

The Hopkins Memorial Lecture.

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WHILE I feel greatly honoured in finding myself in the position to-night of giving a memorial lecture to Sir Frederick Gowland Hopkins, I am conscious that this is a challenge and an opportunity which demand qualities to which I can make no special claim. A man who can be regarded as one of the greatest scientists and, in his own sphere, the greatest scientist of his time, is very rare. A man who is esteemed by everybody and regarded with affection by all his friends, colleagues, and pupils, is by no means common. The combination of both these qualities in one person presents us with a man of such outstanding individuality that the degree of knowledge, skill, judgment, and sympathy required to give his bibliographic lecture is almost unattainable. I can only promise to do my best.

Most of this lecture will be taken up by an account of Hopkins's scientific work, but before beginning this subject I should like to place on record some of his personal qualities, although I imagine that many here are familiar with these. Indeed, one of the thoughts that first passed through my mind in preparing this lecture was the large number of men who could with more justification be called upon to give it.

There are few men with whom one would rather have had a personal chat than Hopkins. This was because, in all conversation, he was kind, unselfish, gentle, sympathetic, and thoughtful, and yet a man of great sense and knowledge. He had a remarkable power of sending a man away much happier than when he came, and, if the subject of discussion was one of scientific research, especially after some depressing experience, a man was much more prepared to carry on with his labours and forget his trouble. This was certainly my experience and must have been common to most other men who had the good fortune to be associated with him in the laboratory. Hopkins had a great stimulating power on young people. A particular instance of this kind happened at an early stage of my undergraduate career at Cambridge, when he introduced to a class taking the Tripos in physiology a discussion on the volume of blood in the body. He succeeded in rousing in me a state of enthusiasm sufficient to make me confine my whole attention to this problem for a fortnight to the detriment of my other work, in order to find a method to decide whether the blood volume was one-thirteenth or one-twentieth of the total body weight. This was an exaggerated reaction of the kind with which I was constantly affected in the few years in which I was intimately associated with him in the laboratory. So long as Hopkins had a small class or a small audience, he had this same power of making them feel pleased with themselves and sending them away happy. With large audiences these qualities were not apparent, because of the smallness of his voice. A good instance of Hopkins's method of talking, either to individuals or to small groups, can be seen in the opening words of a lecture he gave to the Public Analysts of the country in 1906, a discourse which has often been quoted, since in it he outlined the discovery of what came to be known as vitamins. Everybody knows that he had been trained as a chemical analyst and that later he had medically qualified, and that in the year 1906 he was already a Fellow of the Royal Society and a man of real distinction, lecturing before a group of people who were, for the most part, ordinary analysts and medical men. He began this lecture with the words: "Seldom I imagine has this learned society welcomed a visitor less obviously entitled to occupy its time." He then proceeded: "I came to the conclusion that the only real qualifications I had for coming before you were those possessed by an individual who, having been trained for your own profession and having acquired some knowledge of its aims and claims, sought later a training in the profession of medicine and so gained similar knowledge with regard to it. I am, of course, very far from standing alone in this experience but, unlike many who, with the same double training, have practised one or other of these professions with distinction, and still more unlike those who have contrived to practise both, it has been my fate never at any time to practise either." Hopkins, in these few words, stated the exact position but stated it so modestly that most people in the room must have had a feeling of superiority, and yet at that time he had gone a long way to having demonstrated that he was a combination of the ablest analyst and the ablest medical man in the country.

I have thought it well to say these few words of introduction about Hopkins, because nobody could fail to appreciate these remarkable qualities, which were present even when he had reached the highest point of activity and also of fame.

