

**Primary metabolism:
a mechanistic approach**

J. STAUNTON

Oxford Chemistry Series

J. STAUNTON

UNIVERSITY LECTURER IN CHEMISTRY AND
FELLOW OF ST. JOHN'S COLLEGE, CAMBRIDGE

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Editor's foreword

TRADITIONALLY the chemistry of essential life processes (primary metabolism) has been the province of the biochemist while the study of non-essential, species-specific metabolites (secondary metabolism) has provided a fertile field of study for the organic chemist. However, we now know that primary and secondary metabolism are closely inter-related and that a knowledge of both is essential to give a balanced picture of this important area of chemistry. Despite this, undergraduate courses in natural-product chemistry frequently ignore the fundamental life processes which provide the starting materials for the biosynthesis of the more exotic secondary metabolites.

Dr. Staunton is an organic chemist who has made many original contributions to the field of secondary metabolism. In this book he presents the field of primary metabolism in terms of the concepts which are familiar to the organic chemist and which enable him to correlate enzyme-mediated reactions with his knowledge of *in vitro* chemistry. He thus sees that chemical reactions in the living system are controlled by the same fundamental disciplines as other chemical reactions. This treatment is not only reassuring to the organic chemist but it generates new ideas in both biological chemistry and synthetic chemistry based on biological analogies (biomimetic synthesis). The field is thus extremely challenging.

This book together with the companion books in the Oxford Chemistry Series, *Metals and metabolism* (OCS 26) by D. A. Phipps and *Secondary metabolism* (OCS 27) by J. Mann provide the broad coverage of biological chemistry which should find a place in any modern undergraduate course.

J. S. E. H.

Preface

Man has used natural chemical processes, for example, in the two basic industries of brewing and breadmaking, since before recorded history, and so it was inevitable that from its inception one of the main interests of the modern science of chemistry should be the chemical processes of life. Progress was slow until the early nineteenth century when, as a result of improved techniques of purification, natural products were isolated at an ever increasing rate. Since that time an enormous effort has been directed towards the elucidation of the structure and the synthesis of these compounds. The early development of the subject (which we now know as organic chemistry) was largely determined by work in this field. Thus on the practical side the challenge of tackling compounds and mixtures of increasing complexity provided a powerful impetus to the search for improved practical techniques. Even more important, however, was the stimulating effect of natural product research on the development of the theoretical side of the subject. As compounds of established structure increased in number and diversity, patterns of reactivity emerged which could not be adequately explained by existing theory. Modern ideas of conformational analysis and non-benzenoid aromaticity, for example, were sparked off in this way.

Organic chemists were interested not only in the structures of natural products but also in the reactions which lead to their formation *in vivo*—witness the many biosynthetic speculations which can be found in the chemical literature going back to the early years of this century. However, they neglected to follow up this interest by doing experiments, and the foundations of experimental work on the reactions of living systems were laid by a new breed of chemist, the biochemist. As a consequence an unfortunate dichotomy developed in which two closely related branches of chemistry followed parallel courses but with regrettably little cross-fertilization.

This state of affairs continued until the middle of this century when the course of natural product research was profoundly altered by two developments which had their origins outside the subject. First spectroscopic machines were refined by physicists to the stage where they could be applied routinely to the study of organic molecules. As a result the classical methods of solving natural product structures were superseded, and with them went much of the challenge of this line of research. The second major development was the increased availability of isotopes, particularly carbon and tritium. With these the organic chemist could

conveniently seek answers to long-standing questions concerning the biosynthesis of natural products by carrying out incorporation experiments with living systems. Many grasped this opportunity. Having tested the water and found it to their liking they have waded deeper and deeper into the biochemical sea and nowadays it is common to find organic chemists studying biological reactions employing in the process the latest and most sophisticated techniques of practical biochemistry.

This book has been written as an introduction to this area of overlap between organic chemistry and biochemistry which has grown in recent years. It deals with the pathways of primary metabolism, that is, those which are fundamental to life in that they provide the materials and energy used by living cells for their maintenance and growth. Though attention is focused on the chemical aspects of the reactions it is hoped that the treatment will interest biochemists as well as organic chemists.

In the early chapters selected key processes are treated in isolation with emphasis on their mechanism and energetics. In later chapters the biochemical perspective is emphasized to show how the individual reactions mesh together to form a metabolic pathway.

