

# Metabolic Interconversion of Enzymes 1973

Third International Symposium held  
in Seattle, June 5-8, 1973

Organized by  
E.H. Fischer E.G. Krebs H. Neurath  
E.R. Stadtman



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With 235 Figures

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## Preface

The First International Symposium on the Metabolic Interconversion of Enzymes was held in Santa Margherita Ligure, Italy, in May, 1970, under the direction of Professor G. Bonsignore. Because of rapid developments in this field, a second Symposium was organized a year and a half later in Rottach-Egern, Germany (October, 1971) by Professors E. Helmreich, H. Holzer and O. Wieland. At that time, so much new information had accumulated that it was decided to repeat such conferences approximately every other year; the United States was chosen as the next site. This publication reports the Proceedings of the Third International Symposium on the Metabolic Interconversion of Enzymes held at the Battelle Seattle Research Center, Seattle, Washington, June 5 - 8, 1973.

The conferences were originally designed to examine the control of metabolic reactions by covalent modification of certain key enzymes. Covalent, as opposed to allosteric or metabolic, regulation had first been recognized some fifteen years before. Initially thought to represent an added sophistication in regulatory processes possibly reserved to higher organisms, covalent regulation has now been found in both prokaryotes and eukaryotes. Those early studies in covalent modification revealed for the first time the existence of special "converter" enzymes whose purpose in life is to switch other molecules from one state of activity to another. It has since been found that several converter enzymes can act in succession to modulate a single metabolic step: besides allowing for a huge enzymatic amplification, such a "cascade" provides for annex molecules in which the information needed to control intracellular processes can be stored. Through converter enzymes, metabolic pathways can be linked to one another so that a given signal can trigger several physiological processes. An example is the phosphorylation of non-enzymatic cellular components such as histones, ribosomes and proteins related to muscle contraction, by the protein kinases which control carbohydrate metabolism. No doubt other examples will be uncovered. Recently, these conferences have been expanded to include protein-protein interactions, a topic which can no longer be overlooked, and the irreversible modification of enzymes, such as the initiation of metabolic events by limited proteolysis.

The organizers are greatly indebted to the Battelle Memorial Institute, the Fogarty International Center for Advancement in the Health Sciences, and the International Union of Biochemistry whose generous support made this Symposium possible.

They would like to express their gratitude to Dr. Tommy W. Ambrose, Director of the Battelle Seattle Research Center, Dr. W. R. Wiley, Coordinator of the Life Sciences Program of the Battelle Institute, Mr. L. M. Bonnefond, Conference Coordinator and the entire staff of the Battelle Seattle Research Center for making their facilities available to the participants and for the gracefulness with which they attempted to fulfill everyone's needs. Our thanks also go to Mr. A. W. Roecker, Librarian of the Battelle Seattle Research Center, for his invaluable help in preparing and editing this volume, Ms. Vera Swile for retyping the bulk of the manuscripts for photocopy reproduction, and to Dr. Danielle Gratecos for proof-reading them.

Finally, the organizers would like to express their sincere appreciation to the distinguished scientists who travelled from the four corners of the earth to attend this Symposium; their fine contributions form the substance of this book.

Seattle, Washington  
August, 1973

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# Introductory Remarks

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It has been my pleasure and privilege to make some introductory remarks at this symposium on previous occasions — the last time in 1971. Now another two years have come around and I am happy to say that, judging from my reading of the literature, rapid progress continues in the field of interconvertible enzymes.

Dr. Krebs has been kind enough to give me two Tables which he prepared and which show where we stand at present, that is, before we have heard the reports at this symposium which undoubtedly will contain new findings.

TABLE I

## PROTEIN PHOSPHORYLATION-DEPHOSPHORYLATION SYSTEMS

---

### 1. Enzymes

Phosphorylase  
Phosphorylase kinase  
Glycogen synthetase  
Hormone-sensitive lipase  
Pyruvic dehydrogenase  
Pepsinogen ?  
Acetyl CoA carboxylase

---

The enzyme systems reported to occur in a phospho-dephospho form are now seven in number, with one possible newcomer, namely Acetyl CoA carboxylase. Phospho-dephospho forms also imply a kinase and a phosphatase for each of these enzymes. The purification of these conversion enzymes is essential for information on their specificity, kinetics, and regulatory control. This proves to be a formidable task in some cases. For example, O. Wieland and his group reported that the pyruvate dehydrogenase phosphatase of pig heart muscle required an 8500 fold purification until it was homogeneous in polyacrylamide gel electrophoresis.<sup>1</sup> Some of these conversion

---

<sup>1</sup>This footnote refers to articles which appeared in the Second International Symposium on Metabolic Interconversion of Enzymes. Edited by O. Wieland, E. Helmreich, H. Holzer. Springer-Verlag, 1972.

enzymes are rather complex in structure and function, but since they are in many cases the targets of regulatory control, further work is needed.

