SIR RUDOLPH A. PETERS

The faith of a Master in Biochemistry

By SIR RUDOLPH A. PETERS

THE FIRST HOPKINS MEMORIAL LECTURE

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The most unusual honour and trust implicit in this invitation from the Biochemical Society to give the first Hopkins Memorial Lecture is one that can never occur to an individual again. It is tinged inevitably with the heavy responsibility of giving you some impressions of a unique mind and personality, and of the stretch of his scientific influence. Though other countries and other societies will have their heroes, to us British biochemists in the Biochemical Society Sir Frederick Gowland Hopkins will always be our Master biochemist, and it is fitting that his name and influence should be commemorated in a lecture. The explorer in any field must have some vision of what he wishes to do, and the faith to carry this out. Though it may seem paradoxical, in science such faith is necessary; but it must be justified by the realization of proof. Yet if time is to be saved in research, it is important to be a traveller on the right path, and it is particularly true in biochemistry, where the aim must be to understand in chemical terms what is going on in the cell.

In regard to the details of Hopkins's life, we all know that he attained many high honours; among them were the O.M., the Presidency of the Royal Society, the Copley Medal and the Nobel Prize, high distinctions indeed; but the emphasis to-day should lie upon his unique help to us as biochemists. How far did Hopkins have the vision of the way in which biochemistry should go, and how far was his faith sufficiently robust and unwavering to lead him and his pupils to continue in the right path?

The photograph (Plate 1) I find specially characteristic. It is in 1936, and shows Hopkins sniffing, for the first time I believe, the air of New Hampshire on the border of Canada (Quebec) and the U.S.A. Slight but wiry and energetic, though his appearance might well suggest a frailty, his performance belied this. Of his personal and intimate charm much has been written; it is well recorded in *Hopkins and Biochemistry*.^{*} A particularly important though brief account of a very early Cambridge period is given by E. Mellanby, who was a pupil from 1902 to 1907; Lady Mellanby, Professor V. H. Mottram and Professor

^{*} *Hopkins & Biochemistry* 1861-1947. Cambridge: W. Heffer and Sons Ltd.

J. R. Marrack will remember something of this period. Though I started research with Barcroft, I can claim not only to have been on Hopkins's staff from 1919 to 1923, but to have come under his influence 50 years ago, when I came to Cambridge as an undergraduate. From 1910 I was taught by him for two years in the Part II Class, a very direct influence; at that time the subjects of physiology and biochemistry were not divided and we were brought into contact with a group of most original minds.

It was a wonderful experience for any young man to be in that old Cambridge Physiological laboratory during its heroic period, and by any, let alone modern standards, it was old. It occupied the quarters now devoted to the zoology museum, a natural history collection. Part of the laboratories was on the first floor, and another part in the famous cellar (see Plate 2). I think that the University should placard for visitors the steps to this cellar, where much of Hopkins's original nutritional work on rats jostled with that of Keith Lucas and Adrian on nerves, A. V. Hill on muscles, and a cave for the frogs. It reminds one of the Curies' shed, a triumph of determination over environment. In the class, what was remarkable to us as students was that Hopkins had always read the literature, and moreover would have an opinion about it, showing that he had 'appraised it' as he would say. We wondered how he had the time in the midst of his college work, where he even taught anatomy. I think he must have kept this up later, because Dr Marjory Stephenson once said to me that he was a kind of sponge for the literature. We all learnt much from his very individual Part II lectures.

It would be impossible to exaggerate the courtesy in a genuine humility and the patience with which he would consider points of view put to him by even quite junior students. Not always at his best in an elementary lecture, especially when given early, he would make the students his slaves afterwards in the practical class with his polite questions as to their opinions. This intimate respect of them as persons was one of his most engaging traits, and withal most educative—one had to think. This was how his time was rarely his own,

