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Foreword

Inositol lipid-mediated cellular signalling

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In 1955, approximately one hundred years after Scherer discovered *myo*-inositol in a meat extract, approximately twenty years after Henry Dale at the National Institute for Medical Research established the role of acetylcholine as a neurotransmitter, and some ten years after Jordi Folch at the Rockefeller Institute discovered the inositol-containing phospholipids in brain, Lowell and Mabel Hokin, at McGill University, first showed that acetylcholine stimulated the turnover of inositol-containing phospholipids in pancreas and brain cortex slices (see ref. 1 for a personal view of their discovery). Subsequent work showed that this 'phospholipid effect' could be observed in a large number of other systems exposed to a variety of agonists, suggesting a possible role in stimulus-response coupling. At that time one of us (R.G.) was preparing to be a post-doc with Herbert Carter in the Department of Biochemistry, University of Illinois, Urbana where the emphasis was on sphingolipids. These substances were also the subject of my Ph.D. work in Bristol where the chemistry of the inositol-containing phospholipids was being studied by Thomas Malkin's group. My project in Illinois was the structure of a glycosphingolipid from plant seeds (termed 'phytoglycolipid' by Carter), which also contained *myo*-inositol, and thus, from the beginning of my research career, the inositol-containing phospholipids have been one of my prime interests. During this period, the now well-established second messenger adenosine 3′,5′-(cyclic phosphate) was discovered by Earl Sutherland at Western Reserve University, in 1957.

In 1958, I returned to the U.K. after two years in Illinois and changed my line of research to work as a post-doc with 'Kappa' Cornforth at the N.I.M.R., Mill Hill on a synthesis of vitamin B12. During the four years with Kappa, I watched, as an interested outsider, the revolution occurring in the biochemistry of the phospholipids and glycolipids due to the introduction of new analytical techniques. In particular, the mixture of inositol-containing phospholipids was separated and characterised as three individual phospholipids: phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PI-4P), and phosphatidylinositol 4,5-bisphosphate (PI-4,5P_2), primarily by the work of Rex Dawson and his collaborators in Babra-

ham (see ref. 2 for a personal view of this work). The complete structures of these phospholipids were soon established, primarily by the work of Clinton Ballou and his collaborators in Berkeley[3]; at this time, Theodore Posternak also published his classical work on Les Cyclitols[4].

In 1962, I had the opportunity to start an independent research group at Mill Hill and the relatively unexploited area of phospholipid and glycolipid chemistry was my obvious choice as a research field. Our early interest in the vinyl ether-containing phospholipids (plasmalogens) led to the introduction of the allyl and crotyl ethers as protecting groups in carbohydrate chemistry and these have been central to our subsequent work in inositol chemistry. At that time they were put to use in a synthesis of a pentabenzyl ether of *myo*-inositol which was converted into a mixture of diastereoisomeric PIs. Tentative, unsuccessful steps were taken to resolve the optical isomers of the pentabenzyl ether, although chiral material was prepared from perbenzylated galactinol provided by Clinton Ballou, and from here began an interest in optical resolutions and the subsequent exploitation of ω-camphanates as excellent derivatives for the resolution of protected inositols.

