THE ISOLATION AND IDENTIFICATION OF VITAMIN C

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At the time when Barlow's activities were gradually impressing upon medical opinion the identity of infantile scurvy with the adult form of the disease and the fact that their common source lay in errors of diet, the science of nutrition was beginning to reveal the importance of the presence in some natural foods of small quantities of unknown substances essential to life (Lunin¹⁰), which were later to become known as vitamins. Neither the clinician nor the experimental investigator could then foresee the magnitude of the problem to which these observations were leading. The horizon, however, definitely widened when Grijns¹⁹, in continuation of Eijkman's work, suggested that the cause of beri-beri was a dietetic deficiency.

That scurvy was caused by the consumption of an incomplete diet was by then almost universally acknowledged and consequently the discovery that the origin of another disease could be traced to faulty nutrition was not unique. The method of approach, on the other hand, which led to the discovery of this 'deficiency disease' was distinctly novel and was to become instrumental in the progressive activity soon to follow in this field of research.

As far back as 1869, Forster, working in Voit's laboratory at Munich, was investigating the function of mineral constituents in the diet and published his results in 1873¹⁷. In the course of this work he administered to pigeons a diet consisting of caseinogen and starch previously purified so as to remove the inorganic constituents with the result that the experimental birds developed symptoms, including opisthotonus, which were characteristic of what is now recognized as avian polyneuritis. The onset of this condition Forster ascribed to a deficiency of salts, not an unexpected conclusion considering the object of the inquiry and the trend of nutritional research at that period. The attention of the experimenter was then mainly focussed on the mineral constituents of the diets with the object of demonstrating what is now taken for granted, namely, their indispensability. It was, in fact, research of this character which was gradually yielding results destined to lead later to the equally gradual formulation of the vitamin hypothesis. Some twenty years after Forster's description of his experiments, similar observations, this time to be correctly interpreted, were independently made by Eijkman^{12, 13, 14}. The prevalence of beri-beri amongst Europeans in the Dutch Indies impelled the Dutch Government to send out a commission in

1886 to inquire into the cause of this disease. Eijkman was one of the assistants of this commission and remained behind after its departure to pursue the inquiry. During his investigations an epidemic broke out amongst his experimental hens. The symptoms displayed were similar to those described by Forster. Eijkman, however, soon satisfied himself that these symptoms were of polyneuritic origin and he named the disease 'polyneuritis gallinarum.' Furthermore he was struck by the similarity of this disease with that of beri-beri in man which he was studying. With characteristic shrewdness, Eijkman instituted an inquiry into the origin of the epidemic. It was then revealed that during the time when the birds were suffering from polyneuritis they were fed on rejected boiled polished rice from the kitchen of the military hospital which the 'diener' of Eijkman's laboratory (under civil administration) utilized on grounds of economy. Fortunately the cook in the military hospital was replaced by one who was less favourably disposed to civilian activities and the perquisite was stopped. On the consequent re-introduction of unpolished rice the epidemic amongst the birds ceased. The cause of the epidemic was plainly suggested by this coincidence. Further work of Eijkman and his successors, and above all of Grijns¹⁹, showed first that the deficiency of the polished-rice diet was not due to the lack of inorganic constituents and secondly that human beri-beri also resulted from the one-sided consumption of decorticated rice from which a dietetic factor had been removed in the milling. Such striking observations could not remain dormant for long.

It is outside the scope of this paper to trace the numerous and interesting investigations on experimental polyneuritis and beri-beri that followed. The lines on which these researches were pursued made circumstances propitious for the discovery of the production of experimental scurvy and as is usual in human progress they found their man. Axel Holst^{31, 32} in an endeavour to find out whether polyneuritic diets were able to produce the disease in animals other than birds, observed that guinea pigs developed a pathological condition distinctly different from polyneuritis. This condition Holst and Frölich were able to identify with scurvy. Further point was lent to the problem when they found that the scorbutic symptoms failed to appear when the deficient diet was supplemented by antiscorbutic foods. Five years later they published³³ a full record of their brilliant experiments, which will always remain classical.

The results of this pioneer effort were not long in being put into practice. The provisioning of the troops in the great war, especially in certain zones, presented possibilities of 'deficiency disease' making its appearance. This necessitated the careful assessment of the vitamins in raw and treated food-stuffs. Holst and Frölich's work was extended with great rapidity and methods were improved and made quantitative in nature (Chick and Hume^{6, 7}). Moreover, laboratory observations were utilized and found to be of value in the actual prevention and cure of scurvy in the field. The ground was now prepared for the task of the biochemist.

Chemical studies.