exemplifying the Chinese aphorism, 'the Sage has no self to call his own'. I find that I have been forced back to China for this expression, just as Dr and Mrs Needham were in their account! Many of us must have a picture still of the long queue waiting regularly outside his room, consisting as it did of folk from all sides of the University who brought to him their problems for his sympathetic advice. I still treasure a manuscript containing comments by Sir Frederick on a small memo which I wrote on muscle research. I treasure it not only for the shrewd remarks, but also because of an increasing gratitude and appreciation as the years go on of the valuable time he must have devoted to thinking about it. Though he had a core of merciless criticism in fact, he was otherwise so human that any stringency was forgotten. His Tea Club meetings exemplified so well his mastery of the subject, by his ability to put the speaker's remarks into perspective as well as by this kindly critique. He even carried a horror of hurting feelings to a fault, so that it was not always easy to get his real impression. He was indeed patient with his staff. The redoubtable 'Charles', to whom we all turned for help in the advanced class, once starved him of thiophen for some of his lactic acid tests, and he little protested. In fact, it subsequently transpired that there was a full bottle locked up in the cupboard! 'Charles' always felt it necessary to protect stores from the greed of research workers! Hopkins confessed to someone that he never knew what it was to be bored; but he got depressed when research went badly, and grudged the time spent on some of several committees, pointing out that people knew when a committee was cut but not when one cut research. Often his conversation started with the phrase 'worse luck', when he had not time to do something. He once said, a little petulantly for him, 'the truth is that no one has time for research after the age of 30'. I think that this is much less true nowadays with the increased recognition given to whole-time research.

H. C. Rümke, lecturing recently to the Dutch Academy, said: 'The creative man of science revealing things hitherto hidden is much closer to the creative artist than is commonly supposed.' May I expand this a little? In science the artistic is essential, but it is tempered art. The creative here consists in the revelation of what is already there by asking the right question and understanding the meaning of experiments. So far as there can never be perfection, scientific artists must always be at war with what seems to them to be their own inadequacies, and Hopkins was no exception to this rule.

With his assistant, E. J. Morgan, he was always at work, though only a small fraction of this

reached publication; but it gave him a grip on the field and I never knew him not ready to stop and discuss some research result. Of course, a professor was less harassed by administration then, there was less money to spend! There were many delicious human touches. Even now I can see him skilfully avoiding the inconvenient visitor, dodging him with test tube in hand. Then there were the cigarette ends; 'Hoppy' seemed to require the stimulus of starting a cigarette to get his thoughts going; but, this accomplished, the cigarette was laid down and often forgotten, so that there might be several burning at once. It led to a kind of poker-work effect on the floor and even on the professorial desk, as Professor Young has pointed out. His wit was so pleasant too; when he had retired he made a small speech to the then newly founded Nutrition Society, saying that he was now on the shelf, and that this was an uncomfortable place from which to talk! Again, when his portrait was presented to him, he said that he had always wanted to have his portrait painted, but that as he went round College Halls he so often saw a wreck presented on the walls. His own portrait was not this. All these are personal points and they will strike others differently, yet they leave an indelible mark.

In regard to his research and teaching career, his value was recognized early by men such as Archibald Garrod and Michael Foster; but it should be stressed that, for various reasons, Hopkins hardly embarked on his real life's work until he was about 40, and to the end he felt that the fates had cheated him somewhat of time for research. Born in 1861, and numbering among the connexions of his forebears Thomas Gresham, he had an unusual schooling, and then had to escape from a business life in the City of London, as an insurance agent. Eventually, as an assistant at Guy's Hospital, he embarked in educating himself in medicine at the age of 28. Chequered as this early start was, it demonstrates that even in Victorian days there was room for the Hopkins's and Faradays to find their feet. I should not like to have to decide whether the early struggle toughened the later growth. It is interesting to compare this start with that of the famous French physiologist, Claude Bernard, who began in an apothecary's shop. For Hopkins, the Cambridge venture in 1898 was a big speculation and a great financial sacrifice; in this he was loyally supported by his wife, who was a very real partner and strength to him throughout. His professorship did not come until 1914, when his laboratory was located first in premises vacated by the Physiological laboratory, and later in 1919 partly in the Balfour Laboratory in Downing Place. The present Laboratory did not come to him until his sixty-fourth year.

SIR FREDERICK GOWLAND HOPKINS

SIR RUDOLPH A. PETERS—THE FAITH OF A MASTER IN BIOCHEMISTRY

(Facing p. 2)

The first floor belonged to the old Physiological Laboratory, which became part of the Biochemical Laboratory in 1914. Entrance to cellar below.

The steps to the cellar.

To put the biochemical contributions of Hopkins and his school into perspective, I find that I must give some kind of historical sketch of the development of biochemistry, which I attempt with a full realization of the limitations. We have to go back to Benjamin Moore, who was the first Professor of Biochemistry in England at Liverpool, and who gave some of the initial impulse to these studies as an independent discipline. We owe the *Biochemical Journal* to its foundation in 1906 by Moore and Whitley. Moore also contributed much to colloidal ideas of the cell and to knowledge of the influence of hydrogen-ion concentration, e.g. on the development of sea-urchin's eggs.