Throughout the 1960s, Stephen Angyal in Sydney was publishing his pioneering work on the chemistry of inositols and his characterised products have been fundamental for establishing the structures of our intermediates. Also during the 1970s, an impressive volume of work on the synthesis of inositol-containing phospholipids was carried out by Vitaly Shvets and his colleagues in Moscow (for a review, see ref. 5) and they succeeded (1980) in synthesising PI-4,5P_2 before anyone realised its physiological significance. Also throughout the 1960s and 1970s, the biochemists (particularly Rex Dawson, Tim Hawthorne in Nottingham, Bob Michell in Birmingham, and Bernie Agranoff in Ann Arbor) were trying to understand the physiological significance of the stimulated turnover of the inositol-containing phospholipids but with little success since most of their efforts were confined to the major inositol-containing phospholipid PI, and the role of these lipids remained enigmatic. I followed this biochemical and chemical work in order to write reviews[6,7], but made no other contribution to the field. For a lipid chemist, another significant occurrence during this period was the discovery, by Jacques Benveniste in Paris (1972), of another potent physiologically active phospholipid (platelet-activating factor, PAF) whose structure was established by Donald Hanahan in Texas[8]. This phospholipid, 2-*O*-acetyl-1-*O*-alkyl-L-glycerol 3-(choline phosphate), related to plasmalogens, has second messenger properties and some of its physiological actions result from mobilisation of calcium ions by way of the phosphatidylinositol cycle. At this time, with the increasing interest in later decades in changes in the role of intracellular Ca^{2+} levels in the regulation of a variety of cellular functions, Bob Michell, in a seminal review, pointed out the link between inositol phospholipid turnover and increased cytosolic calcium concentration as a result of agonist stimulation[9]. However, at that time, the nature of any chemical link was obscure. Also, Bernie Agranoff introduced the turtle as a representation of *myo*-inositol for those biochemists who were confused with its

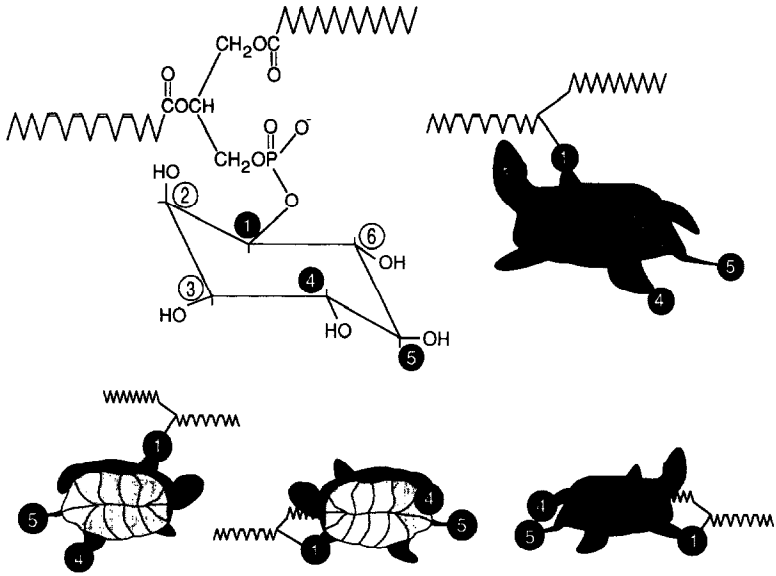

Fig. 1. Phosphatidylinositol turtle diagram.

stereochemistry[10]. The turtles here in Figs. 1 and 3 are reproduced by kind permission of Professor Agranoff.

In 1980, I took some sabbatical leave and was welcomed by Laurens Anderson into his group in the Department of Biochemistry, University of Wisconsin, Madison for six months where I could learn from his considerable experience in the inositol field. The Madison group was the first (1976) to apply tin-mediated alkylation to a *myo*-inositol derivative and this technique, extensively developed in the carbohydrate field, by Serge David and Alain Veyrières in Paris, has been very useful in the regiospecific protection of *myo*-inositol. Laurens had a large quantity of *chiro*-inositol, from natural sources, in store and suggested this as the ideal intermediate for the synthesis of chiral *myo*-inositol derivatives. I did some experiments along these lines aiming to invert one of the axial hydroxyl groups in protected *chiro*-inositol derivatives to give protected *myo*-inositol derivatives, similar to the work subsequently described by Clint Ballou. However, as these *chiro*-inositols were not commercially available (although derivatives are present in large quantities in some plants), we decided to investigate resolutions of derivatives of the cheap and readily available *myo*-inositol when work started in earnest at Mill Hill. Fortunately, our first attempt at a resolution of a (−)-ω-camphanate ester was highly successful and for this we thank Hans Gerlach at the University of Bayreuth for his pioneering work in the use of ω-camphanic acids in optical resolutions.

