THE ACTION OF INSULIN

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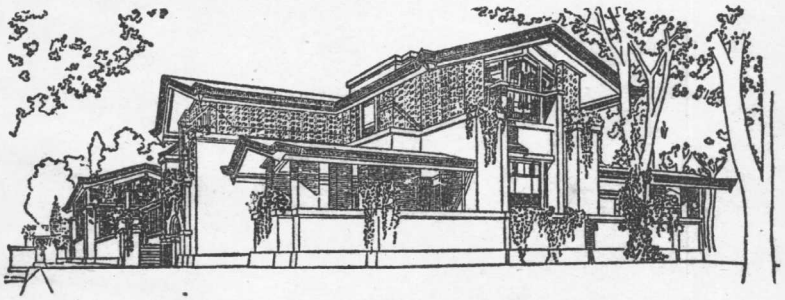

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PREFACE

IN THIS monograph, our aim has been to present the results of recent investigations dealing with the problem of the mechanism of insulin action in a manner which might interest the clinician as well as the research worker. This discussion of the action of insulin should not be considered an all-inclusive review of the subject, but rather a survey of the current state of our knowledge in this field, as we see it. In so doing, we have omitted much pertinent material, at the same time stressing those aspects of the action of insulin with which we have been directly concerned.

To Dr. William C. Stadie, who has contributed so much to present knowledge of insulin action, we would like to express our thanks and warmly acknowledge our indebtedness. We also wish to thank Dr. F. D. W. Lukens and Dr. Richard B. Singer for their helpful criticism.

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CHAPTER I

INTRODUCTION

By

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THE EXPERIMENTAL production in animals, by surgical removal of the pancreas, of a metabolic state closely resembling diabetes mellitus brought into being the problem of the action of insulin. Many phases of this problem have been developed since its classical inception by von Mehring and Minkowski. An early one was concerned with a study of the disturbances of total metabolism in the disease. Experiments were largely confined to the intact animal. This period culminated with the development of the two antithetical theories of under-utilization and over-production—now recognized to be over-simplifications of complex phenomena. The isolation of insulin as the active principle of the pancreas was the crowning achievement of this early work.

But with the astounding developments in enzymology which revealed in detail the complex patterns of intermediary metabolism of foodstuffs, the interest and aims of the metabolist working on diabetes shifted. He now sought to localize the action of insulin to some particular reaction in the metabolic schema. But the development of endocrinology has shown that insulin functions in a milieu of hormones and the elucidation of the chemical action of insulin continues to elude the ardent searches of many

investigators. Thus the problem of the action of insulin has become one of the interaction of hormones with enzyme systems. In time these interactions will yield their secrets to the researcher.

A new phase is just beginning—that of studies of phenomena on the molecular level designed to reveal the molecular mechanism by which hormones and enzymes produce their catalytic effects. Since little is known of these interactions in the case of enzymes alone it is not surprising that the added effects of hormones are shrouded in almost complete darkness. No one can predict the limits to which continued research will lead. But fascinating discoveries will continue to reward the diligent worker. This monograph by Drs. Haugaard and Marsh attempts to summarize our present knowledge of the problem of the action of insulin.

CHAPTER II

ESSENTIALS OF INTERMEDIARY METABOLISM

SINCE the discovery of insulin, more than three decades ago, an extensive scientific literature on the subject of the action of insulin has accumulated. This work has led to an understanding of metabolism in the diabetic organism and of the action of insulin on overall metabolic processes in the intact animal. However, little progress has been achieved in the study of the problem of the mechanism whereby insulin exerts its manifold effects. At present, the precise biochemical mode of action of insulin remains unknown. The study of the mechanism of action of other hormones has similarly met with limited success.

Since hormones are concerned with the regulation of metabolic reactions, progress in the study of hormonal action is dependent on the extent of our knowledge of intermediary metabolism. For this reason we have chosen to begin our discussion with a consideration of some of the current concepts of cellular biochemistry.

ENDERGONIC AND EXERGONIC REACTIONS AND THE NATURE OF ENZYMATIC CATALYSIS

One of the chief characteristics of the living cell is its ability to accelerate chemical reactions which are ordinarily too slow to be easily demonstrable. From these catalyzed reactions, energy is derived which is harnessed by the cell for the performance of work, growth, and reproduction.