Before the Cambridge period, Hopkins's butterfly work and protein studies stamp him as a born investigator. His capacity for devising new chemical tools shows up later in his mercury agent for tryptophan, devised independently of Denigès. All of us who saw him at work will have vivid memories of a beautiful chemical technique as a manipulator; it was a joy to see him titrating. In the earlier nutritional work in Cambridge on proteins and then on vitamins, he exhibited as early as 1906 a remarkable intellectual grasp of the problems of accessory food factors as a whole and produced very convincing proofs of vitamins in his work on the rat published in 1912. We can say this without minimizing in any way the earlier Dutch contributions, the contemporaneous Norwegian and those in the U.S.A. During the first decade of the century, too, he formed the association with W. M. Fletcher which bore such fruit in the research on lactic acid in muscle, published in 1907. Indirectly, this led to Fletcher's pioneer work as the first Secretary of the Medical Research Council. Fletcher's work on carbon dioxide in muscle at the beginning of this century had already overturned the somewhat mystical 'inogen' theory, and their joint research on lactic acid gave point to the study of small molecules. If one assesses the position in 1910-12, it would not be true to say that no one had thought about enzymes in the cell. Hofmeister, for instance, had clearly good ideas of the place of biochemists. Leathes again had published his book on *Problems in Animal Metabolism*; Harden and Young had done their yeast studies, but the efforts were somewhat sporadic, and I do not think that there had been much effect upon the trend of physiological chemistry, which was still running mainly towards the study of excretory products, blood analysis and purely structural chemistry. (Perhaps parts of sugar metabolism should be excepted.)

It was at that point (in 1913) that we have Hopkins in his address to the British Association proposing that we should dare to peep boldly into the chemistry of the cell and follow the dynamic

changes there taking place. It is this address which stamps him as the biochemical philosopher on whose thoughts we depend so much to-day. (I find it curious that at that time a young research worker like myself in that Laboratory did not realize that Hopkins had gone to Birmingham to give it.) Sherrington for one was much impressed; he spoke of the way in which Hopkins described an organized factory manifoldly hydrolysing, pulling to pieces and contemporaneously constructing and reconstructing; he felt himself privileged to see some of the microcosmic world in which the lecturer's thoughts took shape and did his bidding.

With the revelations of the electron microscope now at our disposal, it is hard for us to realize what a fight was needed to establish the position of intracellular enzymes. So few were then known. Even when we (Walker and I) in the 1920's tried to connect enzymes with vesication, there were very few to play with. The fight too has been long to establish the central point that chemical thought should be carried right into the cell. Even now it is not quite over. We can select a few passages from the 1913 address, though I think it should be read as a whole by biochemists. After stressing that studies of metabolism can be made simple, he says: 'I intend also to emphasize the fact that it is not alone with the separation and identification of products from the animal that our present studies deal, but with their reaction in the body, with the *dynamic* side of biochemical phenomena.' He emphasizes that the reactions in the cell, as influenced by enzymes, can be studied *in vitro*. 'We have arrived', he says, 'at a stage when it is logical to conclude that all metabolic tissue reactions are catalysed by enzymes, synthetic as well as in the opposite sense', and then we have the famous remark about the cell: 'its life is the expression of a particular dynamic equilibrium which obtains in a polyphasic system'.

Some touching-up of his views may now be necessary; but it is astonishing how close they come to the modern picture, and how right his ambition was to see these studies grow, even though he was heavily warned off the field when young by an important organic chemist who called it 'super-chemistry'—and how right this was. Though he was apparently so pliant, when it came to the point of defending biochemistry, few could be stouter opponents. Physiology had much to do with launching biochemistry in this country, and at one time I thought him wrong to dissociate biochemistry and physiology; but as the years go on, I believe that it may have been a quite necessary stage in the emphasis of biochemistry as an independent philosophy to be carried on by those with the dual training of chemistry and biology. I was forced to such a view myself in relation to our

International Union. Yet here and there traces of the older attitude are still left. Even in 1939, there were many respected persons who could not believe that a trivalent arsenical might kill because it made a primary attack upon an enzyme system (in this case the pyruvate oxidase system). One respects criticism as the breath of life and scepticism; but there should be limits!

We may now pass to a review of some developments to see how these ideas worked out. In the nutrition field, there has been a steady development; again it was surprising how long it took to displace the idea that deficiency of some factor (vitamin or otherwise) could produce in itself pathological disturbance; so firmly rooted was the notion that disease must be produced by a positive reagent. I can remember arguments about this at a meeting in Cambridge in 1920. Yet Hopkins's ideas of the cell made the deficiency view simple.