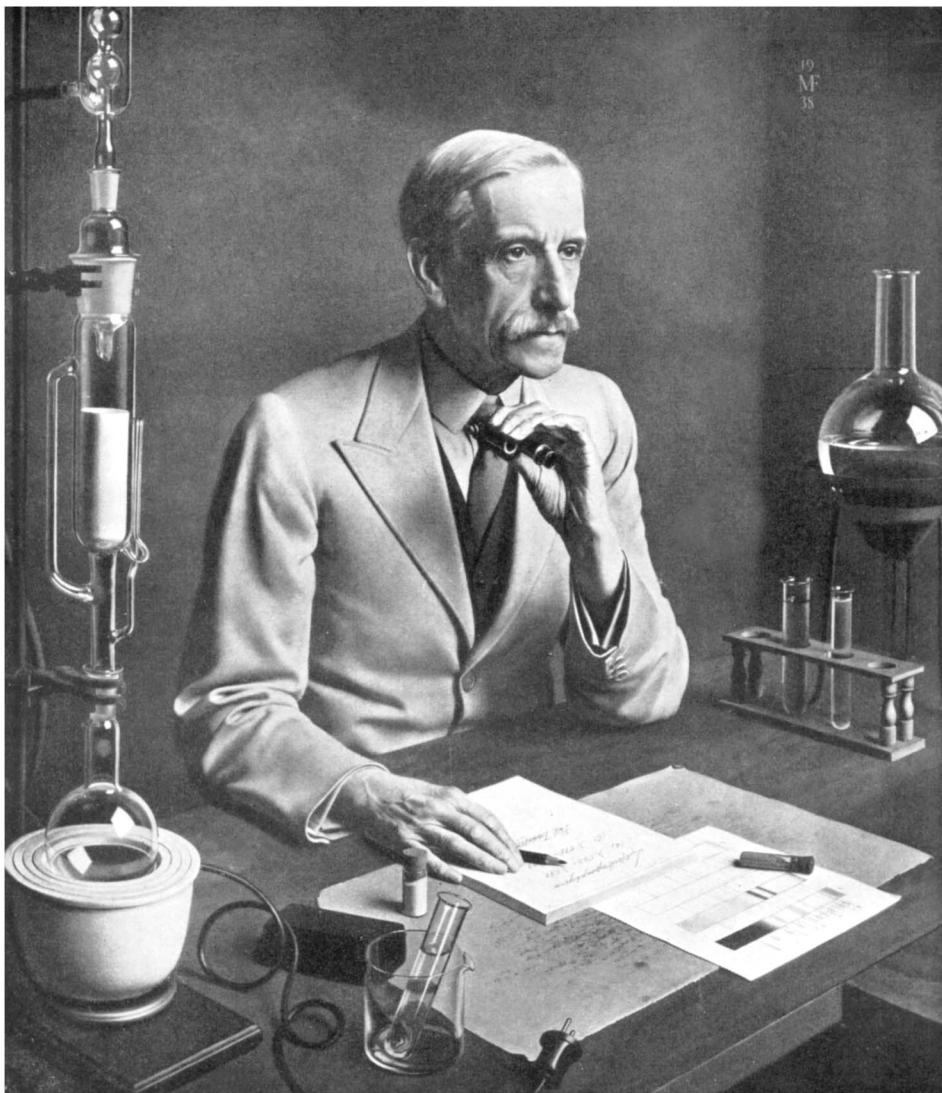
Before discussing Hopkins's scientific research, I wish briefly to refer to those parts of his history which bear upon this work. He was born in 1861, and at the age of 17 was articled to a public analyst, from whom he received his first technical training. In 1883, at the age of 22, he was appointed assistant to Sir Thomas Stevenson, the Home Office Analyst, with whom he stayed for about five years. Then at the age of 27 he became a medical student at Guy's Hospital, and he graduated at the University of London in both science and medicine in 1894. In 1898, when Hopkins was 37, Sir Michael Foster invited him to join the Physiological Department at Cambridge as a University Lecturer, in order to develop the subject of chemical physiology. From 1905 to 1910 he was tutor of Emmanuel College. While he was now in a better financial position, his duties as a college tutor and university lecturer were exacting and must have interfered with his research. In 1910 his pleasure was great when Trinity College offered him a fellowship and appointed him Praelector in Physiological Chemistry, so that he became free from all routine teaching work. In the following year he was made university reader in chemical physiology. In 1914 a special chair of biochemistry was created for him, and a separate biochemical department was formed. In 1921 a large bequest from the Trustees of Sir William Dunn made it possible to provide him with a separate building and staff for the new department. This building was completed and occupied in 1924, and Hopkins immediately started work with a large research staff (of about forty) in this year. It is worthy of mention that before Hopkins got a proper university laboratory and department at Cambridge he had arrived at the age of 63. He continued as professor of biochemistry until 1943, and retired after having held the chair for thirty years. The points, I think, to remember in Hopkins's earlier career are first, that there was practically nothing academic about his original training, except the medical course at Guy's Hospital, and secondly, the great preparation he underwent in first learning the profession of chemical analyst and then following this up by becoming medically qualified. Little wonder that he did not get properly going in his research life until he was approaching the age of 40. On the other hand, it is equally without wonder that a man of his mental calibre and other intellectual qualities having had such a magnificent training should have proved to be one of the most outstanding scientists the country has ever seen.

Hopkins's first entry into the field of research occurred at the age of 17, when he sent the following note to *The Entomologist* for November 1878 :

"*Brachinus crepitans*.—I have observed that the little bombardier beetle has been exceedingly plentiful this year, and I feel interested to know if this has been the experience of others. I caught my first specimen in March, and this was the first I had ever seen here; since then, and till quite lately, they have appeared in great numbers. On the South Downs, near Eastbourne, I also saw several of these insects, though I have no recollection of having observed them there before. Altogether *Brachinus* seems to have been an exception to the general scarcity of his order this year. It is a very sociable insect, and I have seldom seen one without finding others close by. The beetles are very partial to my sugar compound, and have swarmed on trees prepared for moths. *Cotias edusa* has quite disappeared from here this year.—F. G. Hopkins."