At the time of my return to Mill Hill at the end of 1980 (when Dennis Cosgrove published his book[11] on inositol phosphates), there were definite signs that the efforts of the biochemists were beginning to bear fruit, after an induction period of

nearly thirty years, with the realisation that the quantitatively minor inositol-containing phospholipids PI-4P and PI-4,5P_2 were as important as PI itself in this stimulated turnover of inositol phospholipids. Finally, at the end of 1983, Mike Berridge and his co-workers in Cambridge and Germany[12] delivered the vital information: "micromolar concentrations of Ins(1,4,5)P_3 (1D-*myo*-inositol 1,4,5-trisphosphate) release Ca^{2+} from a nonmitochondrial intracellular Ca^{2+} store in pancreatic acinar cells. Our results strongly suggest that this is the same Ca^{2+} store that is released by acetylcholine". The results were confirmed and the production of Ins(1,4,5)P_3 by agonist-stimulated activation of the enzyme phospholipase C, which hydrolysed PI-4,5P_2, was established. Ins(1,4,5)P_3 binds to an intracellular endoplasmic reticular receptor linked to a Ca^{2+} store and binding of this molecule causes Ca^{2+} to flood out into the cytosol. The other product of this bifurcating signalling pathway, a diglyceride (1,2-di-*O*-acylglycerol), was then shown[13] by Yasutomi Nishizuka at Kobe University School of Medicine to activate protein kinase C and thus, overnight, two new second messengers had been discovered. The mapping of the phosphatidylinositol cycle (shown in Fig. 2) began with the discovery of the many different inositol phosphates involved: Ins(1,3,4)P_3 by Robin Irvine and Peter Downes and their co-workers in 1984, Ins(1,3,4,5)P_4 by Steve Nahorski and his colleagues in Leicester in 1985, and Ins(1,3,4,6)P_4 by Stephen Shears et al. in Birmingham in 1987. This work was followed by characterisation of the enzymes involved in their metabolism (see ref. 14 for a review).

The organic chemists were now stimulated to join the game and my own laboratory has concentrated on this subject since 1984. The results of the work of several of the groups of organic chemists involving enantioselective syntheses, syntheses starting from chiral precursors, and syntheses involving optical resolutions were the subject of two major reviews in 1989[15] and 1990[16] and a book in 1991[17]. Allan Reitz, the editor of the latter, said in his introduction: "even though *myo*-inositol looks like a turtle, research has advanced in this area with the speed of a hare". This Thematic Issue shows predominantly progress that has been made in the last year in this field. For the biochemist too, the pace has been hectic and, as pointed out by Lowell Hokin[18]: "the phosphoinositide field is currently the number one field in biochemistry in the number of citations (excluding molecular biology)". I have found it taxing to keep up with the number of review articles on inositol phosphate biochemistry and biology, let alone the original articles.

Recently, new inositol-containing phospholipids have been characterised as phosphatidylinositol 3-phosphate [PI(3)P], phosphatidylinositol 3,4-bisphosphate [PI(3,4)P_2], and phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P_3], primarily by the work of Peter Downes and Lewis Cantley (for a review, see ref. 19). These lipids are formed after the stimulation of a 3-kinase in cells by growth factors and this action appears to be the beginning of a new chapter in signal transduction involving the inositol-containing phospholipids and is at present under very active scrutiny. PI(3,4,5)P_3 is therefore a prime synthetic target for organic chemists.