In relation to the essential conception that intracellular enzymes were responsible for breakdown and synthesis of constituents of the cell, distinct phases can be traced in enzyme research. The first followed on the Pasteur-Liebig controversy; it starts with the Büchners' work on yeast juice and the attempted isolation of invertase by O'Sullivan and Thompson, say about 1896. Until the end of this time, that is until the early 1920's, we had an era when research was engaged virtually in determining various properties of enzymes then known and the limits for their action, as illustrated by research on the kinetics of invertase action. At the end of this period, there was the deliberate attempt of Willstätter and his colleagues to isolate enzymes, an attempt which gave much new technique; but it was left for the biochemist Sumner to prove the protein nature of an enzyme by crystallizing urease. During the early 1920's a step forward was taken by Meyerhof when he demonstrated so clearly that much of glycolysis did not need what had been called hitherto the architecture of the muscle; but that, with all the specificity involved, orderly reactions leading to lactic acid formation could take place in potassium chloride extracts of minced muscle, squeezed through muslin; these experiments played their part in Meyerhof's theory of the 'lactic acid' cycle. During this period too, from 1910-12 onwards, Warburg was studying oxidations; his work early proved that in sea-urchin eggs some oxidations were connected with structure, and later his slice technique opened a field of study wider than that of the tumours at which it was aimed. (It is interesting to note that Wigglesworth in Cambridge developed a slice technique independently.) Warburg became the forceful protagonist of the importance of iron in cell respiration, and in this was the settled opponent of Wieland's view that

oxidation took place by transfer of hydrogen. As will be recalled in what is very familiar ground, Keilin largely reconciled these controversies with his scheme involving cytochrome *c* as the intermediate substance to be reduced through the agency of the dehydrogenases and reoxidized by the influence of oxygen through the cytochrome oxidase, the respiratory ferment of Warburg. In so doing, Keilin showed that cytochrome *c* was oxidized and reduced *in vivo* and produced our first sensible model of the respiratory chain. Warburg also studied his ferment *in vivo*.

Where did Hopkins and his philosophy fit into these developments? In my opinion, it was the thread which gave them coherence. The lactic acid work had laid the foundations of the glycolytic researches; these were also helped much by Embden's hexose phosphate isolation studies in muscle, following upon Harden and Young's classical research on yeast juice. A. V. Hill leant upon the lactic acid work in his studies of heat production in muscle. Above all, those in Hopkins's Laboratory at the time can remember the thrill of glutathione in 1921.

Fig. 1. Sketch plan of the Balfour Laboratory, during the years 1919-24, showing the approximate places in which various research persons worked.

It seems to be appropriate while we still recall it to give some picture of his colleagues in 1919–23 before the present laboratory was built. The research was done then in the old physiological laboratory and also the Balfour, now the music school. The latter was a converted chapel with a floor inserted at gallery level, which I had hurriedly partitioned for Hopkins in 1919 when acting as his adjutant. With the help of Dr Malcolm Dixon especially, I have constructed a plan giving the names of workers and the approximate places where they worked (Fig. 1). Dr Malcolm Dixon, as all will know, has been continuously behind much of the enzyme work in Cambridge since those early days, and it is particularly appropriate that he should be now President of the International Enzyme Commission. The upper floor was the large partitioned research room connected by a small bridge to Hopkins's laboratory (N. K. Adam worked in a small greenhouse). Below there was a small library and a store, and the advanced classroom where several distinguished people did their advanced class in biochemistry: Dixon, Roughton, J. Needham and Mrs Barbara Holmes, for instance*. There were still important researches going on in the old part of the laboratory, M. Stephenson, E. Gordon Young, Jordan Lloyd for part of the time (who kept her protein and colloidal work on gelatin going with clarity and enthusiasm), J. B. S. Haldane, R. A. McCance, R. Hill and R. Fearon. Hopkins's interest in nutrition was waning over this period, and Dr Malcolm Dixon remembers the day when he decided to swing the work towards oxidation. It is important to realize that in spite of the intense individuality of the persons making up this laboratory, Hopkins always maintained a very real intellectual control.

To return to glutathione, it is true that the chemistry took him seven years to clear up; but the impressive point was that a soluble substance with biochemical properties existed, able to react by addition and subtraction of hydrogen, as Wieland postulated. In a final assessment, it was a better proof than that of Wieland.