Fifty-seven years later the *London Naturalist* for 1935 contains the address that Hopkins gave on his election as Honorary President of the London Natural History Society in succession to Lord Grey of Fallodon. In the course of this address he refers again to his original observations on *Brachinus crepitans* :

"I will here venture, hoping for your forbearance, to intrude a fragment of personal history into my remarks. Like Bacot himself and, I suspect, like very many of my audience, I was in my early days an ardent collector of butterflies and moths and (next easiest, I think, for the boy or amateur) of beetles. It was one day in March of the year 1878—it is just 57 years ago—that I first made, with infinite pleasure, the acquaintance of the little bombardier beetle, *Brachinus crepitans*, if that be still its accepted scientific designation. It was plentiful that year and I found it in a northern suburb of London and on the South Downs near Eastbourne. Now, when one day I beheld, without previous knowledge of their abilities, how these insects on being disturbed eject a violet vapour into the air, a most effective act for offence or defence, I felt an intense curiosity to know not only how this volatile stuff could be made and stored but in particular what the stuff could be. I tried a few experiments, putting one bombardier after another into the same test tube, and encouraging each one to shoot. The vapour condensed on the side of the covered tube and I thus collected a little of the material. It was very little, however, and I was a youth



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with no chemical training, so nothing came of my researches. I think, however, that from that time my fate was sealed. Though the designation was not yet invented, I became there and then a biochemist at heart."

Hopkins's next contribution came only after a gap of eleven years, namely in 1889, and, again, the subject concerned insects. He was now able to bring the specialist technique of the analyst to bear on the chemistry of the pigments of butterflies' wings. In the particular case he found that the yellow pigment present in the common English brimstone butterfly could be obtained in solution by simple treatment with hot water. It was not until 1896, however, that research on this subject was fully published by Hopkins in the *Philosophical Transactions of the Royal Society* under the title "Pigments of Pieridæ." It will be remembered that the results of this work were to show that the opaque white substance in the wings of Pieridæ is uric acid, while in the yellow insects the pigment is a related substance, probably identical with one obtained when uric acid is heated with water under pressure, the mycomelic acid of Hlasiwetz. In this work it is possible to see an example of what was a constant occurrence in all Hopkins's publications, namely the establishment not only of a fact or series of facts but also of some idea or suggestion which gave such facts a much wider interest and interpretation. In this particular case he dwells on the fact that in these coloured insects as well as in the white insects the pigmentary substance is actually excreted and that these are examples of normal excretory products being made to subserve the purposes of ornament.

It is a remarkable fact that in 1941, fifty-two years after his first publication on the chemistry of butterfly pigments, Hopkins published another paper on this subject entitled "A Contribution to the Chemistry of Pterins." He was constrained to return to this problem by the fact that Wieland and his colleagues had shown in a series of papers from 1925 to 1940 that these pigments were not uric acid derivatives but belonged to a new group of compounds known as pterins. There is some similarity between pterins and purines, in both their chemical constitution and properties. In recent years the pterins have become substances of great biological interest, one of the most important observations being that a pterin group is found in the folic acid molecule.

Hopkins's work on the chemistry of the pigments of butterfly wings caused him to extend his interest to uric acid problems in man, and in 1893 he published two papers describing a new method of determining uric acid in urine. Whatever developments may have taken place in methods of determining uric acid in later days, I can only say that Hopkins's method was in extensive use for many years after its discovery. This interest in the part played by uric acid in human metabolism he maintained for a good many years, and, even as late as 1898 and 1899, we find him publishing papers on the relation of uric acid excretion to diet. It may be added that all this work was a reflection of the great medical and public interest taken at that time in gout and its relation to uric acid formation. With the decline in incidence and indeed with the practical disappearance of gout from the country, the interest in uric acid has never again been so intense, although the mystery of the disease is still as great as ever.

During his time at Guy's Hospital he also produced a number of other papers, to which I shall just refer in passing, besides the one on the pigments of the Pieridæ and the uric acid publications. With Garrod he published a note on the excretion of hæmatoporphyrin in the urine of patients taking sulphonal, and two papers on urobilin. At this time, also, another chemical problem became apparent in his publications, namely the study of halogen derivatives of proteins. I shall not dwell on this work except to mention that one of these observations—the interaction of bromine with protein—became a further interest in his later work when isolating the amino-acid, tryptophan.

I have mentioned this series of publications for which Hopkins was responsible during the period of his medical qualification and of his early medical years, *i.e.* 1889—1898, in order to show that, although he continued in vigorous research activity and although his work still retained a certain distinctive superiority, it is perhaps questionable whether, if he had remained in this environment, his research would have reached the same high-water mark as in fact it did after he arrived at Cambridge. My own view is that the best thing that happened to Hopkins, both from his own standpoint and from that of the scientific world, was his departure from the medical atmosphere of Guy's Hospital in 1898 to the Physiology Laboratory at Cambridge. Cambridge, and especially that Laboratory, had a great deal to give as well as to receive.

Hopkins had not been long in Cambridge when he got the stimulus which led to his first research of classical importance and which, indeed, brought him at one stride to the forefront of physiological chemists. In a Part II practical class in physiology, one of the tests made by

the students was that known as the Adamkiewicz colour reaction for proteins (proteids, as they were called in those days). Hopkins was scornfully reminded by the class that this test was useless, as indeed it was in this instance, and he felt impelled to find out the cause of failure. It will be remembered that he soon found that the colour reaction was not due to acetic acid itself but to an impurity, glyoxylic acid, and the test is now called the glyoxylic test for protein.

