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PREFACE

In attempting to understand biological processes it is natural that we should be driven back to the study of organic chemistry, which, as Lord Todd points out in the first review in this book, is the chemistry of the substances found in living matter. Indeed it would be easy to fill this volume with reviews on biochemical subjects, and the Governing Body of the British Postgraduate Medical Federation is therefore greatly indebted to the group of medical scientists who have selected the subjects so as to cover several disciplines and fields of scientific interest. It is right, however, that Lord Todd's review should be given priority, and his demonstration that phosphoric acid is so much better adapted for these vital processes than any other acid reminds one of what Starling referred to as 'the wisdom of the body'. There are three other reviews which are primarily biochemical, that by Nordin on the equilibrium of ionic calcium in blood and bone; that by Moss on isoenzymes; and Klyne's explanation of how the shapes of molecules as well as their constitution determine their ability to combine with other substances.

Of the physiological reviews two are concerned with the central nervous system, Oswald's electroencephalographic studies of sleep and dreaming, and Phillips's fascinating recordings of discharges from individual neurones to explain reflex activity in the central nervous system. The others are the elaboration of earlier work on high altitude effects by Pugh, and of the part played by the alimentary tract in the control of body fluid by Black.

Current interest in the problems of immunology is reflected in the reviews by Goffe on active immunization against measles; by Gell on hypersensitivity reactions; by Pepys on the immunological response to vegetable dust; and in a fascinating study by Miller of the many functions of the thymus gland.

Another group includes reviews of pathological processes, four of which deal with morbid anatomical subjects, namely Symington's survey of the pathological physiology of the human adrenal cortex, which he has linked to the clinical manifestations of hypercorticalism; Marshall's account of the cerebral circulation in cases of 'stroke'; Poole's on the structural aspects of thrombosis; and Johnson's interesting review of the reaction of epithelium to injury which he contributed to the series of lectures on the Scientific Basis of Dentistry. Among the pathological subjects must be included two reviews on microbiology, Mary Barber's comprehensive account of the semisynthetic penicillins; and Gordon Smith's review of the zoonoses, the infections transmitted to man from other animals, to which he adds a plea that there should be somewhere a 'Department of Zoonoses' in which medical and veterinary workers could combine with entomologists, animal ecologists, meteorologists and others to pursue the very necessary research into these infections.

Finally there are two reviews dealing with radioactivity, Stevenson's critical assessment of the importance of radiation-induced genetic changes; and Belcher's review of the many ways in which radioactive isotopes are being used for the investigation of physiological and disease processes. This catalogue of the many and varied subjects dealt with in these reviews, necessarily limited to only twenty out of the thirty lectures in the thirteenth series on the Scientific Basis of Medicine, is intended not merely as an elaborated table of contents, but as an indication of the extent to which this volume may be used as a source of information and a stimulus to clinicians as well as to research workers in many branches of medical science. To all of our lecturers, and especially to those who have prepared reviews for publication in this book, we offer our most grateful thanks.

J. PATERSON ROSS
Director, British Postgraduate Medical Federation

LIST OF PLATES

NOTE

The reviews printed in this volume
are based on lectures delivered on the following dates:

I.	16 October 1962	XI.	27 November 1962
II.	1 November 1962	XII.	29 November 1962
III.	18 January 1962	XIII.	10 January 1963
IV.	24 January 1963	XIV.	8 November 1962
V.	7 February 1963	XV.	22 November 1962
VI.	5 February 1963	XVI.	13 March 1963
VII.	19 February 1963	XVII.	18 October 1962
VIII.	12 February 1963	XVIII.	25 October 1962
IX.	4 December 1962	XIX.	30 October 1962
X.	20 November 1962	XX.	15 November 1962