If there be any who wonder why the chemistry took so long, I would advise them to look at the original paper, where they will see how difficult the

substance was to prepare, what pains were taken by Hopkins to get analyses done, and how closely they agreed with the suggestions of structure. Let them also think of the difficulties of isolating mixed disulphides, and remember that this had to be done without paper chromatography. It is indeed 'superchemistry'. Quite recently, Dr P. F. V. Ward in my Laboratory has gone through a similar experience in trying to purify a synthetic fluorocitric acid, which analysed well, but showed on a silicic acid column the presence of anything from 5 to 10 substances!

Some discoveries are made by logical inference. Several are nowadays the product of more than one mind, and this research may be expected to increase with the multiplication of special disciplines and techniques. But there will always be a place for the individual. Hopkins's discovery (with S. W. Cole) of tryptophan (I am told) was started by the awkward questions asked by a student in the advanced class. In the case of glutathione, Hopkins seems to have looked for acetoacetic acid in muscle with the nitroprusside reaction and come across the sulphydryl compound instead. The point of course was, and it is almost absurd to mention it, that he realized it was different. Most intelligent experiments are worth doing because they so often reveal the unexpected; but it is perhaps better to be not too much like the French physiologist Magendie, who is said to have made an idol of facts alone.

The scientific impact upon biochemistry of the isolation of this soluble sulphydryl substance was great and its significance continues to increase. It was the key which unlocked much of the work on intracellular enzymes, especially when coupled with the Thunberg dehydrogenase technique. Hopkins's efforts with pupils to find the 'fixed SH' in tissue preparations with which glutathione SH interacted, apparently unsuccessful at the time, contributed their quota to the present knowledge of SH enzymes. Professor Sidgwick once said (1928) that biochemists said their prayers to pH; perhaps he would now add SH, because SH as well as pH dominates biochemistry. So glutathione has become the ancestor of much research today. But to say this is not to detract from the brilliance of subsequent SH developments, which have been quite independent, and have involved much originality and new technique; our recent symposium on glutathione† is enough to demonstrate this. Nor should we be too complacent; even now can we say in *chemical terms* just why the SH in triose phosphate dehydrogenase is especially sensitive to iodoacetic acid and little so to a monosubstituted tervalent arsenical; or why the SH in

† *Glutathione*, Symposium No. 17, Biochemical Society. In the Press.

* An amusing story is told of these times. The workers on proteins and those trying to grow *Aspergillus* could not understand why the incubator seemed to do their specimens harm. Two of them therefore watched one night to see what happened. It was then noticed that one research worker came into the laboratory in the evening, hung his coat on the iron bar of the gas incubator, which accordingly responded with a large rise in temperature; when he left two hours later, he removed his coat and the incubator returned to normal; so by the next day the temperature was down to normal.

aconitase and isocitric dehydrogenase is insensitive to iodoacetic acid, and very easily attacked by disubstituted tervalent arsenicals?

In the 1920–30 decade we can notice that, following the work on glutathione, much effort in the Cambridge Laboratory was devoted with the dye technique to the reactions of dehydrogenases. In work on xanthine oxidase, for instance, Hopkins pushed forward such researches and encouraged oxidation–reduction studies at the microscopic level. I am afraid that these studies left many physiologists, and others, little impressed with the possible impact of the great gains in the differentiation of specific intracellular enzymes, though their attention did soon focus upon the enzyme cholinesterase. The studies of Quastel and colleagues on succinic dehydrogenase and its inhibition by malonate were a first example of competitive inhibition, and in this may be reckoned as the precursor of antimetabolite studies. It was a moment of great excitement for us in Oxford when we found about 1930 an effect of vitamin B₁ (thiamine) *in vitro*, because this led to the connexion of intracellular studies with pyruvic acid, with vitamins and cellular oxidation; and it definitely related this biochemical lesion with pathological changes. These researches, I really think, sealed the connexion of the intracellular enzyme with physiological events, oxidations and vitamins, and we were fortunate that a prolonged research should have matured in this way. Other B vitamins later took their place in coenzymes. At this time (1930) too, the earlier background research on parts of the tricarboxylic acid cycle was in progress by Szent-Györgyi and others to lead ultimately to the formulation by Krebs of his citric acid cycle. In the later 1930's, the progress of research in the glycolytic field led to the independent formulation of coupled phosphorylation by D. M. Needham and Meyerhof with their colleagues. Arising from this we have come to the realization that adenosine triphosphate is a special kind of fuel and of the special position of oxidative phosphorylation; at present the biochemical fire is blazing in so many places that it is now difficult to discover the Hopkins thread.