He then proceeded with the assistance of Sydney Cole to apply his supreme analytical skill to discover what exactly it was in protein that gave the purple reaction with glyoxylic acid. Hopkins was always happy with a colour test when on the scent, and, indeed, if there were no colour test at hand, he usually proceeded to discover one. With the aid of the glyoxylic test he and Cole soon found the right reagent, namely mercuric sulphate in dilute sulphuric acid, for precipitating tryptophan from protein digests and separating it from the other amino-acids. The decision that this substance was the hypothetical compound tryptophan, which reacted with bromine water to give a rose-red colour, was an interesting and most important chapter of this work. (It will be remembered that Hopkins and Cole thought at first that this substance was skatoleaminoacetic acid and that it later turned out to be indoleaminopropionic acid.)

It is characteristic of Hopkins that he did not rest after this chemical triumph or simply pass on to another subject. His mind was characteristically directed at once to the meaning of this new chemical grouping in protein, and he wanted to know if it had some special duty to perform in the animal economy. With Edith Willcock, later Mrs. Stanley Gardner, he proceeded to do feeding experiments on young mice with synthetic diets of pure substances, and, by using zein made from maize as the sole source of protein, he obtained a tryptophan-free diet. Control mice he left on this diet and to the diet of other mice he added either tyrosine or tryptophan. Thus evidence was obtained that, although the tryptophan did not make these mice grow, it added considerably to their length of life and obviously played an important nutritional rôle in animal metabolism. This was one of the earliest experiments (1907) directed to showing the importance of quality of diet, and it formed one of the essential classical tests which brought this aspect of nutrition to the great prominence of later years.

One of the most important investigations in which Hopkins participated was that into the part played by lactic acid in muscle metabolism. This work was done in 1905—1907, and at this period I was closely associated with Hopkins and, indeed, shared with him the small room in which it was done. It will be remembered that W. M. Fletcher, who had previously worked on lactic acid in muscle, collaborated with Hopkins, and in my view he was responsible for inducing him to join in this further investigation. It would be wrong, however, if I left the impression that Fletcher was the major partner in this work. This was certainly not so, and I am quite sure that, not only as regards the ideas and philosophy behind the work but also as regards the actual labour involved in the investigation, Hopkins more than played his part. Many people would perhaps consider that this particular research of Hopkins did not occupy the major position in his scientific history that I consider is its due. It is true that, unlike most of his other work which was nearly always that of breaking new ground, this investigation on lactic acid was rather of the nature of development of a recognised problem. It was known or at least stated that lactic acid appeared in muscle under various conditions of activity, but the whole subject was a medley of contradiction. The merit of the Fletcher and Hopkins investigation was not only that, by careful, quantitative work, order was brought out of chaos, but, more important still, that the results were of such great interest and the issues, especially as set out in the publication, so important that they stimulated all manner of further research on muscle physiology, biochemistry, and biophysics, which continued for many years.

You may remember that one of the great difficulties met with early in this work was that, not only when muscle was stimulated to activity but even when it was treated in any way, mechanical or chemical, it responded by producing lactic acid. Nursing muscle by rest, and especially pampering it by surrounding it at the same time with an atmosphere of pure oxygen, caused the lactic acid to disappear or to be greatly reduced, but both the appearance and disappearance of this substance depended upon the tissue cells being alive. Thus Hopkins in this research was dealing with a chemical problem of actual living tissue, and it was this experience (the only one of its kind in his career, so far as I can remember) which helped him on to the road of what became his later, greatest interest—the chemical dynamics of the living cell.

The first problem in this work was to obtain lactic acid out of resting muscle without getting the back-kick of further production—a reaction which had spoiled much previous work on the subject. This was done by rapidly freezing and grinding the dissected muscle in cold alcohol which dissolved out the lactic acid present. It is unnecessary to give the detailed results of this work, but it may be useful to point out that the actual attempt that Fletcher and Hopkins

made to explain these strange results in terms of muscle function and metabolism as understood at that time is an interesting chapter in physiological chemistry. It was obviously impossible for them to make a serious interpretation of the results without a much fuller knowledge of other simultaneous chemical and physical changes of muscle, such as its glycogen content, oxygen intake, carbon dioxide production, and the energy and heat changes. The authors realised this and stated that their work and its discussion were only in the nature of a preliminary communication. A time came, however, in 1915 when the same authors, in giving the Croonian Lecture to the Royal Society, got the opportunity of discussing their results in the light of much further knowledge which had proceeded from research that their work had undoubtedly stimulated. They were prepared by this time to accept a wider framework in which to fit their lactic acid results—the Croonian Lecture, “The Respiratory Process in Muscle.”

By this time also A. V. Hill and Parnas, working independently, had studied the wider problems of muscle metabolism raised by the Fletcher and Hopkins lactic acid work. They had investigated and attempted to correlate such things as heat production, oxygen uptake, and carbon dioxide formation, together with lactic acid production and disappearance under different conditions. In the Croonian Lecture, Fletcher and Hopkins took up all these new facts and discussed them in a masterly way. Their general conclusion was that lactic acid was a major factor in the wider problem of energy production in the body from carbohydrate and in the special case of muscular contraction. The emphasis they placed on lactic acid in muscular contraction can be seen from the following quotation :

“In the evolution of muscle it would appear that advantage, so to speak, has been taken of this phase in carbohydrate degradation and that, by appropriate arrangement of the cell elements, the lactic acid before it leaves the tissue in its final combustion is assigned the particular position in which it can induce those tension changes upon which all the wonders of animal movement depend.”