As the title indicates, a mechanistic approach is adopted in the treatment of reactions. Since mechanism is interpreted in different ways in the various branches of chemistry, it seems wise to explain here in what sense the word is used in this book: we shall consider what intermediates are or might be involved in each process and also the nature of the electronic shifts which take place when one intermediate is converted to another. This approach has been used with great success to systematize the diverse reactions of organic chemistry and has brought considerable benefits to the teaching of that subject. In the present context it will be used to show how many apparently unrelated chemical processes are in fact closely related in mechanism; to emphasize the relationship between biochemical processes and analogous chemical reactions; to show that by considering the mechanism of biological reactions organic chemists can design novel chemical processes which are of use in synthetic organic chemistry.

One aspect of mechanism which will not be treated in depth is the question of reaction kinetics. In other words we shall not consider in detail which stage of a multistage reaction is rate determining or how the individual steps might be catalysed by the enzyme. These considerations are of course essential to a complete understanding of the mechanism of any process, but for most important biological reactions we can still do no more than speculate. Needless to say speculation on such matters is rife in the chemical literature; it is hoped that having read this introduction the reader will feel more competent to assess any ideas on these topics he may meet in more comprehensive treatments of the subject.

Finally it gives me pleasure to acknowledge my debt of gratitude to Drs. J. S. E. Holker and D. G. Buckley who read the manuscript and made many helpful suggestions which lead to its improvement; to Mrs. E. George who prepared an impeccable typescript for the printer; and most of all to my wife, Ruth, who bore with patience and fortitude many months of book widowhood.

Cambridge
May 1977

J. S.

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1. An introduction to metabolism

THE CHEMICAL reactions of living systems, so long regarded as the exclusive preserve of the biochemist, have attracted increasing interest among organic and inorganic chemists in recent years; and those chemists who have entered the field as active investigators have found a rich harvest of fascinating chemical problems ripe for solution.

The aim of this book is to provide an introduction to the subject in the form of a chemical approach to metabolism. Since it will be assumed throughout that the reader knows little or no biochemistry, it is appropriate to begin with an explanation of what is meant by that term. Metabolism is the collective word for all the chemical reactions which take place in living systems. The most telling way to appreciate its meaning is to spend some time studying a metabolic pathways map. Charted on the map are all the more important reaction pathways whereby organic compounds (metabolites) are interconverted in living cells to produce the complex molecules of life on the one hand and the waste products of metabolism on the other.

A map will probably be available for reference in your laboratory or library, or one can be purchased very cheaply. It would be useful though not essential to have a map available for easy reference while reading this book.

Reaction to the map will vary from person to person. The complete novice will probably be reminded of one of those children's puzzles where one is required to trace a pathway through a maze from start to finish. However, this maze has extra complications, in that it has no obvious starting point, and exit points are equally hard to find; almost always when one gets to the outside of the maze an arrow inconveniently points the way back to the middle and usually one can trace a full circle to return to the starting point by a different route. When viewed in this way, as no more than an interconnected set of chemical reactions, the pathways map seems a certain prescription for chaotic muddle, and yet in practice the whole system operates in a precisely ordered manner. This order is imposed by the metabolic machinery of the living cell. Clearly, therefore, before we embark on a detailed consideration of the chemical basis of metabolic reactions, we need to consider the biological environment in which they operate.

The first point to make in this connection is that not all the pathways shown on the map will be in operation in every living cell. Many animals,

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man included, consume both carbohydrate and protein foods and therefore require the pathways to metabolize both types of compound. On the other hand a yeast cell, growing on glucose as the sole source of carbon, will use the pathway of glucose metabolism, but the pathways associated with the breakdown of proteins will not be needed and will not operate. Similarly the end-products of metabolism can vary from one cell or organism to another. For example, the main fate of glucose in man is oxidation to carbon dioxide and water, whereas in a yeast growing under anaerobic (oxygen-free) conditions the glucose is converted mainly to carbon dioxide and ethanol. Such differences in the pattern of metabolism (i.e. pathways in operation) can also occur within a given organism between the cells of one part and another. In man, for instance, the pattern of metabolism in a liver cell is very different from that in a brain cell. So the metabolic pathways map does not show a set of reactions which is in operation in every living cell at all times. Instead it presents a composite picture built up from pathways which take place in a wide range of living systems. Only the more important pathways are included and therefore while a given cell may not use some of the pathways shown on the map it may use other pathways which are not shown.