Such a short sketch is a dangerous over-simplification; it leaves out so many distinguished persons who worked in the present laboratories and important fields like the steroids; still it may suffice to indicate how justified Hopkins was in his address to the British Association in professing his faith in the value of a ruthless exploration of specific intracellular enzymes and in studies of the life of the cell carried out on small molecules with the firm belief that the logic of chemistry could be used in interpreting biochemical events.

From a perusal of his writings over this period, though he had stressed the colloidal nature of his

'dynamic equilibrium' in his address, he seems to have travelled more to the idea that the specific enzyme was all important in the control of intracellular events. Here he may have been influenced by the organization of enzymic reactions in muscle minces, which we now know would still contain much organization at the level of mitochondria and microsomes. Doubtless he felt rightly (as did many others) that accurate knowledge of events in cells must wait upon a deeper and more intensive study of enzymes in isolation, and that the wisest policy was to encourage this as a sound basis for reconstruction. He was certainly much surprised, as I know from a letter, when Lundsgaard showed that iodoacetic acid could produce a muscle which contracted without formation of lactic acid; even now do we really know how to relate the biochemistry to the physiological events of contraction? It is hard to know whether in his inspired phrase 'dynamic equilibrium' he really pictured the extraordinarily rapid interchanges of which we are now aware through the use of radioactive isotopes. At any rate, it was the inspired guess of genius.

In parenthesis, I might mention a personal impact. Having thought that I knew something, after several years' work, about the behaviour of pyruvic acid in the old-fashioned brain brei, I was truly astonished when working with Professor and Mrs Chain in Rome last summer to find the extent of the reactions in which the ¹⁴C from labelled pyruvic acid had taken part. In a two-way paper chromatogram as scanned by their two-dimensional scanner, the substances labelled were hexose phosphate esters, phosphoglycerate, citrate, α -oxoglutarate, glutamate, alanine, aminobutyrate and some other unidentified substances. The Chains and colleagues have already published work of a similar type starting from glucose.

D. D. Woods pointed out, in his Memorial Lecture on Marjory Stephenson, that she stressed in relation to microbiological studies the different levels of organization in bacterial cells, all of which could under appropriate conditions be worth while objects of research. In their enthusiasm, I think that some biochemists have claimed too much for their isolated studies; this Hopkins would not have done, because with his background and intellectual equipment he never forgot the importance of the biological outlook—it was part of him. No doubt in his efforts to teach that the inside of the cell was within reach of the experimentalist, he was led to overstress the small molecule. It is hard to know what would be meant now by the colloidal system of coexistent phases, because colloidal science has much changed. Some of us, nevertheless, in the 1930's did begin to put forward views that the cell was even more organized

chemically than previously supposed; perhaps we could say shortly that it had a real geography. In essence, this amounted to saying that the same mode of thought should be applied to the cell as one applies to the carbon atoms in a benzene molecule. In my own case, these attempts at conscious co-ordination arose especially from the Hardy-Langmuir conceptions of surface orientation of molecules, which gave substance to the idea that even a monomolecular film of a fatty acid could behave on one side as a dissociable acid and on the other as virtually a paraffin. Such ideas really transformed some aspects of colloid chemistry, and it further became known that the nature of a metal surface could influence chemical changes taking place near it. It seemed that there could be an organization on these lines inside the cell, taking part in the co-ordination of cell reactions. This is not the place to elaborate such views, which I regarded as an extension of dynamic biochemistry; but they involved more emphasis upon the geography than Hopkins liked—I think probably because he was alarmed that such thoughts harked back to the mystical and entirely sterile idea of the 'inogen molecule'. But in his later Linacre lecture he warned of 'organization of an undreamt of complication'. Where I think that the views differed was in the suggestion that the enzymes of the cell themselves might be under the control of some mosaical structures. I feel sure that Sir Frederick would have been fascinated to follow the electron micrographs of mitochondria and microsomes and the later suggestions that some of these may be fragments from some larger sheaths. The remarkable thing is that they can be studied at all.

Dr de Duve and his colleagues, and others, have been carrying these studies further. In my Laboratory Dr V. P. Whittaker has recently collaborated with Dr K. M. Smith of the Molteno Institute to find that the particles which have the cholinesterase and acetylcholine, and which Miss Hebb has shown to contain the choline-acetylase, separate from the mitochondria in the Spinco centrifuge, and therefore are separate from the enzymes of the tricarboxylic acid cycle. Evidently we have some way to go in unravelling these complications.

