the mere raising of the metabolic rate. A study of the effect of these polyphenols on the metabolism of tissue slices, as studied by the Warburg technique, has provided a series of researches. Some of the early work was done by me and my colleague, G. D. Greville, and we were able to show that unlike other stimulators of metabolism the poly-nitro-phenols stimulated both respiration and glycolysis. This occurred in normal and in tumor tissue (12, 15). During the 20 years that have elapsed since these results were published, the whole question of tissue metabolism and oxidation has made very great advances, but recent work on the action of the poly-nitro-phenols has emphasized the correctness of our original view, namely, that they act as a general stimulus to the action of all the oxidative systems in the tissue.

The pituitary and the control of gastric secretion

In the year 1924 my colleague F. Dickens and I were interested in studying methods for the production of insulin. The standard technique of the day was the alcohol process worked out by the original Toronto research workers. We had been interested in studying the properties of insulin picrate, and we were able to show that it was differentially soluble in a 70 per cent aqueous acetone solution. We, therefore, developed a process (10, 11) in which picric acid was applied direct to the mixed pancreas, and the insulin picrate was extracted with 70 per cent aqueous acetone. The hydrochloride was regenerated from the extracted insulin picrate and it was found that the yield was very high indeed. This method

was used successfully in commercial production of insulin, but in the long run modifications of the alcohol processes proved to be more efficient and in any case produced a substance capable of much greater purification so that the acetone–picric acid process, as it was called, became redundant and was only used for the estimation of insulin content of tissues.

In the early 1930's there was a great awakening of interest in pituitary hormones, and it was decided in my laboratory to apply the acetone–picric acid process to the pituitary to see what effects could be obtained from extracts prepared by this method. The original process was applied to pituitary glands obtained from the slaughterhouse and a good yield of hydrochloride was obtained. This substance was readily soluble in water and was a white non-deliquescent powder. Its properties were described by Noble, Smith, and myself in a paper (16). We investigated the general effects of this hydrochloride when administered to laboratory animals (18). Our first experiments were conducted on rabbits of 2 to 3 kg. weight, and an injection of 150 mg. of the material was arbitrarily chosen as a suitable commencing dose. It was quite obvious that the effect on the animals was marked. The first find was severe prostration, from which the animal recovered in a few hours. Most of the animals recovered, but we noticed that they ate nothing for some seven to ten days and the feces contained altered blood. After this period the rabbits ate voraciously and regained the weight they had lost, very rapidly becoming normal. It was apparent that something very dramatic must have taken place in the alimentary canal to produce these marked changes.

We decided to investigate the alimentary canal when we imagined the lesion, if any, would be at its height, some fifteen to twenty-four hours after the injection. On opening the abdomen of the rabbit killed under these conditions, it was immediately seen that there was a marked change in the stomach. The fundus, as seen from without, was plum-colored and was obviously the seat of a severe lesion. On removing the stomach and washing out the food residue a most remarkable lesion was evident. The esophagus, duodenum, and distal portion of the stomach appeared the normal pale color which we associated with the mucous membrane in this region. The fundus, however, presented a dark red, mottled appearance with a fibrinous exudate on the surface (Fig. 2).

Marked edema of the walls of the stomach beneath the mucosa usually accompanied the lesion; in fact the whole appearance was very similar to that seen in the post-mortem examination of human subjects who have taken a large dose of some severe corrosive poison.

This experiment was repeated over fifty times and the result was always the same, namely, a severe lesion confined entirely to the acid-bearing area of the stomach. A careful examination of the other organs of the body showed that they were unaffected. The problem arose, therefore, as to the nature of the mechanism producing this lesion, whether it was a pharmacological effect or whether it represented the exaggeration of some naturally operating control of gastric secretion from the pituitary body.

It was at this stage of our investigations that we were fortunate in being joined by Dr. Windsor Cutting, who spent some years in our laboratory and was present during the unraveling of this extremely complicated story. The following account is based on a series of publications in the *Proceedings* of the Royal Society appearing in 1937 (7, 8, 9, 19). One of the first questions to arise concerned the nature of the gastrotoxic factor. Experiments very quickly showed that the toxic factor was confined to the posterior lobe of the pituitary, since extracts of the anterior lobe had no effect whatsoever. Careful investigation of the oxytocic and vasopressive fraction showed that the former was without action, while the whole effects of our acetone–picric acid process extract could be obtained by the pituitary preparation containing only the vasopressive substance. We therefore concluded that our toxic factor was either the vasopressive itself or a substance associated with it. Investigations in recent years have shown that the action is given by the pure vasopressive substance; therefore we must now conclude that the results originally described by us in 1934 were due to the administration of an excess of vasopressive factor.