It will be easier to appreciate fully the development of the chemistry of vitamin C by recalling the appearance of the problem in the

early stages of the inquiry. The only method of establishing the presence or absence of the vitamin was the biological method which, as is well known, is time consuming and often subject to untoward delays and complications. The choice of reagents that could be used in the chemical study of the vitamin was limited since toxicity had to be considered in connection with the testing. Negative results could not always be interpreted as such, because the inactivation of more or less concentrated preparations in the process of manipulation had to be considered owing to the labile nature of the vitamin. These difficulties were to a great extent overcome as the chemical properties were gradually revealed by patient research. In fact, the concentration of the active principle was only achieved in intermittent stages as its conditions of inactivation became known. Although the fractionation and the study of the chemical and physical properties occurred concurrently, for the sake of clarity the two branches of research will be treated separately and not in chronological order.

The citrus fruits were always considered as specifics against scurvy. This fact was confirmed by experiments showing the lemon and the orange to belong to the most potent natural sources known at the time. It was only comparatively recently that the mango (Perry and Zilva⁴²), paprika (Svirbely and Szent-Györgyi⁴⁹) and the anterior lobe of the pituitary (Gough and Zilva¹⁸) were discovered to be markedly more active. The lemon particularly appeared to lend itself to chemical work since the bulk of the solid matter in its juices, namely, the organic acids, was found to possess no antiscorbutic activity. The removal of this inactive residue yields in consequence a solution the potency of which can be raised by concentration at low temperature and pressure. Such preparations were found to be active when tested on animals and human beings (Harden and Zilva²¹, Harden, Still and Zilva²⁰, Zilva^{62, 64}). After removal of the acids from lemon juice it was shown that the sugar contained in the residue could be fermented without impairing the activity of the juice (Zilva⁶³, Lepkovsky, Hart, Hastings and Frazier³⁹). Alcohol precipitated further impurities after which precipitation with basic lead acetate yielded a very active fraction (Zilva⁶⁵). It is now known that these preparations sometimes approximated very closely indeed in activity to that of pure ascorbic acid, but were characterized by instability and, not being crystalline, offered little possibility of chemical identification. Lemon juice is a source from which the crystalline vitamin can be obtained only with the greatest difficulty. Later the process was modified by precipitating the active principle with neutral lead acetate at pH 7.2 (Zilva⁶⁷). Concentrates were also obtained by basic lead acetate precipitation from other plant juices such as swede juice (Zilva⁶⁶) and cabbage juice (Bezssonoff⁴).

Data which afforded some illuminating information on the chemical character of the antiscorbutic factor were obtained by means of diffusion experiments. Holst and Frölich had already proved that the vitamin could diffuse through a parchment membrane, which implied that the active principle was not a colloid or associated with anything in colloidal dispersion.

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A technique of differential dialysis devised by Brown⁵ offered further possibilities of studying this problem.

Brown's technique consisted in dialysing solutions through collodion thimbles without the application of pressure. By soaking the thimbles in alcohol of various strengths, different degrees of permeability were obtained. Thus a thimble soaked, say in 30 per cent. alcohol, allowed substances of lower molecular dimensions to pass through, whilst impeding the passage of larger molecules which could pass through a thimble soaked in stronger alcohol, such as 90 per cent. In other words a molecular sieve with meshes of varying sizes was obtained by regulating the strength of the alcohol in which the collodion was soaked. Applying this technique to the study of vitamin C (Zilva and Miura⁷³) it was found that membranes which permitted a free passage to substances of small molecular dimensions such as sodium chloride retained the antiscorbutic factor during a period of three to four days. Only thimbles through which semicollodial substances, such as dyes, passed allowed the vitamin to dialyse. In extending this investigation it was demonstrated (Connell and Zilva⁹) that the active principle diffused through membranes of somewhat lower permeability than that which permitted the passage of dyes, the size of the active molecule appeared to be not far removed from that of a hexose and further the rates of diffusion of the antiscorbutic factor, of the nitrogenous residue and of the reducing sugars were different.

These results indicated that vitamin C was in all probability nitrogen-free and that the size of the active molecule whether associated or free was that of a hexose. On the identification of the vitamin nine years later, it was found indeed to be a hexose derivative.

As mentioned above one of the greatest difficulties in the fractionation and purification of the vitamin was its lack of stability and it was therefore, of paramount importance to throw some light on the mechanism of this inactivation.