In Table II are assembled some phospho-dephospho forms of nonenzymatic proteins. Here we have much less of an idea what the functional significance of these two forms might be. Again we meet the problem of characterizing the interconversion enzymes. The protein kinases, some of them activated by cyclic nucleotides, play an important role in the phosphorylation of these proteins. When these phosphorylations involve protamines, other nuclear proteins and ribosomal proteins, one suspects that some important function or regulation of the genetic apparatus is served by these phosphorylations. Similar considerations apply to most of the other proteins listed here. A large amount of work remains to be done which undoubtedly will yield new and important results.

TABLE II  
PROTEIN PHOSPHORYLATION-DEPHOSPHORYLATION SYSTEMS

---

II. Nonenzymic proteins

Nutritive proteins

Casein

Phosvitin

Histones and protamines

Nonhistone nuclear proteins

Ribosomal proteins

Myofibrillar proteins

Actin

Troponin (TN-I, TN-T)

Light myosin chain

Connective tissue proteins (collagen, etc)

Membrane proteins

Proteins of cell membrane

Proteins of sarcoplasmic reticulum

Neurotubular proteins

---

Other covalent modifications are shown in Table III. The regulation of *E. coli* glutamine synthetase through adenylylation-deadenylylation has been the subject of several reports at previous meetings by Stadtman and Holzer and their collaborators. The  $P_I$  and  $P_{II}$  regulatory proteins which effect this interconversion may in the case of  $P_{II}$  occur in two forms as suggested by the incorporation of a uridyl moiety from UTP into  $P_{II}$ .<sup>1</sup> Another adenylylation-deadenylylation system from *E. coli* involving aspartate kinase has recently been described by Niles and Westhead (1973). The last entry in the Table refers to the remarkable effect of diphtheria toxin described by Hayaishi and his group in catalyzing a transfer of adenosine diphosphate ribose from

NAD to aminoacyl transferase II from rat liver.<sup>1</sup> Very likely, I have overlooked some recent findings in this brief survey.

In previous years, I have tried to bring up some specific problems for discussion by asking some questions.<sup>1</sup> One of these questions regarding regulatory control of glycogen deposition has not received a complete answer and for this reason I would like to bring it up again, but approach it from a different angle.

TABLE III

## PROTEIN NUCLEOTIDE-DENUCLEOTIDE SYSTEMS

<u>Nucleotide</u>	<u>Enzyme</u>
AMP	E. coli glutamine synthetase
AMP	E. coli lysine sensitive aspartate kinase
UMP	P <sub>II</sub> - regulatory protein (glutamine synthetase)
ADP-ribose	Aminoacyl transferase II

The background is as follows. Potentially glycogen degradation in liver through phosphorylase can be at least 10 times as fast as its synthesis via UDPG-glucosyl transferase. Another point is that the liver generally contains a considerable amount of the phosphorylase in the active form. There can be little doubt that when glucose is injected or ingested and the liver begins to deposit glycogen, there must be a decrease in active phosphorylase and an increase in active glycogen synthetase. Two mechanisms have been described which could do this.

TABLE IV

## SUMMARY OF EFFECT OF HORMONES ON GLYCOGEN METABOLISM

<u>Agent</u>	<u>Target</u>	<u>End Effect</u>
Epinephrine } Glucagon }	Adenylate cyclase + }	Phosphorylase +
Cyclic AMP	Protein kinase + }	Synthetase -
Calcium ions	Phosphorylase kinase + Phosphorylase phosphatase - }	Phosphorylase + (in contracting muscle)
Insulin	Adenylate cyclase - ? }	Synthetase +
Glucose (increasing)	Phosphorylase phosphatase + }	Phosphorylase -
Glycogen (increasing)	Phosphorylase kinase + Synthetase phosphatase - }	Phosphorylase + Synthetase -

+ denotes an increase and - a decrease in the activity of the respective enzymes.