Coincidental with the elucidation of the phosphatidylinositol cycle has been the

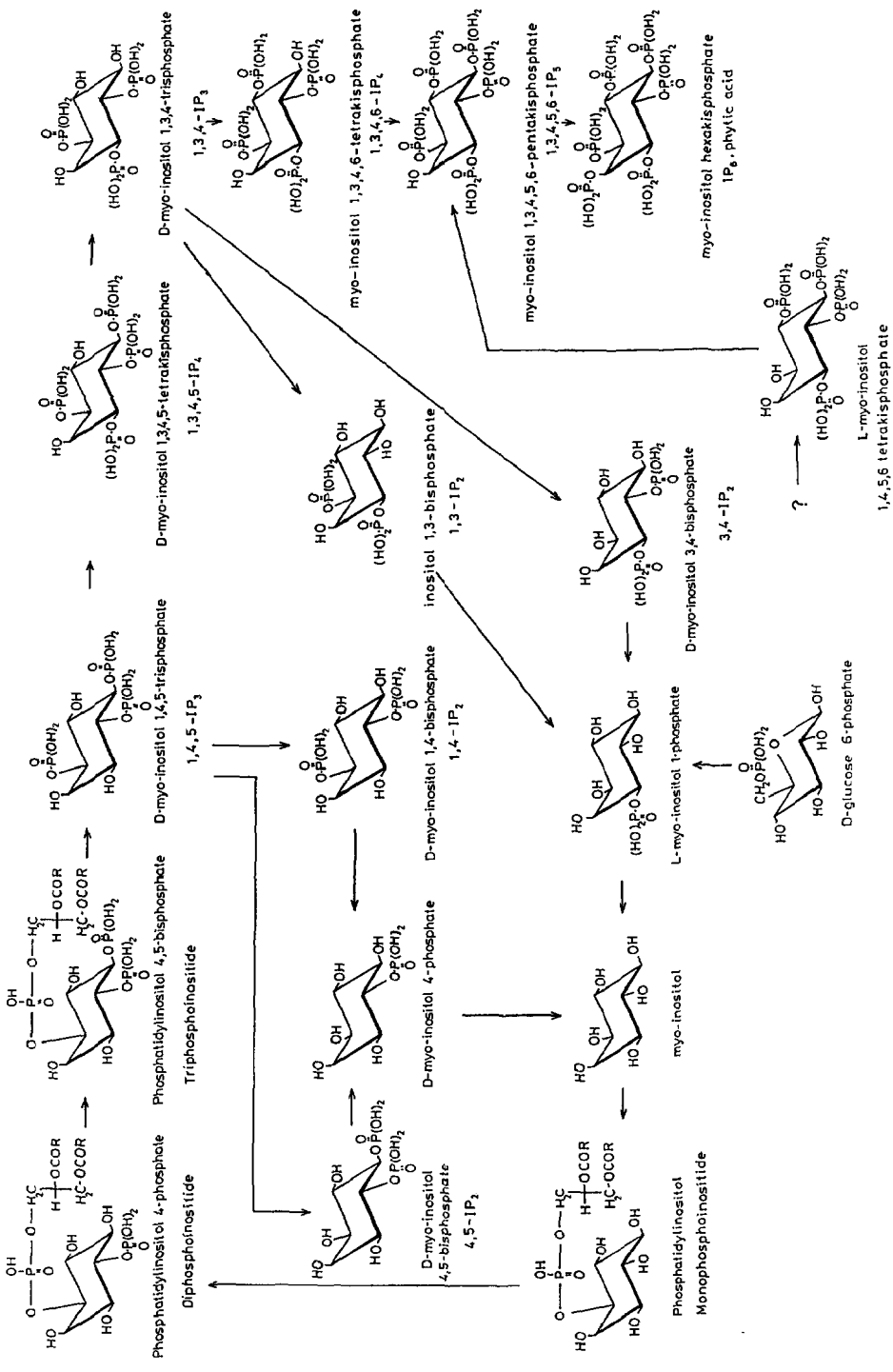

Fig. 2. The phosphatidylinositol cycle.

discovery and characterisation of the 'lipid anchor' which holds proteins in the plasma membrane. This too is based on phosphatidylinositol and structural work has advanced sufficiently (particularly from the work of Mike Ferguson in Dundee who has recently reviewed the field[20]) for this to be a suitable area for organic synthesis.