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I

Phosphates in Vital Processes

LORD TODD

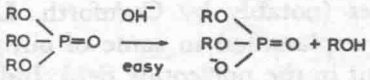
IT is perhaps not unreasonable that an organic chemist should have the privilege of opening a series devoted to the scientific basis of medicine. For from its very beginnings organic chemistry has been intimately associated with medicine and its links with the latter are today stronger than ever. We may recall that organic chemistry was first defined as the chemistry of the substances found in living matter, and interest in these substances was at the time largely stimulated by the desire to find drugs for the treatment of disease. The explosive growth of organic chemistry during the latter part of the nineteenth and the first half of the twentieth century has coincided with the rise to power of experimental medicine—and this is no mere coincidence. Throughout its history, and despite the importance of other aspects, it is fair to say that most of the major advances in organic chemistry have stemmed directly or indirectly from the study of substances found in living matter, and that inevitably these advances have, through biochemistry, physiology and pharmacology, profoundly influenced the practice and the development of medicine. One could, of course, exemplify this influence by giving an account of advances in chemotherapy, in hormone therapy or in many other ways, but it seemed to me in considering my subject that it might be appropriate to look rather deeper and to discuss some purely chemical matters in which I myself have been interested and which I think show something of the way in which organic chemistry is providing a basis for understanding the character of biological processes—an understanding which is ultimately necessary for medical progress even if the immediate relevance is perhaps less obvious

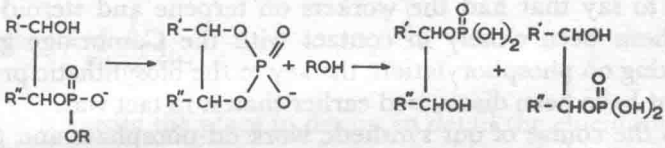
than that of, say, a new drug for the treatment of trypanosomiasis.

On this basis then I should like to discuss some features of the chemistry of the phosphate esters. The frequent occurrence of organic phosphates in biological materials was early recognized and with the passage of time biochemical studies have made it clear that phosphates are of prime importance in the functioning of living organisms—they are involved not only in the transfer and storage of energy, but are key intermediates in both synthetic and degradative processes, and many complex natural molecules such as those of the nucleic acids, coenzymes and phospholipids contain phosphate ester groups. Yet although their importance in nature has long been recognized it is true that until comparatively recently organic chemists paid little attention to the chemistry of the organic phosphates and as a result many biochemical facts remained without any reasonable explanation. My own interest in the phosphates began with the need to study and to devise new techniques for phosphorylation so as to open up the field of nucleotide and nucleotide coenzyme synthesis. But as this work progressed we began to see that some of our findings offered an immediate interpretation of some puzzling biochemical phenomena and we have in the past year or two devoted considerable effort to the detailed chemistry of the organic phosphates with a view to clarifying various biological problems by providing chemical analogies and indicating which types of reaction are possible and which are not. For we must remember that there is no magic about the chemical reactions which nature employs to effect her purposes.

Let us first consider an example in which a knowledge of the simple facts regarding the hydrolysis of phosphate esters was instrumental in opening up a whole array of extremely important fields in biological studies. The neutral triesters of phosphoric acid are readily hydrolysed by alkali to the diesters; but the latter are normally resistant to further hydrolysis by alkali, presumably because the formal negative charge on the anion hinders approach of the negatively charged hydroxyl ion to the phosphorus atom. But there is one type of phosphodiester which does not show this resistance and which is indeed very easily hydrolysed by alkali to the mono-ester. The characteristic

feature of this type is that one of the esterifying groups bears a *cis*-hydroxyl in the α-position.



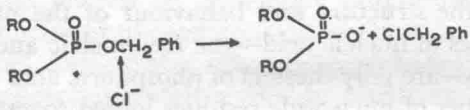


In such cases the spatial proximity of the hydroxyl group permits attack on phosphorus despite the negative charge on the anion and we get very easy hydrolysis via a cyclic intermediate, the phosphoric acid group invariably remaining attached to the glycol residue. Equally of course, and for a similar reason, phosphotriesters contining a *cis*-α-hydroxyl hydrolyse with extraordinary ease.