Since 1915 an enormous number of facts, especially on the chemical side, have been obtained as regards metabolic changes accompanying muscular contraction, but we still do not know the answer to many of the essential problems. In recent years interest in lactic acid has tended to diminish. This is due in part to the discovery that muscle can show contractions for a limited time even in presence of iodoacetic acid, a substance which inhibits one of the dehydrogenases necessary for glycolysis and therefore prevents lactic acid formation. Moreover the discovery of creatine phosphate and, more recently, of the adenosine triphosphate-actomyosin system, has focused attention on these as being the factors more closely linked with muscular activity. The breakdown of carbohydrate is, of course, still believed to be necessary to restore energy to the system—to rewind the mechanism as it were—but even here, pyruvic acid rather than lactic acid seems to be the primary product. It is held that lactic acid can arise by reduction from the pyruvic acid, but only to a significant extent when oxygen is excluded or, *in vivo*, when the muscle is working so strenuously that the blood-flow cannot supply enough oxygen to maintain fully aerobic conditions

Although lactic acid tends to be regarded as of secondary importance in muscular contraction, the work of Hopkins and others on this acid was at any rate an essential step leading to the more recent discoveries. Moreover, the general technique which Hopkins used in his experiments on muscle has been a model for many other workers, not least his insistence on isolating the substance under investigation as a pure, analysable specimen.

In consequence of the influence on his further work, it is important to observe that in 1915 Hopkins was still greatly interested in lactic acid oxidation and regarded this, from the point of view of production of energy in the body, as one of the major problems. It will be seen in a moment that he placed acetoacetic acid, one of the products of fat metabolism, in the same category as lactic acid as holding an important biological secret from the point of view of oxidative change and the supply of energy, and it was this combined interest in oxidation and in these small molecules that brought about his entry into the great field of tissue oxidations.

Hopkins has been greatly honoured for his work on vitamins, and it may be expected that this work will be discussed at length in this lecture. I do not, however, propose to spend much time on it, partly because the work is already so well known. It can be said at once that the scientific world was ripe for the leadership and direction given by the Hopkins publication in 1912 of “The Importance of Accessory Food Factors in Normal Diets.” Hopkins himself often said to me in the years 1905—1907 that he thought the whole subject of nutrition was on the point of being revolutionised. Curiously enough, in spite of the lecture published in the *Analyst*

in 1906, which has become world-famous because in it he helped to foreshadow the importance of these accessory food factors, and, in spite of the fact that it was in this year that he published his work on the nutritional value of tryptophan, it never seemed to occur to him that these were the basis of the discoveries that were to revolutionise the science of nutrition; at least that is the impression left on my mind in looking back to those years. The fact is that this particular field of feeding young animals on synthetic diets of purified substances had been so long cultivated by previous workers, extending back to the work of Lunin in 1881, that a study of the literature leaves the actual new discoveries which were made by Hopkins in this work as less impressive than would be expected. It was rather his biological insight and his remarkable power of writing about the subject that impress all readers with the conviction that here we had at last a leader in physiological chemistry who had placed the subject in its proper, important perspective. In this work Hopkins's main facts were twofold: (1) That young rats on synthetic diets of purified substances consisting of protein, fat, carbohydrate, and salts will soon stop growing, but that if 2—3 c.c. of milk per rat per day are added to the diet growth will be resumed; if, on the other hand, young growing rats are on the diet plus the milk, and the milk is removed, then the rats soon stop growing. (2) That milk contains something which in minute quantities stimulates growth, and that this, in turn, causes an increase in appetite; on the contrary, cutting out the milk from the diet removed the growth stimulus, and cessation of growth then leads to a loss of appetite. It must be quite clear that, according to this work, it is not primarily the fact that the animals eat less that makes them grow less or that eating more makes them grow more, but the fact that the growth stimulus is present or absent and the appetite tends to adjust itself accordingly. There are times, it is pointed out, when the animals are eating much more than would be expected from their rate of growth and that on other occasions they are eating much less than would be expected. Another point emphasised is the quantitative aspect of the experiments.

The whole subject moved so rapidly from this time that little critical attention was paid to the detailed experimental facts as demonstrated by Hopkins. The growth curves that he showed in the publication were, however, reproduced in all parts of the world, but it is a matter of some interest to know that neither Hopkins himself nor anybody else was able to repeat the particular experiments—that is, the growth stimulus supplied by 2—3 c.c. of milk per rat per day. There was apparently some unknown condition in the experiments essential for their successful repetition (a state of affairs not uncommon in biological research). This fact, although of no real practical importance, because of the further development of the subject in America especially by Osborn and Mendel and also by MacCollum and his colleagues, greatly troubled Hopkins for many years, and it was only in 1945 that he returned to the subject and claimed to have found out the cause of the difficulty of repeating the early work. The secret apparently was that in the earlier work he used potato starch in his synthetic diet and this had led to a condition later known as refection. In refection, undigested starch grains collect in the cæcum and become a medium for growth of bacteria which produce water-soluble vitamins. It is possible under such conditions to get good growth in young rats without any additional vitamins. It was under such conditions that Hopkins was able to show the action of these small quantities of milk. The main point is that, in any case, this investigation of 1912 had a remarkable influence on research in this subject in all parts of the world.

