INSULIN ACTION

edited by

IRVING B. FRITZ

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Banting and Best Department of Medical Research
University of Toronto, Toronto, Canada

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DEDICATION

It is a pleasure to dedicate this book to Dr. Charles H. Best, the codiscoverer of insulin and Head of the Banting and Best Department of Medical Research at the University of Toronto from 1941 to 1968. In addition, he has provided an excellent and vigorous closing paper "Perspectives: Past and Future" at this fiftieth anniversary meeting.

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FOREWORD

The fiftieth anniversary of the discovery of insulin is an important time to take stock of past accomplishments and to look forward to new achievements. The discovery of insulin epitomizes the dependence of a major breakthrough in the relief of human suffering on the painstaking accumulation of fundamental scientific knowledge, coupled with the imagination and enthusiasm for its clinical application. The discovery of insulin provided a major incentive for the expansion of research in wide areas of basic biological science and was a major initiative in the development of clinical studies along scientific lines.

In this symposium attention has been focused on an understanding of the mechanisms of insulin action and on the relationship of insulin to the complex interplay of factors concerned in the control of intermediary metabolism. It is through accumulation of such knowledge that one must look forward to future clinical applications.

A. L. Chute, Dean
Faculty of Medicine
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PREFACE

In 1921 Banting and Best succeeded in maintaining depancreatized dogs with pancreatic extracts, and they subsequently sparked investigations which led to the purification of their insulin preparations. These preparations have saved the lives of untold numbers of patients with diabetes mellitus. In the fifty years which have elapsed since those crucial experiments were initiated, enormous progress has been achieved in all areas embracing insulin chemistry, metabolic-endocrine physiology, and diabetes, but no new finding in the insulin field has thus far been able to match in scope the excitement which was engendered by the initial discovery by Banting and Best.

It is to pay homage to this discovery that a Symposium on Insulin Action was organized to be held in Toronto in 1971. It was felt that if justice were to be done to the subject, it was important to rekindle the fire of enthusiasm for future advances rather than simply to examine the ashes of this magnificent past bonfire. To this end, invitations were issued to leading insulin researchers to participate in a symposium at which emphasis was to be directed toward a detailed analysis of the mechanisms of insulin action. In our organization of the symposium, we considered a thorough coverage of the nature of the insulin-receptor interaction to be of primary importance. It also appeared equally necessary to examine the sequence of events leading to ensuing changes in rates of metabolic processes.

For the first portion, it was obvious that a knowledge of the chemistry of insulin was essential. We were most fortunate in having several representatives from the Oxford group (Drs. Dorothy Hodgkin, Guy Dodson, and Tom Blundell) present to explain the three-dimensional structure of insulin crystals. In addition, Dr. Edward Arquilla was invited to discuss the portions of the insulin molecule that are functionally important biologically, and to relate these findings to those of the Oxford group by inferring whether insulin in solution displays the three-dimensional properties of crystalline insulin. Other related chemical properties were to be considered by Dr. D. F. Steiner in his discussion of proinsulin.

For the part of the program devoted to insulin-receptor interactions, it was also of obvious importance to evaluate all that could be ascertained concerning the nature of the putative receptor and the plasma membrane in

which it is presumably located. For this discussion, contributions by Drs. P. Cuatrecasas, G. I. Drummond, T. Kono, G. V. Marinetti, and C. C. Yip were sought. Since the discussion among the participants concerning this and other controversial areas was likely to be useful, it appeared important to include questions and comments in the published proceedings of the symposium.

For the second portion, concerned with the coupling of the activated receptor to metabolic events downstream, it was easier to envisage an analysis of the metabolic events than it was to evaluate the coupling mechanisms about which essentially nothing is known. It appeared desirable to cover the effects of insulin on cyclic AMP levels, the control of adenyl cyclase activity, and the control of cyclic AMP phosphodiesterase activity in detail. Accordingly, contributions from various speakers mentioned above, as well as from Drs. R. L. Jungas, J. Larner, C. R. Park, and M. Vaughan were sought. We assumed that there would be considerable interest in ascertaining to what degree the actions of insulin could be associated with mechanisms resulting in lowered levels of cyclic AMP in various cellular compartments, and therefore attempted to leave adequate time for discussion of this topic.

Finally, analysis of insulin actions on the metabolism of glycogen, lipids, and protein was to be covered by Drs. M. Halperin, C. H. Hollenberg, L. Jefferson, M. E. Krahl, J. Larner, J. Tepperman, and I. Wool.