Now we see in progress from several sides the postulation of very long molecules of nucleic acid to act as templates for the genes. Dr E. F. Gale studies such problems in bacteria. At the same time, the structure of a protein in terms of the order of its constituent amino acids has become revealed for the first time in Hopkins's old laboratory by Sanger, where Professor Chibnall turned especial attention to studies of the proteins, and where the present Professor actively en-

courages studies on the structure as well as the function of some of the hormones. It is interesting too that two pupils of this Department (I refer to Martin and Synge) devised paper chromatography, which played a prominent part in the elucidation of the structure of the protein, and is so extensively used now that our Society meetings are often leopard-like! The wheel has come full circle; the giant molecule is having its revenge, but it is a Pyrrhic victory; for we have retained enough of Hopkins's philosophy to realize that it is not too mysterious to study in chemical terms. Perhaps we have become too courageous!

Our more purely chemical friends are often puzzled when we say that biochemistry is a separate discipline; they see us using essentially chemical techniques in our work, even if we often have to shape our own tools. Wherein then is the difference? It is mainly in the mental attitude; in the fact that, even though we have to concentrate upon isolations, we should have eyes all the time upon the application of chemical facts in life. Subsidiarily, however, there is the strangeness of the chemical medium. So much takes place at pH 7.0, and Nature in the course of evolution seems to have learnt more than any chemist yet knows about chemistry at this pH and comparatively low temperatures; further, organisms have learnt to defend their pH as part of their internal environment. Our respiratory centre is sensitive to a change of about 0.01 pH. In 1919 I remember that in the advanced class we looked at some vinegar eels which had been brought to me by a zoologist from one of the College High Tables. We were amazed to find that these beasts put up with a concentration of some 0.01N-hydrochloric acid. This fired Hopkins, who was disappointed that the class, with their narrower range of knowledge, was not so excited. Incidentally, this organism also stands very deep freezing. I believe that we ought to think more about pH even than we do. Many years ago, I proved that the apparent pH at the interface between an aqueous and lipoid phase was shifted as compared with the bulk pH, and I still feel that some of the mysteries raised by pH optima for some enzymes far removed from the pH of the cell are to be explained in this area. We also know that buffers can alter the pH optima of isolated enzymes in solution.

Quite recently, Dr R. M. C. Dawson in my Laboratory has found a relevant phenomenon in connexion with his studies on phospholipids. It has been recognized for some time that two steps were needed to take off the two fatty acids in lecithin (or kephalin) by enzymes known as phospholipase A and B. Working with a phospholipase B preparation from *Penicillium notatum*, Dawson found that activation of this for phospholipase A

activity could be induced by adding to the substrate various acidic lipids, one of which is cardiolipin. The net effect is to convert one enzyme activity into two. This action appears to lie in the surface field as the activation goes parallel with decreasing lipid solubility of the lecithin and an increase of hydrophilic properties. With Dr A. D. Bangham, of the Pathology Department, this effect has been traced to a change of the electrical charge of the substrate particles from positive to negative. This then gives a new and concrete example of how surface chemistry may alter conditions in cells, and is clearly of much theoretical interest. Much more knowledge is wanted in the surface field, even on the technical level, as a basis for the technique of chromatographic column separations.

There are still important gaps in our knowledge of the chemistry of these microheterogeneous systems. Taking pH as an illustration, a simple calculation which I made years ago shows that, at the neutral point, particles like the mitochondria or the virus even will have on average much less than one dissociated hydrogen ion. I know that the thermodynamic argument is that there will be a field for a fraction of the time; but I find this rather unreal. The explanation seems to involve some unknown point, which may be connected with the speed of adjustment of this type of equilibrium, or it may even be that Nature takes advantage of an aqueous background which is not ionized. I think that we must be on the look-out for the unexpected reaction in this type of system, especially as so few molecules seem to be involved at one time, a point stressed by Krebs. We have found that aconitase is vastly more sensitive to fluorocitric acid when it is inside the mitochondria than in isolated solutions.

The sweep of modern biochemistry must astonish us all; witness the detailed knowledge of biosynthesis by enzymes now being obtained. Hopkins had always enormous belief in the potentialities of living matter, a belief justified by almost daily events. Bacteria hit back by becoming resistant to penicillin; flies by putting up with large concentrations of DDT. Even rabbits have learnt how to become immune against myxomatosis, and this has raised a big economic problem for Australia and even here, where rabbits used to do £50 million damage a year to crops. Knowledge of these biochemical mechanisms is wanted for practical reasons, but even more to complete the intellectual picture. My short session in an Institute of the Agricultural Research Council has focused for me the extraordinary biochemical adaptations in the ruminant, where in the rumen Pasteur's '*vie sans l'air*' is in progress all the time and enables these animals to survive where we should perish.