At a meeting of the Biochemical Society in 1986, I was introduced by Robin Irvine to a young, phosphate-orientated chemist called Barry Potter then at the University of Leicester (who was obviously in a hurry). We began a successful collaboration in which the Mill Hill group provided protected *myo*-inositol derivatives and Barry's group put on phosphate (and thiophosphate) groups and deprotected the products, which allowed rapid progress in the early stages of this research. Our work represented the first use of a P(III)-based phosphorylation strategy using highly reactive phosphoramidites. Fortuitously, a still better method for the preparation of phosphate esters using phosphoramidites became available around this time[21] and this has revolutionised synthesis in this area. I will leave it to Barry to describe in more detail the biochemical and pharmacological problems that have been investigated with these and related products, and the expectations for the future in this area.

My (B.V.L.P.) first exposure to *myo*-inositol and its polyphosphates was during a coffee break in 1984 at the Max-Planck-Institut für Experimentelle Medizin in Göttingen, Germany, where I was doing postdoctoral work in molecular biology and DNA synthesis, when Fritz Eckstein came in with a review by Berridge and Irvine[22] and asked us all how on earth could one contemplate protecting the six hydroxyl groups of the molecule in a regiospecific fashion for phosphorylation. We all examined *myo*-inositol, and agreed that, yes indeed, this was a difficult problem and with the recognition of this we went back to the 'softer' option of oligonucleotide synthesis in solution! At least deoxyribonucleosides only possessed two hydroxyl groups and differentiation between them was straightforward. It was also at this time that the new P(III) phosphoramidite methodology was really starting to make an impact in DNA synthesis.

Subsequently, I left Germany for a 'New Blood' Lectureship at Leicester University. There in 1986, to my good fortune, I became acquainted with Steve Nahorski and we recognised an opportunity for a combined chemical–biological attack on what was by now a burgeoning area of second messenger research, but still one with precious little chemical input. Ins(1,4,5)P_3 had by then still not been chemically synthesised. With my subsequent introduction to Roy Gigg and the realisation that he had solved the regiospecific protection problem, it seemed appropriate to combine forces as Roy has described, especially since, at that time, phosphorylation of protected inositols using conventional P(V) chemistry was proving to be troublesome.

Since the mid 1980s, progress in this field has been little short of breathtaking. It is not surprising, therefore, that, in this special issue on current second messenger research, the majority of papers are concerned with aspects of the

polyphosphoinositide pathway. Chemically, the first synthesis of Ins(1,4,5)P_3 was reported in 1986 by Ozaki et al.[23]. Subsequently, many other groups, including ourselves[24], have synthesised this molecule as well as many of the other important stereoisomers and positional isomers of the higher and lower polyphosphates, using a battery of different approaches[15–17]. The synthesis of these molecules has meant that a number of important difficulties have had to be addressed, namely: the regiospecific protection of inositol in order to afford suitable intermediates for phosphorylation; the optical resolution of such intermediates; the polyphosphorylation of intermediates possessing vicinal diol functionalities, where it is essential to avoid the facile formation of 5-membered cyclic phosphates; deblocking of fully protected polyphosphates, without concomitant migration of phosphate groups; and finally, purification of the final water-soluble polyphosphate. Essentially, these problems have all been overcome and it is now, at least in principle, possible to synthesise any inositol polyphosphate. Much effort, however, is still being expended in the development of novel routes to optically active inositol phosphates and improvement upon the established methods developed to combat the above problems. Several groups have used other naturally occurring inositols as starting materials for synthesis. A notable achievement is that of Ley et al.[25] with their synthesis of Ins(1,4,5)P_3 starting, imaginatively, from benzene.