The recognition of these facts provided the key to the understanding of the structure and behaviour of the nucleic acids. The two types of nucleic acid—the ribonucleic and deoxyribonucleic acids—are poly-diesters of phosphoric acid consisting of a large number of nucleoside residues joined together by phosphodiester linkages. The conclusion that both types of nucleic acids are linear polyesters with a recurring 3′:5′-internucleotidic linkage, as propounded by Brown and Todd in 1951, rests essentially on their recognition that the behaviour of ribonucleic acids towards hydrolytic agents is essentially that of phosphodiesters containing *cis*-α-hydroxyl groups, whereas deoxyribonucleic acids show the stability of normal phosphodiesters. This recognition of the chemical structure of the nucleic acids provided a base for the brilliant biochemical and biophysical achievements of recent years which have greatly furthered our understanding of the nature of the genes and viruses. Even now these achievements are unfolding a whole new picture of many matters vital to medicine—the nature of mutagenesis and the mechanism of protein synthesis to mention but two out of many fascinating fields that now lie before us.

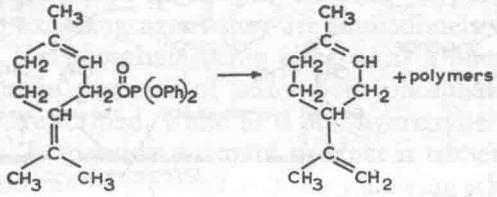
We may follow up this mention of phosphate hydrolysis by considering briefly a case in which a biosynthetic process when elucidated by a brilliant combination of chemical and biochemical studies (notably by Cornforth, Lynen and Bloch) found a ready explanation in some of our simple phosphate work carried out in the nucleotide field. Indeed it is probably true to say that had the workers on terpene and steroid biosynthesis been closely in contact with the Cambridge group working on phosphorylation, the key to the biosynthetic process might have been discovered earlier than it in fact was.

In the course of our synthetic work on phosphate and polyphate esters it was found necessary to devise methods for removing one benzyl group from a phosphotriester to yield a phosphodiester without having recourse either to hydrolysis or hydrogenation. In such phosphotriesters the CH_2 of the benzyl groups is strongly electrophilic, the effect of the phenyl group upon it being reinforced by that of the phosphorus atom. It is thus readily attacked by anions, debenzylation being thereby effected.

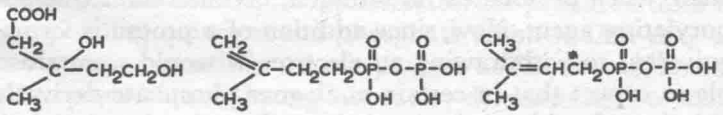


It is not indeed necessary to use an actual anion to bring about this reaction although, in the laboratory, it is usually convenient to do so. Other nucleophiles such as tertiary bases are equally effective and the method can be used to remove allyl groups as well as benzyl groups. Indeed, provided that the two R groups in the triester are such (e.g. phenyl) as to make the diester anion produced by the fission one of high stability, even methyl groups can be removed by this process, which we usually refer to as anionic or nucleophilic fission. An example of considerable interest in connexion with the relevance of these observations to the biosynthesis of terpenes and steroids is provided by the observation of one of my former colleagues, Dr. F. A. Atherton, that geranyl diphenyl phosphate, in which the nucleophilic olefinic group is or can be spatially close to the

phosphate residue, undergoes cyclization (and more complex polymerization) with great ease.