Gratitude is offered to the Canadian Medical Research Council for providing generous support for travel expenses of speakers and for many other expenses associated with the symposium. It is a pleasure to express appreciation to the officers of the University of Toronto, who graciously donated full use of the Medical Sciences Building Auditorium, together with its excellent audiovisual facilities, during the three days of the symposium. Sincere thanks are also extended to the Dean of the School of Medicine, Dr. A. L. Chute, for his introductory remarks of welcome and for his strong moral support of this venture, from conception to completion. Grateful appreciation is tendered to the various discussants and chairmen of the sessions, including Drs. A. M. Fisher, R. E. Haist, G. Hetenyi, J. Logothetopoulos, and H. Schachter for their full participation and cooperation. I am especially indebted to the speakers for their sterling presentations and for their willingness to submit manuscripts of their talks so quickly after the completion of the symposium. Finally, it is not possible to express fully my sense of gratitude to Mrs. Jackie Campbell, because it is she who attended to an endless number of details concerning the arranging of accounts and programs, and it was she who laboriously produced an accurate transcript of the discussion, together with a final typing of all the edited discussion and manuscripts. Without her efficient, accurate, and cheerful help, I doubt if I would have had the stamina to have completed the charge of the University of Toronto

"Committee for the Fiftieth Anniversary of the Discovery of Insulin" to explore the possibility of publishing the Proceedings of the Symposium on Insulin Action.

Irving B. Fritz

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CHAPTER I

THE ARRANGEMENT IN THREE DIMENSIONS OF THE ATOMS IN INSULIN MOLECULES AND CRYSTALS

Dorothy Crowfoot Hodgkin

with

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INTRODUCTION

We have a tremendous problem to consider today, the problem of the nature of the biological activity of insulin. Fifty years ago a preparation of the hormone was obtained from the pancreas which proved capable quite literally of saving victims of diabetes from imminent death. Photographs of the first patients brought in for treatment illustrate the characteristic wasting away of the tissues of the body caused by the disease (wasting which is hardly ever seen now), and their astonishingly rapid restoration after the hormone had been administered. How insulin affects these changes is our problem today.

It is my part in this discussion to describe the present state of our knowledge of the structure of the active molecule, the arrangement in three dimensions of the atoms as observed in insulin crystals.

The first extracts of insulin made by Banting and Best and used for clinical tests were very far from pure. It was common during the first treatment of patients to observe small abscesses at the point of injection which

appeared to be due to the presence of proteins other than insulin. A critical stage in the purification of the hormone (still continuing today) was the preparation in 1925 of the first crystals of insulin (Abel, 1926). Banting and Best were both very young when they isolated insulin, 30 and 22 years old respectively, at the beginning of their scientific careers. J.J. Abel was quite old, 67 and near retirement, when A.A. Noyes suggested that he should take a sabbatical year at Pasadena and try to crystallize insulin. Abel gathered a group of young graduates and, with their help, successfully grew crystals of insulin in the characteristic rhombohedral form which he identified correctly. However, his initial success was followed by a confusing period; for several years some insulin preparations gave crystals easily, others not. The confusion was resolved in 1934 by the researches in Toronto of D.A. Scott and A.M. Fisher, his research student (here today), who showed that the rhombohedral crystals required for growth zinc or some similar divalent metal (1934). Scott had noticed that zinc was ordinarily present in the pancreas and deduced that it might be necessary for crystallization. From this point on, the different pharmaceutical firms used zinc to crystallize their insulin: it was one of the first samples of micro crystals prepared by Boots Pure Drug Company which was given to me by Professor Robert Robinson in 1935. From this preparation, single crystals of insulin were grown by Scott's method large enough to give interpretable diffraction effects by X-rays (Crowfoot, 1935).

From the first X-ray photographs of single air-dried insulin crystals, the edge of the unit rhombohedron was defined as 44.3 Å long. Density measurements combined with solvent loss on drying led to an estimated molecular weight for the protein in the unit rhombohedron of 35,700+, close

+ Note The figure first reported, 37,600, was rather higher owing to too low an estimate of the water present in the crystals.

to the first estimate of 35,100 for the insulin molecular weight in solution measured by Svedberg and Sjögren in the ultracentrifuge in 1931 (Brandenburg, Private Communication). Since the crystals had trigonal symmetry, the measurements showed that the unit was divided into three equivalent parts of weight approximately 12,000. Only gradually in the following years did evidence gather that the weight of the insulin molecule was half this figure, of the order of 6,000, corresponding with the amino acid sequence established by Sanger and his coworkers in 1958 and synthesized in the early 1960's. The distinction made little difference to the problem of structure analysis presented by the zinc insulin crystals to the X-ray crystallographer. Whether in one or two insulin molecules, there were still a calculated 512 carbon atoms, 152 oxygen atoms, 130 nitrogen atoms and 12 sulphur atoms to be placed in three dimensions in the insulin crystal structure.

The possibility of solving the crystal structure of insulin by the study of an isomorphous series of crystals containing different heavy atoms was envisaged in a letter written by J.D. Bernal in 1935 (Hodgkin and Riley, 1968). It is clear from this letter he had been reading Scott and Fisher's paper, which gave analytical data on zinc, cobalt and cadmium insulin, and that he thought the differences between zinc and cadmium insulins should be explored. At the time the letter was written, not even the structure of one small organic molecule had been found by the method of isomorphous replacement. Many years had to pass in which considerable developments occurred in the measurement of the intensities of X-ray diffraction effects, in electronic computers, in experience in the X-ray analysis of other protein crystals, before in 1969, an interpretable insulin electron density map was calculated (Adams et al., 1969).

The crystals used in the X-ray analysis were of pig insulin, grown by methods developed by Schlichtkrull (1958) in the preparation of slow