The decrease in free energy ($-\Delta F$) associated with a chemical reaction is a measure of the maximum amount of energy which is available for the performance of work. Reactions in which there is a total decrease in free energy are called exergonic. Such reactions may occur spontaneously and the energy derived may be utilized for synthetic processes and work. Endergonic reactions are associated with an increase in free energy. They do not occur spontaneously but require energy to proceed.

In the cell, chemical reactions take place continuously in such a way that the energy derived from exergonic reactions is utilized to carry out endergonic processes. Overall synthetic reactions such as the formation of glycogen, fat, or protein are endergonic. The energy required to carry out these processes in the cell is obtained by linking the synthetic reactions to the oxidation of hydrogen to water, a strongly exergonic reaction.

Although the value of ΔF for a given chemical reaction indicates whether it is possible for such a reaction to occur spontaneously it does not yield information about the rate at which the reaction actually does occur under given experimental conditions. To answer this question it is necessary to consider the activation energy of the reaction involved. It is a familiar observation that an increase in temperature of 10° C will double or treble the rate of a chemical reaction. The occurrence of a reaction depends upon the successful chance collision of two molecules or groups capable of reacting. A rise in temperature increases the number of collisions and hence the reaction rate. Yet actual calculation shows that a 10° rise in temperature will increase the average kinetic energy of most systems by 3% (1), hardly enough to explain a two-fold increase in reaction rate. The explanation is that not all collisions between molecules result in a reaction. Only collisions

between "active" molecules; i.e., molecules having a total energy (chiefly vibrational) in excess of a certain amount do result in a reaction. The amount of energy required to change one mole of average molecules into activated ones is called the activation energy, E. It can be shown that a 10° rise in temperature doubles or triples the number of activated molecules.

An excellent discussion of the significance of the activation energy in chemical reactions has been given by Haurowitz (2). It may be said that in a given reaction the overall change in free energy is not altered by a catalyst. However, the catalyst decreases the activation energy by altering the pathway. Thus the reaction (*1*) $A + B \rightleftarrows AB$ may proceed by the pathway (*2*) $A + B + C \rightleftarrows ABC \rightleftarrows AB + C$. If the activation energy necessary for reaction (*2*) is lower than that necessary for reaction (*1*), C will act as a catalyst and the reaction rate will be accelerated. From this point of view the action of an enzyme consists of changing the pathway of a reaction from one requiring a high activation energy to one requiring a low one.

The work of Michaelis and Menten (3) led to the concept that enzymes combine with their substrates to form an enzyme-substrate complex which is subsequently broken down to the free enzyme and the products of the reaction. The velocity of the overall reaction is determined by the rate of decomposition of the enzyme-substrate complex. The intermediary compound corresponds to the formation of ABC in equation (*2*) above. Direct proof for the existence of enzyme-substrate complexes has been obtained by Stern (4) and by Chance (5) for the enzyme catalase. A mathematical expression of this concept of enzyme action is the Michaelis-Menten equation in which the law of mass action is applied to the formation of the enzyme-substrate complex. The dissociation constant K

for the reaction enzyme + substrate⇌enzyme-substrate is equal to

$$\frac{\text{(concentration of free enzyme) (concentration of substrate)}}{\text{(concentration of enzyme-substrate complex)}}$$

By appropriate mathematical treatment (6), this constant can be calculated for a given reaction from measurements of the reaction velocity as a function of substrate concentration. The Michaelis constant is a measure of the affinity of the enzyme for the substrate.

THE STEPWISE UTILIZATION OF ENERGY

The energy provided by the reactions of intermediary metabolism is used by the cell for the performance of work, including growth and reproduction as well as mechanical work such as muscular contraction. The overall process involves the oxidation of foodstuffs to CO_2 and H_2O. The energy so generated is not released all at once as it is when sugar is burned in air. Instead, the original assimilated foodstuff molecules undergo a series of intermediate reactions. In each step, energy is absorbed or liberated by the synthesis or cleavage of the chemical bonds present in the intermediate compound. Some of these steps are oxidative; i.e., hydrogen atoms (electrons and protons) are removed from the substrate. The electrons are transferred by a series of respiratory enzymes and eventually cause the reduction of oxygen in the presence of hydrogen ions to form water.