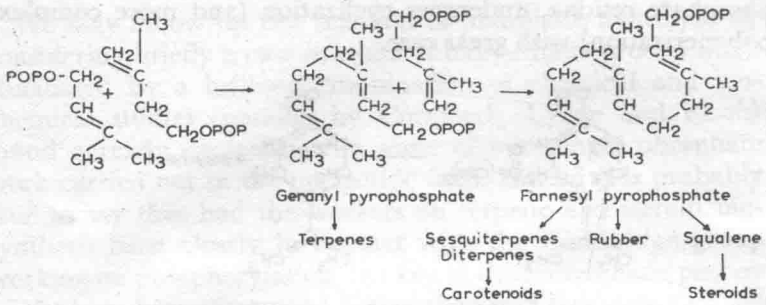


This is hardly the place to discuss in detail the elucidation of the biosynthetic pathway to the terpenoids and thence to the steroids and I shall only indicate in general terms that portion which is relevant to our present theme. The ultimate precursor in living organisms is acetic acid, from which is first synthesized mevalonic acid and thence *iso*pentenyl pyrophosphate which can be regarded as an 'active isoprene'; from it first geranyl pyrophosphate and then farnesyl pyrophosphate are synthesized and thence the polyterpenoids, carotenoids, steroids and rubber. The process used is strictly analogous to the nucleophilic fission described above. *Iso*pentenyl pyrophosphate, like other similarly constituted compounds, can undergo isomerization to 3:3-dimethylallyl pyrophosphate. In the latter we have an allyl ester capable of attack (at the asterisked carbon in the formula given below) by a nucleophile; the unsaturated g.oup in the *iso*pentenyl pyrophosphate form provides such a nucleophile and the two isomers will thus react with one another to produce geranyl pyrophosphate with expulsion of pyrophosphate (1 mol.). One would expect that in this biosynthetic process ionization of the pyrophosphate residues in the two reactants would have to be suppressed and this is probably one of the functions of the enzyme protein involved. The further course of the biosynthetic process is outlined roughly below.



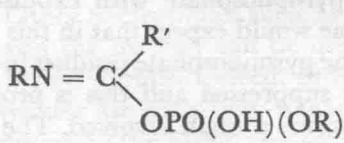
Mevalonic acid | Isopentenyl pyrophosphate | 3:3-dimethylallyl pyrophosphate

B



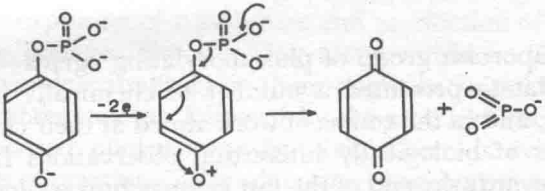
The two topics we have discussed are examples of those in which laboratory experience and biochemical facts coincide to give a full understanding. But I should like now to turn to one or two cases where we have not yet reached this position but where the chemical behaviour of certain phosphates in the laboratory suggests explanations for biological phenomena. The first which I shall consider is oxidative phosphorylation.

Research into methods for the preparation of phosphate and polyphosphates has, during the past fifteen years, produced a variety of phosphorylation procedures which fall into two distinct groups, (*a*) those proceeding by a bimolecular displacement mechanism (usually based on anhydride-like derivatives of phosphodiesters or on phosphoramidates) and (*b*) those involving metaphosphate intermediates (usually based on 'activated' phosphomonoesters). One of the commonest methods of type (*b*) is to convert a phosphomonoester into an imidoyl phosphate



which, when protonated on nitrogen, becomes an active phosphorylating agent. Now, since addition of a proton is formally equivalent to withdrawing an electron, it would seem reasonable to expect that in certain analogous phosphate derivatives oxidation should result in their acting as phosphorylating agents. We were able to demonstrate that this is indeed so and

that particularly interesting examples are to be found in the quinol phosphates. Quinol monophosphates are very resistant towards hydrolysis in the absence of air or oxygen, but in presence of an oxidizing agent they are immediately converted to a quinone, the phosphate being released as a phosphorylating entity. Thus in presence of water both phosphate and pyrophosphate are formed, while in a non-hydroxylic solvent such as dimethyl formamide the main product is trimetaphosphate. The reaction can be pictured as in the following scheme:



The above formulation is purely illustrative, for the process may indeed be a one-electron oxidation, but the essential feature is that the monomeric metaphosphate produced is regarded as the phosphorylating entity. This oxidative phosphorylation process is not confined to hydroquinone phosphate—it is common to all quinol phosphates.

These results are of considerable interest in connexion with the biological problem of oxidative phosphorylation in the respiratory chain, particularly since we have shown that this procedure can be used in the laboratory to synthesize both adenosine diphosphate (ADP) and adenosine triphosphate (ATP). Since several quinones, e.g. members of the vitamin K and coenzyme Q (ubiquinone) group, are probable participants in the processes of oxidative phosphorylation associated with the respiratory chain, it seems reasonable to postulate that they participate in the manner above indicated, viz. as phosphate esters of their reduced forms. This, of course, poses the further problem of how these quinol phosphates are produced from the corresponding quinones and phosphate anion so that they can function continuously in a cyclic process. So far, no one has been able to provide a convincing laboratory demonstration of such a reductive phosphorylation, although numerous theories have been advanced to explain it. Here there is clearly need for more