The lesion can be produced no matter how the vasopressive factor is introduced, whether by subcutaneous, intravenous, or intracisternal injection. Provided the pituitary extract is given in the form of the standard acetone–picric acid preparation, it will also produce the lesion when given by mouth. With regard to the type of animal, we were able to show that typical lesions could be produced in the monkey, cat, rabbit, guinea pig, rat, and mouse.

These results have been confirmed by a number of other workers (4, 26), and lesions have also been shown to be present in the dog (27) when the extract is given in adequate dose. As I have already stated, in all animals it is found that the lesion is confined to that area of the stomach in which oxyntic cells are present. If the extract is administered to an animal undergoing an acute experiment in which the stomach is exposed, a very interesting series of changes can be seen. Injection of the material intravenously produces a blanching of the entire stomach mucosa. This is followed about an hour later by marked dilatation and engorgement of the capillaries. Shortly afterward exudation and hemorrhage appear. A careful histological examination of sections taken during this period confirms microscopically the dilation. Figures 3, 4, and 5 show the microscopic appearance one hour, three hours, and six hours, respectively, after injection of pituitary extract (x60). Very early changes occur in the oxyntic cells, and blurring of the cell outline and pyknosis of the nuclei are common. As already stated, there is no sign of a lesion anywhere else in the mucosa of the stomach, esophagus, or duodenum. Only in one instance did we find an isolated lesion in the pylorus, and on section this was found to be an area of isolated rest of oxyntic cells. In the rabbit we found occasionally punched-out ulcers—in one case definitely showing a perforation sealed by a piece of omentum (Fig. 6). The interesting point is that even after so severe a lesion, complete healing can occur and the animal will show no change whatsoever.

Attempts to imitate the action of the pituitary extract with other drugs proved to be unsuccessful, with one exception. While atropine, pilocarpine, adrenalin, and histamine in the largest doses possible have never shown any lesions, the administration of normal hydrochloric acid into the stomach of the anesthetized animal produced a typical lesion. The injection of intense vasoconstrictors such as barium chloride produced a typical lesion, and therefore we concluded that an essential part of the mechanism was a diminution in the blood supply caused by vasoconstriction.

As previously stated, other organs of the injected animals appeared to be unaffected. We were, however, able to demonstrate another peculiar finding. A number of animals injected with the extract showed a very severe anemia. This after all is

not surprising when one considers the extensive nature of the lesion and the persistence of melena for some days after the injection. Together with Noble (17) I made a detailed study of the anemia. This appears on the fourth to fifth day and the red blood cell count may be reduced to as low a figure as a million red cells per cu. mm. This is associated with a leucocytosis of up to 50,000 white cells per cu. mm. Hemoglobin falls considerably lower than the diminution of the red blood cell count would lead one to expect. Reticulocyte response usually sets in on the fifth to sixth day and continues up to the eighth. Reticulocyte counts as high as up to 50 per cent were observed. A study of a stained smear shows a very interesting series of changes. The cells appear to be well filled with hemoglobin, but anisocytosis is present to a very marked extent. Large numbers of macrocytes and a few microcytes are seen in the smear. Also nucleated red cells have appeared. It is interesting to note that one cannot produce this type of change by experimental hemorrhage. The whole picture is very similar to that seen in pernicious anemia of the human subject, the anemia being very definitely of the macrocytic variety. A return to this subject was made during the war and it was shown that these changes occur in some 20 per cent of animals injected. Owing to the difficulty of obtaining stock at this period, these experiments were not continued, but they certainly raise the interesting question as to whether there is some centralized control of hemopoiesis.

Effect of posterior pituitary extract on gastric and intestinal secretion

Experiments were made mainly on rabbits and cats. These were anesthetized with nembutal, and a glass cannula was stitched into the most dependent part of the stomach, the pylorus being tied off. In the cats used for the chronic and prolonged experiments, a permanent external gastric fistula had been previously created, and it was found that with training it was possible to collect gastric juice in a pure form after the stomach had been emptied. The effect of various stimuli on gastric secretion was studied. It was found that histamine was one of the most suitable. In order to obtain a more physiological reaction we also studied the effect of the administration of insulin. It had previously been established (28, 31, 25, 5) that the production of a sudden hypogly-