For example, the oxidation of lactic acid consists of a transfer of two electrons and two protons to a coenzyme; in this case, diphosphopyridine nucleotide (DPN). This reaction is catalyzed by a specific dehydrogenase. The reduced coenzyme in turn oxidizes a second coenzyme, alloxazine adenine dinucleotide, again with the help of a specific protein enzyme. The protons (i.e., H^+) transferred

to the coenzyme are exchangeable with protons in the environment so that what actually occurs is a transfer of electrons. The final links in the chain of enzymes are cytochrome C and cytochrome oxidase. These constitute an enzyme system containing iron in a porphyrin prosthetic group. The iron is alternately oxidized and reduced. The final step consists of the oxidation of cytochrome oxidase by molecular oxygen. This series of reactions represents a pathway which is widely utilized by many cells for the oxidation of a variety of substrates. However, the particular reactions and coenzymes involved vary with the type of cell and with the substrate oxidized. Some substrates, for example, do not react with DPN but are oxidized directly by an enzyme containing alloxazine adenine dinucleotide as the requisite prosthetic group. In some cells, cytochromes other than cytochrome C are present.

The important consequence of carrying out the oxidation of a substrate by a process such as the one described is that a maximum amount of energy may be obtained for useful work. Each of the reactions involved take place in a nearly reversible manner so that very little energy is wasted.

The essential principles involved in cellular oxidation are treated in detail in the book edited by Lardy (1). An excellent series of lectures by Dixon (7) is also pertinent to this discussion. The problem of electron transfer in biological systems is discussed by Geissman (8).

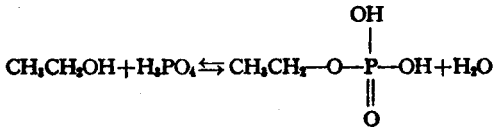
THE MOBILIZATION OF ENERGY BY MEANS OF THE ENERGY-RICH PHOSPHATE BOND

The researches of Harden and Young (9) on alcoholic fermentation in yeast and of Meyerhof (10) and others on glycolysis is mammalian muscle conclusively demonstrated

the importance of phosphorylated intermediates in cellular metabolism.

The importance of the high energy phosphate bonds lies in the realization that the chemical bond between the organic part of the molecule and the phosphate radical may contain larger or smaller amounts of potential energy. The compounds containing phosphate bonds of high potential energy serve as a store of energy which is readily available when needed. The continuous synthesis and breakdown of high energy phosphate bonds constitute an important mechanism of intermediary metabolism.

Let us consider the esterification of alcohol by phosphoric acid:



In this reaction, ethyl alcohol is phosphorylated. The reverse reaction would be called dephosphorylation. There are other types of phosphate bonds which are not true esters. The most important of these are:

1. Carbonyl phosphate. Example: glucose-1-phosphate.
2. Guanidine phosphate. Example: creatine phosphate.
3. Carboxyl phosphate. Example: 1,3 diphospho-glyceric acid.
4. Enol phosphate. Example: Phospho-pyruvic acid.
5. Pyrophosphate. Example: The two terminal phosphate bonds in adenosine triphosphate.

The four last-mentioned of these bonds are high energy phosphate bonds. The free energy contents of some of these bonds are listed below:

Alcohol phosphate—400 calories per mole.
Carbonyl phosphate—2,000 calories per mole.

Pyrophosphate—10,500 calories per mole.
Carboxyl or enol phosphate—14,000 calories per mole.

The theoretical development of the concept of the energy-rich phosphate bond is based to a great extent on the work of Lipmann (11). The subject has been reviewed by Kalckar (12), and Oesper (13) has discussed the difficult and interesting question of the relation between the molecular structure of the compounds involved and the energy content of the phosphate bonds.

In the cell, few compounds are phosphorylated by a direct reaction with inorganic phosphate. Instead most of them are *transphosphorylated* by reacting with a compound, usually ATP*, containing an energy-rich phosphate bond. The transformation of glucose to glucose-6-phosphate, the initial step of glucose metabolism, is such a reaction:

$$\text{Glucose} + \text{ATP} \xrightarrow[\text{Hexokinase}]{} \text{Glucose-6-phosphate} + \text{ADP}$$

In this reaction, energy is supplied by the high energy phosphate bond of ATP and the resultant glucose-6-phosphate has a low energy phosphate bond. The first step in the utilization of glucose—the hexokinase reaction—is a transphosphorylation reaction and it involves the expenditure of energy by the cell. It seems paradoxical that the first step in the utilization of a foodstuff by the cell should require energy instead of yielding energy. However, the subsequent reactions of the glucose phosphate more than make up this deficit, since these reactions regenerate energy-rich phosphate bonds in excess of the number initially introduced.