Biologically, the Ca^{2+}-releasing receptor first purified by Snyder et al.[26] has now been cloned, sequenced, and, in reconstituted form, demonstrated[27] to gate Ca^{2+} in response to Ins(1,4,5)P_3. Inositol phosphate metabolism has been shown to be highly complex and, although initially Ins(1,4,5)P_3 is acted upon by only two metabolic enzymes, 5-phosphatase and 3-kinase, to give Ins(1,4)P_2 and Ins(1,3,4,5)P_4, respectively, a plethora of metabolites is formed subsequently, some of which can be utilised to build up higher polyphosphates such as IP_5 and IP_6. The role, if any, of many of these metabolites is often still unclear and the subject of active investigation. The complex nature of inositol phosphate metabolism is still providing regular new targets for chemical synthesis. Ins(1,3,4,5)P_4, in particular, has proven to be an enigmatic molecule. It was suggested first that it might have a role in gating the entry of extracellular Ca^{2+}, an important part of cellular Ca^{2+} homeostasis. This putative role has enjoyed mixed fortunes over the years[28] and Ins(1,3,4,5)P_4, which has its own receptor, is still the subject of considerable debate and controversy[29].

The discovery of the polyphosphoinositide pathway of cellular signalling is undoubtedly one of the most important recent events in cell biology and has stimulated much academic activity. Notably also, there has been significant industrial interest, since receptors which bind second messengers, enzymes which metabolise them, and the generative pathways responsible for the formation of such molecules are naturally seen as potential targets for rational drug design. It is clear, however, that a drug-design strategy based upon an intracellular receptor and a polyphosphorylated ligand must address the very considerable problem of membrane permeability for synthetic entities. It is not surprising that a central goal

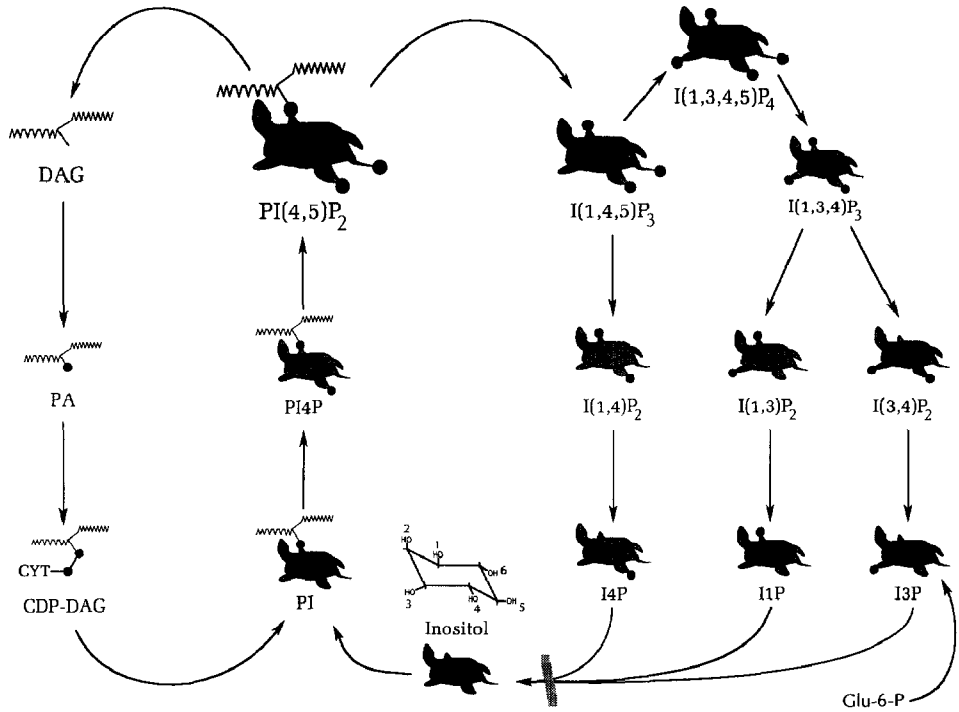

Fig. 3. Turtle-based metabolism of IP_3.