Based on the findings made nearly 10 years ago that insulin activates glycogen synthetase, Larner and Villar-Pilasi (1971) believe that insulin secretion after a



glucose load is the main regulatory mechanism. The other mechanism is based on the observation by Hers and his group that glucose as an allosteric inhibitor makes phosphorylase  $\alpha$  more accessible to the action of its own phosphatase.<sup>1</sup> In the intact animal injected with glucose, phosphorylase  $\alpha$  decreases during the first 2-3 minutes and this is followed by a rapid rise in active synthetase. This glucose effect was found to be independent of insulin, since it occurred in alloxan-diabetic rats or in rats treated with anti-insulin serum (De Wulf, 1971). Larnier and his group, on the other hand, could not detect any effect of glucose alone on the level of active glycogen synthetase in depancreatized dogs maintained with insulin. As far as I know, this lack of agreement in experimental results has not been resolved.

It seemed to me of interest to approach the problem in a different way and to ask the question whether we can say anything more definite about the primary target of insulin action. As you can see, I have rather boldly assumed that insulin acts to inhibit adenylate cyclase and it thus becomes a direct antagonist of epinephrine and glucagon. A number of investigators, notably Sutherland and Park, have measured the cyclic AMP level in various insulin sensitive tissues. The general findings were that insulin lowers the cyclic AMP concentration, especially when it is elevated as the result of epinephrine or glucagon injection or in diabetes. Effects of insulin on the basal level of cyclic AMP were not regularly obtained (Jefferson *et al.*, 1968). Larnier and Villar-Palasi (1971) emphasize the fact that they could obtain an increase in active glycogen synthetase in muscle after insulin injection without changing the level of cyclic AMP. Because of the difficulties involved in dealing with concentration relationships in intact tissues, the effects of insulin on cyclic AMP can at best provide indirect evidence of an interaction with adenylate cyclase.

Direct evidence for such an interaction has been provided by a number of authors (Hepp, 1972; Flaviá and Torres, 1973). Illiano and Cuatrecasas (1972) worked with partially purified preparations of liver and fat cell membranes that exhibited adenylate cyclase activity and had specific receptors for glucagon and insulin. In both preparations insulin at concentrations as low as  $10^{-11}$  M (2  $\mu$ units/ml) depressed the activity of adenylate cyclase stimulated by glucagon or epinephrine. The effectiveness of insulin at these low concentrations agrees well with the dissociation constant ( $5 \times 10^{-11}$  M) for insulin and its membrane receptor determined by Cuatrecasas (1971). An inhibiting effect of insulin on the basal adenylate cyclase activity could not be demonstrated with liver cell membranes but was consistently observed at 20  $\mu$ units/ml with fat cell membrane preparations. No effect of insulin on degradation of cyclic AMP by phosphodiesterase could be demonstrated in these preparations. Negative results were reported by Rosselin and Freychet (1973).

There is other evidence suggesting a relation of the insulin binding site to the activity of adenylate cyclase. Certain plant lectins (concanavalin A and wheat germ agglutinin) compete with insulin for its binding site and like insulin, they also inhibit the basal and the epinephrine-stimulated adenylate cyclase activity in fat

membrane preparations. These lectins also show an insulin-like activity on glucose transport and epinephrine-stimulated lipolysis (Cuatrecasas and Tell, 1973).

I will not discuss the question here whether the different biological effects of insulin can all be explained by its interaction with cell membrane receptors since this would take us too far afield. Rather, I would ask what effects would be expected if adenylate cyclase activity were inhibited. We are probably far from knowing all the biological effects of cyclic AMP (and cyclic GMP), but we do know something about its interaction with protein kinase. Cyclic AMP activates the protein kinase by binding to an inhibitor protein, causing dissociation of an enzyme-inhibitor complex. Within its effective range of concentrations, cyclic AMP would thus regulate the amount of active protein kinase and this in turn would determine the ratio of active to inactive phosphorylase and glycogen synthetase, such that insulin would promote inactivation of the former and activation of the latter. It thus seems to me likely that a double control is exerted over glycogen synthesis which involves both the insulin effect described by Larner and the glucose effect described by Hers.

Knowledge of the structure of adenylate cyclase and the link between the different membrane receptors and the catalytic unit that makes cyclic AMP is basic to an understanding of the mechanism of action of different hormones which may also include insulin. The site and mechanism of action of insulin has been one of the most elusive problems of regulatory biology and has been and still is rendered difficult by lack of reproducibility of experimental results. Perhaps one can hope that we have come near to the solution of this important problem.

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