Apart from the Croonian Lecture of 1915, there was an interval of nine years between the vitamin paper in 1912 and his next really important contribution on "An Autoxidisable Constituent of the Cell," published in 1921. The war was undoubtedly responsible for the interruption of Hopkins's research activities during this period, and from the point of view of scientific discovery this can only be regarded as a calamity. I wish, however, to call attention to his remarkable address in 1913 on "The Dynamic Side of Biochemistry," an address given as President of the Physiological Section to the British Association. Every biochemist or would-be biochemist ought to read this address.

By this time Hopkins had attained to his fullest stature as a biochemical philosopher, and it was his outlook on the chemical processes of living tissues which he then announced that bore such remarkable fruit in later years. I should like just to indicate some of the points of this discourse. In the first place he states his view that the raw material of metabolism is so prepared as to secure that it will be in the form of substances of small molecular weight. These views were of course much more striking then than they are now. Not only does he continue to discuss this point at length, but he deprecates the view of the physiologist that biological phenomena occur within a biogen or living molecule, or, in the case of muscle, inogen, where all directive power can be attributed in some vague sense to its quite special properties. Instead

of such vague directive powers, he points to the importance of the new idea of endo-enzymes as the universal agent of the cell, and in support of this view refers to Buchner's discovery of zymase and cell-free alcoholic fermentation, to the arginase of Kossel and Dakin, to Dakin's enzyme which converts pyruvic aldehyde into lactic acid, and to the "small army" of enzymes known to play a part in the breakdown of nucleic acid. He then proceeds to discuss the question as to whether so large a part of the chemical dynamics of the cell, as comprising simple metaplastic reactions catalysed by independent specific enzymes, can be regarded as feasible, and fully accepts the implications.

When, after the war, he was able to get down to work again, the reality of the teachings of his 1913 lecture are obvious. In that lecture he advocated greater use of the direct method of attack to separate from tissues further examples of the simpler products of metabolic change, no matter how small the amount in which they may be present. Putting his faith, as usual, in a colour test, he began to practise this teaching by applying the nitroprusside reaction (in the modification developed by his pupil Rothera) in order to detect the presence of acetoacetic acid in tissues of animals which had been deprived of carbohydrate. You will remember that instead of finding this substance he immediately came up against the "philothion" of Rey-Pailhade. What was certainly the same substance had been shown to react to this colour test by Hefter and Arnold, and had become a subject of great interest to these workers. It was Hopkins's first main task to isolate this substance, which he later called glutathione. Here again he had an opportunity, which he seized with both hands, of showing his great analytical skill. In passing, we note that once more he made a substance of hypothetical interest, as in the case of tryptophan, into a tangible one of great practical and experimental importance and thereby opened up a new world of discovery.

Turning now to work on the problem of biological oxidations which received a great stimulus by the discovery and isolation of glutathione, Hopkins's actual entry into this field was heralded by the publication of two papers, one published with Morgan and Stewart in 1922 on xanthine oxidase, and the other, already referred to, on the isolation of glutathione. These two papers, I think, contain his most significant personal contribution to the literature of oxidations, not only because of the intrinsic importance of the discoveries described but also because of the tremendous influence they had on other workers both in his own department and throughout the world.

During the early 1920's, the study of biological oxidations was dominated by two rival and apparently incompatible theories. One theory, associated mainly with the names of Wieland and Thunberg, explained the oxidative breakdown in the tissues of stable substances like lactic and succinic acids as being due to activation of pairs of hydrogen atoms (in reality activation of the substrate itself whereby hydrogen atoms are "loosened") by the agency of tissue enzymes called dehydrogenases. This theory rested mainly on the fact that such oxidations can take place in the complete absence of oxygen, provided some suitable alternative hydrogen-acceptor such as methylene-blue is present. The other theory, due to Warburg, was that biological oxidations are brought about by an iron-containing catalyst which activates oxygen. This view was based mainly on the activity of model catalysts containing iron deposited on charcoal, and on the inhibiting action of cyanide on the charcoal model and on most tissue oxidations, due it was suggested to its inactivating the catalytic iron.