Fortunately, although the pattern of metabolism can vary markedly from one cell to another, the task of building up a composite picture of the metabolic pathways is considerably simplified by the fact that there is a remarkable constancy within the pathways wherever they operate. Thus glucose is oxidized to carbon dioxide and water in the cells of a vast range of widely different organisms including animals, plants, and microorganisms. The process follows the same pathway throughout the range even though the enzymes that catalyse individual steps may show significant variation in structure from one organism to another. A further simplifying feature comes to light when the pathway by which glucose is oxidized to carbon dioxide and water in a mammal is set alongside that by which glucose is converted to carbon dioxide and ethanol in yeast. Although it leads to a different final product, the pathway in yeast follows exactly the course as that in the mammal for most of the way. Therefore, in designing the map it is necessary to show only one pathway, that corresponding to mammalian metabolism, with a branch at the appropriate point to show how ethanol is formed in yeast.

The functions of metabolism

In principle we could now begin to trace our way through the maze by selecting a particular type of living cell for study. The starting point for our analysis of the pathways map would then be determined by the food stuff of the chosen cell and the exit point would correspond to the material excreted from this cell. However, if we are to understand fully

the chemical basis of metabolism we need to do more than just trace the flow of material along the various metabolic pathways. We need to explain in addition how the cell benefits from the flow and this brings us to a consideration of the various functions of metabolism.

The metabolic pathways can be classified according to their function into two main categories which together encompass the great majority of the pathways shown on the map. These are the catabolic pathways (catabolism) and the anabolic pathways (anabolism). The immediately obvious difference between the two types of pathway lies in the nature of the chemical change which they bring about. Thus a catabolic pathway has the effect of breaking down its starting material into smaller molecules whereas an anabolic pathway results in the biosynthesis of a complex molecule from smaller precursors.

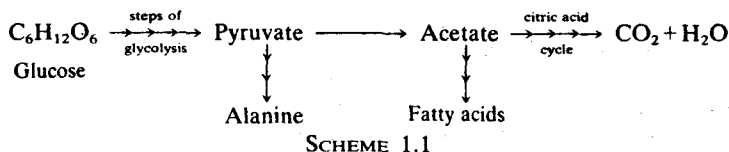
The function of the anabolic pathways is straightforward: they provide the means of synthesizing the complex molecules needed to build, maintain, and reproduce the living cell. The starting point for an anabolic pathway may be a compound taken in as a part of the food material of the cell: an amino acid for example. However, for many metabolic pathways the starting material is not present as such in the food, in which case it has to be produced by the metabolism of the cell. This brings us to one of the main functions of the catabolic pathways: they serve to degrade the primary carbon sources taken in as food to produce the precursors of biosynthesis. A second vital function of catabolism is the provision of chemical energy in a form suitable for powering the various energy-requiring operations of the cell or organism. A third function is the conversion of waste material to a chemical form suitable for excretion from the cell or organism. The first two functions are the ones which will concern us most and taking them into account one can see that the pathways of catabolism and anabolism are not independent but closely integrated: the catabolic pathways service those of anabolism by providing the starting materials for biosynthesis and also by providing the chemical energy which is necessary for the successful operation of most biosynthetic pathways.

The pathway of glucose catabolism

This interrelationship can best be illustrated by considering the function of a particular catabolic pathway. The pathway of glucose catabolism has been selected for this exercise because it takes place in organisms of all types and is unquestionably the most important of all the catabolic pathways. Thus it provides an impressive number of metabolites which also serve as starting materials for vital biosynthetic pathways. In addition it is beautifully adapted to provide the living cell with useful chemical energy, and thus the breakdown of glucose provides a

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major source of this vital commodity. In view of its importance the pathway of glucose catabolism is considered to be one of the vital 'central pathways' of metabolism, and it is not surprising that it is awarded the place of honour at the centre of the metabolic pathways map.

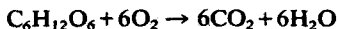


The pathway is shown in outline in Scheme 1.1. It is divided into two stages, one glycolysis, and the other the citric acid cycle, which operate in succession. The reason for this division will become clear when we consider the pathway in detail in Chapter 8. For the moment we shall concentrate on the fate of the two intermediates, pyruvate and acetate. The scheme indicates how one can serve as precursor for the biosynthesis of the amino acid alanine and the other as precursor of the fatty acids. Thus acetate is considered to be as the starting point for fatty acid biosynthesis. At first sight this may appear an arbitrary choice for one can trace a continuous pathway from glucose to fatty acids. What, then, is the basis for dividing the sequence into a catabolic stage (as far acetate) and then an anabolic stage (from acetate to the fatty acid)?