Turning to our relations with other sciences, to the physiologist we must often still appear to pursue with our minces and particles a course so far removed from life that there is no hope of application. It must give them the kind of feeling that some of us have when we look at very modern art; I mean that kind of picture which depicts a mixture of playing cards and bits of limbs. Of course we can challenge this; in essence it is not different philosophically from the attempt to synthesize the physiological reactions of the whole animal from (say) a perfused-heart preparation.

But the point that I want to make particularly is that the behaviour of the intracellular enzyme must often be an active intruder in physiological experiments on the so called 'normal'. We are well aware of the importance of the cholinesterase type of enzyme in the functioning of nervous tissues; but in my view it is not only this type that matters, nor is our function as biochemists merely concerned with setting the right biochemical background for normality in the physiological experiment. I can focus my point best by asking you to listen to a rat heart poisoned with a long-chain fatty acid, fluorodecanoic acid, where the fluorocitric acid synthesized blocks the enzyme aconitase. You will hear that, as compared with the normal beat at 550/min., the heart is slowed and irregular. Whatever be the intermediate steps, the primary block here is in the enzyme aconitase, one of the enzymes of the tricarboxylic acid cycle, i.e. in the working of an intracellular enzyme; so that this explanation can be traced to a root in the Hopkins 1913 address. These matters are of course subtle and are mixed up with biophysics. But it is this type of experiment which convinces me that we must be on the look-out for the intrusion of intracellular biochemistry into some physiological experiments, and not only those concerned with the maintenance of stability of membrane potentials. What even is the final use of a membrane theory without knowledge of the constitution of the membrane which may itself be undergoing adjustments during life?

In pharmacology, pathology and medicine, it is certain that we have the clearest arguments for our importance. Biochemical change must precede pathological disturbance, and effects upon enzymes more often than not induce pharmacological action, whether this be by combination with active centres or by antimetabolite effects.

In conclusion, some general remarks seem necessary. There are still misunderstandings as to what biochemistry is about; fortunately, we can comfort ourselves with Emerson's saying that 'to be great is to be misunderstood'. In particular, objection has been taken to the term 'high energy bond', used in biochemistry to denote a molecule

with a valuable form of instability; the grounds of the objection are that the term does not mean this in chemistry. Surely someone could retort equally that the use of the term 'nucleophilic' in chemistry has always been most puzzling to the histologist, who has used nucleophilic stains since time immemorial.

There is some evidence also that the centuries' old clash between vitalism and mechanism is taking on a new dress with a redefinition of vitalism—not as the entelechy of Driesch, but as a statement which appears to reduce to the idea that the state of organization in living matter is too complicated for expression in present chemical terms. It seems to be somewhat arbitrary to set a limit to the degree of complexity which physical and chemical knowledge may be held ultimately to embrace, and I doubt whether many chemists would do this. Perhaps in so far as living organisms become individuals, this will always set part of their activities outside statistical laws; certainly individuals or small groups count in the world's history. But surely our task is to continue to apply chemistry and physics to the inside of the cell until this fails us. Then, as Hopkins said, from time to time we may meet the spectre of vitalism. What biochemists have accomplished in the past is enough to show that any chemist entering the field with due humility will still accomplish much, and to justify the sustained faith and effort to put our feet on the

right path. (I do not think that humility matters much, because Nature humbles us all.)

One thing is certain: now that we know a little more about the chemical nature of constituents of the cell, we are only just in a position to embark on the next stage, and it will take long even with present techniques to progress to our final goal. We are likely to remain inspired by the belief that if we put the mixture together properly the machine will go, if you like 'live'. Even if this happens, and it will be a long time before it could, we must be cautious in our philosophical interpretations and not go beyond our facts. Much of practical importance will emerge in our pilgrimage; but, best of all, the intellectual pursuit is worth while. *Labor ipse voluptas*. As Hopkins indicated in 1933, biochemistry is a better intellectual discipline than many of our friends in the humanities would think.

May I finish with some words from Joshua Reynolds, in which he refers to the pictorial arts: 'Study therefore the great works of the great masters, for ever.... Consider them as models which you are to imitate, and at the same time as rivals with whom you are to contend.'

I am indebted to Mrs B. E. Holmes for the photograph of her father, and to many others for their advice. The photo was enlarged from a group of three taken by a member of Harvard University, believed to be Dr Dill.