has been the design of potential inhibitors of phospholipase C. Significant efforts have thus been focused upon the synthesis of modified inositols, especially fluoro-inositols[30], which it is hoped will be taken up by cells and incorporated into phospholipid[30,31]. This may be a route to the development of antiproliferative agents. Another area of significant activity has been in the development of inhibitors of inositol monophosphatase[32], the enzyme responsible for converting Ins(1)P, Ins(3)P, and Ins(4)P into free *myo*-inositol. This enzyme is Li^+ sensitive and it is hoped that the development of inhibitors which mimic the potent pharmacological action of Li^+ in the treatment of manic depressive disorders may provide compounds of potential therapeutic utility in neurodegenerative disorders.

It is certainly clear, now that many of the synthetic problems have been overcome, that the focus of synthetic activity is moving invariably towards structurally modified inositol phosphates with novel biological properties as potential receptor agonists, antagonists, and enzyme inhibitors[33,34]. Inositol 1,4,5-trisphosphorothioate was the first analogue to be synthesised[35] and is finding many biological applications. This area is still at an early stage of development, but a number of relatively potent Ins(1,4,5)P_3 5-phosphatase, 3-kinase, and Ins(I)P monophosphatase inhibitors are emerging, together with tentative leads for antagonist design. Although Ins(1,4,5)P_3 receptor antagonists have been identified[36,37], there is still no small molecule antagonist — a clear challenge for the future.

Equally, there has been significant progress in the synthesis of caged compounds, photoaffinity analogues, fluorescent probes, and inositol polyphosphates and analogues linked to affinity matrices, which are finding roles in cell physiology and receptor and enzyme labelling and purification. Current interest in inositol polyphosphate analogues will undoubtedly yield a rich harvest of synthetic pharmacological tools, with the aid of which the polyphosphoinositide pathway of cellular signalling can be persuaded to yield up more of its secrets. Some of the exciting progress made in this area is reported in this current compilation from some of the most active research groups. Crucial to the success of these ventures will be interdisciplinary collaborations by chemists with cell biologists, biochemists, and pharmacologists. If the current pace of progress is maintained, we can expect an exciting future!

We choose to close in verse with a poem capturing the historical flavour of the development of the PI signalling pathway written by Tim Hawthorne, presented by him at his recent retirement party, published in *The Biochemist*[38], and reproduced here by kind permission of Tim and The Biochemical Society.

A Professor's Lament
Inositol, I sing to thee,
At least from 1953,
From Hokins, Mabel and L.E.,
To P.L.C. and Sue Goo Rhee.
Thy brain inositides did Folch discover;
Their structures he could not uncover.
In Berkeley though, there was another,
To Clint Ballou they gave no bother.
The biosynthesis was not too tough
For Kennedy and Agranoff,
While Kai and Salway found it true
that PIP kinase made PIP_2.
And Dawson made it clear enough,
That PIP_2 really was hot stuff.
But what was all this fuss about?
We needed a guru to find out.
Enter Michell with calcium gate –
a theory which I loved to hate,
Yet calcium seemed to be the key,
As Berridge and John Fain could see,
With blow-fly spittle and 5-HT.
But was it PI or PIP_2
Receptors sent their message through?
Abdel-Latif could see it true,
And Kirk agreed, it was PIP_2.

At last the message came alive,
It was trisphosphate, one-four-five.
Berridge was known throughout the land,
With Robin Irvine's skill to hand.
Said they, "Will single message suit yer?
With two we'd rather face the future",
And so they turned to Nishizuka.
His Kinase C with glyceride,
Supplied by their inositide
Could open vistas, far and wide.

There's more to tell if we had time,
New phosphates all along the line,
And far too many an isozyme.
In single cells you may be troubled,
To see that calcium levels wobbled,
As you may learn from Peter Cobbold.

To seek the truth must be our goal,
Though yet we do not see it whole,
You must agree, inositole
Has kept one scientist off the dole.

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