In fact the division can be justified on a number of grounds. Firstly, on chemical grounds, for the reaction sequence from glucose to acetate is definitely degradative in character, whereas from acetate to the fatty acids the sequence is unquestionably synthetic. Secondly, there are important biochemical grounds for the division, in that acetate is a branch point in the metabolic sequence from glucose to the fatty acids and therefore any mechanism for controlling the rate of fatty acid production must operate after that point if it is not to interfere with the rate of conversion of glucose to carbon dioxide and water. Thirdly, acetate can be generated in the cell from many compounds other than glucose so it is logical as well as convenient to consider the biosynthesis of fatty acids as if it started at that point. Finally the energetics of the pathway provide a further basis for making the division, for chemical energy is released in the conversion of glucose to acetate but a considerable input of energy is required in the conversion of acetate to fatty acids.

Bioenergetics

The energetics of metabolism will constitute a major theme of the following chapters and so it will provide a helpful background if at this



SCHEME 1.2

stage we examine in general terms how the chemical energy released in the course of glucose catabolism is harnessed for use in other spheres.

The overall chemical process is one of oxidation at the expense of molecular oxygen as indicated in Scheme 1.2. Surprisingly, however, oxygen does not react directly with any of the intermediates of glycolysis or the citric acid cycle. Instead the oxidative steps of the pathway are carried out by various coenzymes. (A coenzyme is a compound which acts in collaboration with an enzyme to bring about a metabolic reaction. Sometimes the role of the coenzyme is solely catalytic but frequently (as in the case under consideration) the coenzyme functions effectively as a reagent and is transformed as a consequence of the reaction into a different chemical form.) The general principles of how these coenzymes operate will be illustrated by examining the role of the one most frequently involved, namely the nicotinamide coenzyme, NAD^+ . For the purpose of this outline treatment it is not necessary to know the structure of the coenzyme or the details of how it acts, but merely to accept that it can act as a dehydrogenating agent in a variety of metabolic reactions. In the process two hydrogens are abstracted from the substrate (which is therefore oxidized) and the coenzyme is reduced to its dihydro derivative, NADH . For example, one of the redox reactions of the citric acid cycle, the oxidation of malic acid to oxaloacetic acid, is shown in Scheme 1.3. The presentation of this reaction follows the standard biochemical convention. The double headed arrows signify that the reaction can take place readily in either direction in the living cell. In the pathway of glucose catabolism the direction of operation is from left to the right. Note also that the reduction product of NAD^+ is shown as NADH rather than NADH_2 . This is because the dihydro derivative of the coenzyme exists in the deprotonated form at pH 7. The reason for this will become clear when we meet the full structure. Following standard practice the proton will not be shown in subsequent reaction schemes of this type.

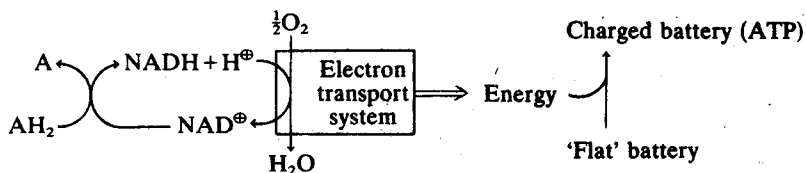
In the complete conversion of a molecule of glucose to carbon dioxide and water several molecules of the coenzyme are reduced in this way. In order to keep the process going on a continuous basis it is necessary that



SCHEME 1.3

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the NADH be reoxidized to NAD^+ and this is where oxygen comes onto the scene. The reoxidation takes place at the expense of molecular oxygen in a specially organized enzymatic apparatus called the electron transport system as illustrated in Scheme 1.4. The coenzyme in its oxidized form is then ready to function once more as an oxidizing agent at one of the redox reactions of glucose catabolism in association with the appropriate enzyme. Thus the nicotinamide coenzyme functions effectively as a hydrogen carrier by shuttling backwards and forwards between the enzymes of glycolysis and the citric acid cycle on the one hand and the electron transport system on the other, and, although glucose is oxidized at the expense of molecular oxygen, the process takes place in a roundabout way.



SCHEME 1.4

The chemical by-product of the reoxidation of NADH in the electron transport system is water but the really significant by-product from the metabolic point of view is the large amount of energy released (220 kJ mol^{-1}). The chemical energy is not just dissipated as heat but part is conserved for use elsewhere. To understand how this is achieved it is necessary to introduce a second coenzyme ATP. The structure and mode of operation of this compound will be covered in the next chapter; for the moment it is sufficient to picture it as a chemical battery which can be 'charged up' with energy at one point and then discharged to do useful work at another. In addition to bringing about the reoxidation of NADH the electron transport system has the remarkable ability to harness the energy liberated in the process to effect the regeneration of ATP as indicated in Scheme 1.4. Thus the electron transport system can be viewed as the power-house of the living cell in the sense that it 'burns' NADH as fuel and harnesses the resultant energy to recharge the chemical battery.