This was the general background when Hopkins was carrying out his pioneer work on oxidations. He published his work on the discovery of glutathione in 1921. In this paper he mentions by the way that one of his reasons for following up the sulphur compounds of tissues was an attempt "to discover if vitamins were to be found among sulphur-containing compounds"—surely a striking example of pre-cognition, for the vitamins thiamine and biotin were isolated and proved to contain sulphur in the years 1926 and 1936 respectively. Hopkins showed that the new sulphur compound, glutathione (which later proved to be a tripeptide), could exist in a reduced, sulphhydryl (or thiol) form ($G\cdot SH$), and in an oxidised, disulphide form ($G\cdot S\cdot S\cdot G$), these two forms being interconvertible. He suggested that the function of glutathione within the tissues might be that of a catalyst, the disulphide acting as the hydrogen acceptor in being reduced, and then passing on the hydrogen to oxygen during its spontaneous reoxidation by oxygen. As he says, the substance would then be fairly spoken of as a co-enzyme, playing an important part in the chemical dynamics of the cell. This seems to have given the first hint that intermediate hydrogen transport might be a process proper to living tissues—a conception which is to-day fundamental to biological oxidation, being involved as it is in the action of catalysts such as cozymase and the flavoproteins.

There is no time to go into detail of his later work on glutathione—his experiments showing

that alternate oxidation and reduction of glutathione does actually take place in the tissues, that the liver of well-fed animals has a greater reducing power towards the substance than that of fasting animals, that the oxidised tripeptide can reversibly change the "fixed SH" groups of tissue proteins into the disulphide form, and that the SH groups are regenerated by reduced glutathione. The last discovery led to the idea that certain hydrolytic enzymes such as papain which are activated by sulphhydryl compounds are dependent for their activity upon the presence of SH groups in the protein of the enzyme. Hopkins investigated this question in the case of some of the dehydrogenases and showed that the activity of succinic dehydrogenase was dependent upon such SH groups, the enzyme being inactivated by oxidised glutathione and reactivated by reduced glutathione. Peters suggested that such SH groups might be part of the pyruvic acid oxidising enzyme, and this conception, coupled with Voegtlin's observations on the affinity of thiol compounds for arsenic, was the basis for the splendid researches of Peters and his colleagues leading to the development of British Anti-Lewisite ("BAL"), which might have played a vital part in the war, and is now, in peace, likely to be important in medicine—for example, in counteracting the toxic effects sometimes occurring during treatment with arsenic and other metals.

I believe it would be true to say that, as an intermediary tissue catalyst in the sense envisaged by Hopkins, glutathione has so far proved a disappointment. The work of Keilin indicates that a large proportion of the oxidation of the tissues occurs *via* the cytochrome system in which glutathione is apparently not required. Moreover, the specific activating effect of glutathione on the enzyme glyoxalase has lost some of its interest now that methylglyoxal is no longer regarded as an important intermediate in the breakdown of carbohydrate to lactic acid. It may be that the chief importance of glutathione lies in its power to keep the various sulphhydryl-containing enzymes in an active state. It may well be, too, that other important activities of glutathione remain to be discovered. Meanwhile the importance and fruitfulness of the ideas put forward by Hopkins as a result of his work on glutathione can hardly be exaggerated.

Referring for a moment to Hopkins's other early paper on oxidations—the one published with Morgan and Stewart in 1922 on the enzyme xanthine oxidase which oxidises the purines hypoxanthine and xanthine to uric acid—there is only time to mention that this paper, apart from the facts which it describes, is full of the seeds of ideas for further work on fundamental properties of enzymes. Many of these seeds fell on good ground, and as a result about a score of papers dealing with this enzyme were published during the succeeding years by members of Hopkins's department. Many of these papers throw much light on fundamental enzyme problems such as specificity of action, methods of enzyme purification, enzyme dynamics and the action of inhibitors, and so on. Moreover, the actual experimental technique used in most of these papers, as in much of the work on tissue oxidation in general, was that used by Hopkins in the 1922 paper, namely the Thunberg methylene-blue tube for anaerobic work side by side with the Barcroft differential manometer for aerobic experiments. Use of this double technique in 1924 led Fleisch in Cambridge and Szent-György in Groningen independently to suggest that both the Wieland and the Warburg mechanism were involved in most biological oxidations. The former controversy was finally resolved by Keilin's cytochrome work, involving dehydrogenation at one end of the chain of reactions and oxygen "activation" by the cytochrome oxidase at the other end.

To sum up, Hopkins, in his work on biological oxidation, as in his other work, constantly stresses the dynamic side of the problems, not merely *what* substances are at work, but *how* they work, and the phrases "cell dynamics" and "dynamic equilibrium" constantly recur in his papers and lectures. In spite of the wide range which his researches covered, and of the many new fields which his work opened up, there is an essential unity about his work. Here is just one example of the linking up of two different aspects of his work, the vitamins and tissue oxidations. Two of the most important oxidation catalysts of the hydrogen-carrier type, namely the pyridine dinucleotide cozymase and the *isoalloxazine* dinucleotides (in the form of flavoproteins) which function as carriers in the manner he first suggested, are now known to contain vitamins in their molecules. Presumably we need these vitamins (nicotinamide and riboflavin) in order to build up these carrier catalysts. Similarly, in a more recent paper he described experiments on the inter-relationships between the oxidation of glutathione and vitamin C.

One may think of Hopkins as working on a giant jigsaw puzzle in which, while fitting together sometimes one, and sometimes another, group of pieces, he constantly attempted to bring these different groups of pieces together in an endeavour to fit them into the master pattern. The measure of his greatness lies not merely in his own work and writings, but in the inspiration he

has been to countless workers in biochemistry who have spread all over the world, and who now continue building on the pattern as he left it.