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* ATP Adenosine triphosphate
ADP Adenosine diphosphate

There are four main cellular reactions involving phosphate bonds (see Drabkin [14]). They are:

1. Phosphorylation. Direct addition of inorganic phosphate.
   Example: Glyceraldehyde phosphate + DPN + phosphate $\rightleftharpoons$ Glyceric acid diphosphate + H_2 DPN
2. Dephosphorylation. Splitting of the phosphate bond.
   Example: Glucose-6-phosphate + HOH $\xrightleftharpoons[\text{phosphatase}]{}$ Glucose + phosphate
3. Transphosphorylation. Transfer of phosphate from one compound to another.
   Example: Glucose + ATP $\xrightarrow[\text{hexokinase}]{}$ Glucose-6-phosphate + ADP
4. Phosphorolysis.
   Example: Glycogen + n phosphate $\xrightleftharpoons[\text{phosphorylase}]{}$ n Glucose -1-phosphate.

Although the details have not been worked out as well for the metabolic reactions of protein and fat, there is ample evidence that phosphate plays an equally important role in their metabolism.

The energy accumulated in ATP is the driving force for synthetic reactions. As we have seen, this energy is obtained from degradative reactions which are mainly oxidative. It is important to note that the energy obtained in oxidative reactions is not only generated in the initial dehydrogenation of the substrate but that the subsequent reactions of the respiratory enzymes give rise to the production of energy-rich phosphate bonds. This had been inferred from theoretical considerations (15) and was recently demonstrated experimentally by Friedkin and Lehninger (16).

There is yet another aspect of the conversion of metabolites into phosphorylated intermediates which should be considered. Drabkin (14) has pointed out that since the cell

contains approximately 70% water (40 molar), reactions yielding water which take place inside the cell do so against an overwhelming concentration gradient. Consider, for example, the synthesis of glycogen. The direct formation of glycogen by the polymerization of glucose would involve the production of water. According to the law of mass action such a reaction would be difficult to accomplish since the high concentration of water in the cell favors the reverse reaction. In the cell this reaction is accomplished by a preliminary phosphorylation of glucose to glucose-6-phosphate, followed by transfer of the phosphate to the 1-position. Glycogen is finally synthesized from glucose-1-phosphate, a reaction involving the production of inorganic phosphate rather than water. Since the concentration of phosphate in the cell is low this reaction is much more easily accomplished.

THE INTERMEDIARY METABOLISM OF CARBOHYDRATES, FAT AND PROTEIN AND THE CONCEPT OF THE METABOLIC POOL

As a result of extensive work by Schoenheimer (17), Stetten (18) and others with isotopically labeled metabolites, a dynamic concept of the nature of metabolic reactions has developed. Before this work it was thought that fat, for example, was stored in stationary depots, to be mobilized only when needed. It is now clear that continual synthesis and breakdown of fat occurs even in the fat depots (19). It is apparent that a constant interchange of molecules between fat, carbohydrate and protein takes place. There is a metabolic pool of intermediate compounds which supplies molecules for oxidation and for the synthesis of fat, protein, glycogen and all the other necessary components of the cell. There is no sharp distinction between structural elements of the cell and substances used for the pro-

duction of energy by oxidation. However, the rates at which different substances in the cell are broken down and rebuilt may vary considerably.

The phosphorylated intermediates in the pathway of glycolysis constitute a metabolic pool. Regardless of the rate of glycolysis, the concentration of each intermediate at any given time is approximately the same. While the rate of formation of end products may vary widely, the concentrations of the constituents of the metabolic pool are relatively constant. What does change with the rate of glycolysis is the speed with which the intermediates are formed and broken down. This is called the turnover rate.

The Krebs cycle is a fundamental sequence of reactions which begins with the reaction of oxaloacetate and a two-carbon unit formed from acetate or pyruvate to form citrate. The chemical nature of the two-carbon fragment has recently been established as the acetyl derivative of coenzyme A (20). It is at this point that the interrelation between carbohydrate, fat and protein metabolism becomes apparent. The acetyl-coenzyme A may be derived, ultimately, from fatty acids and amino acids as well as from carbohydrate. Details of fat and protein metabolism will not be considered here, except to indicate that fatty acids are broken down into two-carbon units which may form acetoacetic acid, or may be oxidized or resynthesized into fat. It is interesting to note that CO_2 is not merely a waste product, but may be incorporated into organic compounds by animal tissue even though excess CO_2 is eventually released.

ALTERNATIVE METABOLIC PATHWAYS

The cell provides many pathways for a given metabolite to follow. For example, acetate may be oxidized via the Krebs cycle, may condense to form acetoacetate, or may