Needless to say, this simple treatment of the subject cannot tell the whole story, and to avoid confusion later two very important deficiencies should be mentioned. Firstly, NADH is not the only 'fuel' acceptable to the electron transport system: in one of the steps of the citric acid cycle succinic acid is dehydrogenated to fumaric acid; this process takes place directly in the electron transport system without the assistance of NADH and the resultant energy is harnessed for ATP production. Secondly, the

electron transport system is not the sole means of harnessing the energy liberated in the course of glucose catabolism, for ATP generation takes place as an integral part of certain steps on the pathway.

Thirdly, to keep the energetics of metabolism in perspective, it should be remembered that glucose is not the only carbon source used for energy production in living cells; other food materials including fats and proteins are degraded in a similar way to produce NADH as fuel for the electron transport system. Finally we have to bear in mind, that the original source of all the energy available to living organisms is the radiant energy of sunlight. This energy is harnessed by the photosynthetic apparatus of green plants to bring about the synthesis of carbohydrates such as glucose from carbon dioxide and water. So, viewed from the perspective of the biosphere as a whole, glucose is seen not as the starting point of metabolism but as an energy storage compound: having been synthesized in the plant kingdom as a means of storing the energy of sunlight, it is subsequently degraded to produce chemical energy, either in the cells of the plant which produced it or in the cells of another organism which has consumed the plant.

The scope of the chemical approach

This brief survey of metabolism is intended to set the scene for later chapters where key metabolic processes are considered in detail. Obviously, in treating the various reactions we shall be interested in the question of reaction mechanism. In addition, however, we shall need to consider the factors which govern the net direction of flow of material along metabolic pathways. Therefore, in the treatment of the chemical basis of individual reactions the question of equilibrium and free-energy change will loom large, because it is this aspect of chemical reactivity which governs the favourable direction of operation of the process under consideration.

The early chapters will deal with the energetics and mechanism of a selection of metabolic reactions one by one. Then in later chapters some of the more important metabolic pathways will be traced step by step to show how the individual reactions mesh together to produce a coherent and smooth running system.

Before closing this introduction it is desirable to mention certain other aspects of metabolism which will not be covered. The first is the problem of how the pathways are regulated, that is to say how the rate of operation of individual pathways is controlled. For example we have seen earlier that pyruvate (formed from glucose) can either be oxidized to carbon dioxide and water to provide energy, or it can be used in the biosynthesis of the amino acid alanine. It is essential for the smooth functioning of metabolism that the rate of operation of each pathway

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should be capable of independent variation according to the requirements of the cell while both pathways are in operation. The way in which this is achieved, by controlling the rate of reaction at a key step in each reaction sequence (for example by varying the amount of enzyme available to catalyse the reaction), is a crucial feature of metabolism. Fortunately, although this is a serious omission in terms of biochemistry, it will not vitiate the chemical approach to metabolism adopted in this book. We shall be concerned with the chemical processes which pump the materials along the pipeline; the control mechanisms functions effectively as a valve and in this sense it is a completely independent problem.

A second aspect of metabolism which we shall largely ignore is the question of the intramolecular location of the enzymes. The interior of the cell is not homogeneous, but is divided into sub-cellular compartments. For a full understanding of the controlled working of metabolism it is essential to know where enzymes are located, and how metabolites are transferred at the appropriate stage from one compartment to the next. Again this is a question of organization rather than chemical reactivity, and so, generally speaking, we shall only refer to the location of an enzyme when it helps to explain the chemistry of the process under consideration.

Finally, it has been decided with regret that there will not be room in this short book to give a comprehensive treatment of the subject of enzyme catalysis. The field is so vast it would need a complete separate volume to do justice to this topic even at the introductory level. So in spite of the fact that this is an aspect of metabolism which is actively investigated by chemists as well as biochemists, the coverage in this book will be restricted to reactions in which coenzymes are involved.

Even with these important omissions we are still left with a wide and fascinating brief: the mechanism and energetics of metabolic reactions. It is hoped that having read this book the reader will be stimulated to explore these subjects in more depth and also to range more widely into the equally fascinating biochemical aspects of metabolism which will not have been covered.