Hopkins's own research, while it must impress everyone familiar with this subject as being a remarkable contribution to knowledge and one of revolutionary effect, is only a part of his enormous achievement in the world of science. How great his influence was can be seen in the fact that, at the beginning of the present century, there was literally no biochemistry and but little physiological chemistry in this country, whereas when he retired from his Chair at Cambridge I think it is not unreasonable to claim that British workers in biochemistry could look those of other countries in the face and at least claim equality with them. In 1900 physiological chemistry and biochemistry were almost a German monopoly: in that year the thirty-first volume of the *Zeitschrift für physiologische Chemie* was published. It was not until 1906 that the first volume of the *Biochemical Journal* came into existence. It is difficult to mention a single British biochemist as a contemporary of the great Continental workers who were active in the closing decades of last century, such, for instance, as Hofmeister, Kossel, Emil Fischer, Hoppe-Seyler, Hammarsten, and Salkowski, although, at that time, British physiologists were outstandingly good. Only with the appearance of workers like Hopkins, Dakin, Barger, and Harden did this subject get a proper start in this country. It is not my purpose to discuss any further this development of biochemistry in England, but it can at least be claimed that Hopkins and his school at Cambridge formed the backbone of this evolution.

It would also be easy to show Hopkins's great influence in biochemistry by referring to the large number of his pupils who have been elected to University chairs in this subject. Or again, much could be written of the splendid output of research of many of his pupils and assistants, both in this country and abroad, a large part of which has, naturally, been in the field of enzyme chemistry. An idea of the productivity in research of his pupils can be obtained from the volume "Perspectives in Biochemistry" (Cambridge, 1937) which was published in honour of his seventy-fifth birthday by a group of them, but this aspect of Hopkins's influence could be very greatly expanded. I am tempted to discuss at length some of this impressive work, but shall content myself with referring in passing to one only of these discoveries of a pupil, which at the present time is having very great repercussions. I mention it because it seems to me that such a discovery could only have come from one working in association with a biochemical leader whose views on the dynamics of cell chemistry, particularly in regard to enzyme action, were those of Hopkins.

It may be remembered that in 1928 Quastel in collaboration with Wooldridge, working on the assumption that an enzyme could be regarded as possessing an active centre whose groups were arranged in a definite configuration which determined its specificity, found that malonic acid inhibited the action of succinoxidase. This and other like instances led to the idea of *biochemical competition*: that analogues derived from a natural substrate by a slight change in constitution were similar enough in shape to the substrate to be loosely held by the enzyme at its active centre and yet did not fit sufficiently well to react further. These analogues, therefore, competed with the natural substrate for the enzyme surface, and, according to their concentration and affinity for the surface might occupy all or part of it, so slowing down or stopping the enzyme action. In 1941 this theory was extended by Fildes and Woods to explain the sulphanilamide action on bacteria. They postulated that *p*-aminobenzoic acid was essential for the metabolism of micro-organisms, and that sulphanilamide, owing to its structural similarity, competed with it for an enzyme surface. Another instance is the antagonism, demonstrated by Woolley, shown in animals by 3-acetylpyridine to its analogue nicotinic acid, and its power thereby to produce in animals a vitamin deficiency similar to pellagra. This kind of explanation may be expected to account for the pellagragenic action of maize when nicotinic acid is deficient. The same theory of biochemical competition has had many other wide extensions in recent years and will probably be found to be the basis of a large number of new chemotherapeutic, nutritional, and biological phenomena. Such a theory has the merit of explaining how substances may exert therapeutic, toxic, or other biological effects which are conditional on other circumstances and may be reversible, a kind of mechanism for which physiologists have long been looking. This subject can, I think, be regarded as a most important product of Hopkins's teaching and philosophy.

I am conscious that in this lecture I have omitted to deal with many of Hopkins's qualities and experiences which deserve mention. In other cases, I have possibly been, by commission or omission, unbalanced in my description; but whatever the failings may be, I hope I have succeeded in conveying my certain opinion that Hopkins was not only one of our greatest scientists but also a man of remarkable personality. Apart from the interference caused by the

years of the two wars, which was disastrous to his proper work, it can be said that his research investigations were carried out almost to capacity. He was not only blessed with a long, active life, but throughout his life he had extremely good health, perfect home life, and for many years conditions which allowed the fullest employment of his skill. To this extent all of us and indeed the whole world have profited, and the least we can do is to express our unbounded gratitude. I have not mentioned all the high honours that were conferred upon him, but I think everybody will agree that, whatever the honour conferred upon Hopkins, he himself honoured by accepting.

There is one final suggestion I should like to make. In the course of time some form of memorial will undoubtedly be established in his honour. It seems to me most important that it should take a form which will ensure that his spirit and his teachings will be carried on through successive generations of workers in biochemistry. This might well be attained by the foundation of a lectureship by the Chemical Society, possibly in conjunction with the Royal Society and the University of Cambridge, the main condition of the lectureship being that each lecturer should choose a part of Hopkins's own work for discourse in the light of knowledge as it has developed at that particular time.