Holst and Frölich emphasized in their pioneer work the instability of vitamin C. They found, for instance, that certain vegetables (white cabbage, dandelion) lost much of their potency in the process of cooking. The juices were even more thermolabile and deteriorated quickly also at room temperature. Acid juices, on the other hand, were more stable both when heated and when stored. They demonstrated that this increase in stability was due to acidity since citric acid extracts of cabbage juice were markedly more stable than the untreated juice. Further work on the subject indicated that the loss in activity incurred during heating could not be due entirely to thermal degradation. Delf¹¹ observed that when swede and orange juices were heated at temperatures above 100° C. in a closed autoclave the loss of activity was much reduced. She, therefore, suggested that the rate of destruction of the vitamin was affected either directly by retarding oxidation or indirectly by the production of stabilizing bodies. That the former was the case was proved simultaneously by two workers as an indirect outcome of different investigations. Hess²⁶ and Hess and Unger²⁷ found that the addition of hydrogen peroxide to raw milk under conditions which prevented the growth of bacteria had a deleterious influence on the antiscorbutic activity of the milk, from which it was concluded that this destruction was due to oxidation especially as they also found that neutralized milk or tomato juice loses antiscorbutic value on shaking in air. The other line of evidence emerged from the study of the effect of ultraviolet light. It was found that when the ozone generated

by the lamp did not come in contact with the antiscorbutic solution the activity was hardly impaired (Zilva⁵⁸). On the other hand, contact with ozone even at room temperature or the passage of air for several hours destroyed the vitamin. When the decitrated lemon juice, used in these experiments, was boiled in an atmosphere of CO₂ for two hours no marked diminution in the antiscorbutic activity could be recorded (Zilva^{59, 60}). It, therefore, became clear that oxidation was the main factor concerned in the destruction of the antiscorbutic factor. Even heating decitrated lemon juice in an autoclave for one hour at 40 lb. pressure (143° C.) destroyed only about one-half of the vitamin (Zilva⁶⁰)—a loss most probably due not to thermal degradation but to the presence of very slight traces of oxygen in solution.

The destructive effect of oxygen on vitamin C having been established it became clear that this reaction was controlled by another factor, namely, the hydrogen-ion concentration. The observations of Holst and Frölich that the antiscorbutic activity of extracts disappeared in alkaline much quicker than in acid solution could now be co-ordinated with the oxidative process of inactivation. It was shown (Zilva⁶¹) that decitrated lemon juice when made $\frac{N}{20}$ alkaline and exposed to the air at room temperature lost about 80 per cent. of its potency in half an hour. On the other hand, when the same solution was kept in the absence of air no loss in activity took place. Moreover such drastic treatment as aspirating air through a boiling solution of lemon juice as acid as pH 2.2 greatly delayed the destruction of the active principle. These experiments, therefore, established the fact that the destructive oxidation of the vitamin was deterred in acid and greatly accelerated in alkaline solution. Other factors conducing to the oxidation of vitamin C will receive later reference.

Another characteristic property of active solutions was their power of reducing a number of reagents. Bezssonoff^{2, 3} found that vegetable extracts containing the vitamin reduced phosphomolybdotungstic acid (Folin reagent for phenols). Even concentrates from which many impurities had been removed were observed to reduce ammonical silver nitrate and to decolorize potassium permanganate in the cold (Zilva^{63, 65}). These results were so striking that it became evident that they occupied a pivotal position in the scheme of vitamin C research. An early attempt to correlate these two reactions with antiscorbutic activity (Connell and Zilva⁶) failed to establish a parallelism. Both these reagents, however, are reduced by a great number of substances and consequently this negative result could not be considered as final. A more specific reagent phenolindophenol* was eventually found which made it possible to determine conveniently the reducing capacity of antiscorbutically-potent substances with precision (Zilva⁶⁸). The reagent is decolorized by antiscorbutic solutions in the process of reduction and by this reaction the connection between reduction and vitamin activity was investigated. Two main features came to light in this inquiry. One was, that although a certain parallelism between antiscorbutic activity and reduction existed, there were, nevertheless, marked deviations. The second observation showed that when decitrated lemon juice was oxidized with indophenol and administered to the

* Substituted indophenols fulfil the same object.

experimental animals immediately, the major part of the vitamin activity was retained by the solution. If, on the other hand, the oxidized antiscorbutic was allowed to remain for about twenty-four hours before dosing it became almost inactive (Zilva⁶⁹). The writer's interpretation of his results was that vitamin C itself did not reduce indophenol but that the decolorization of the indicator was due to a reducing substance closely associated with the active principle, which tended to prevent oxidation.

About this time the hope of isolating the vitamin was realized but in a manner which had not been foreseen. Moreover, the antiscorbutic character of the substance thus isolated was not appreciated until some years later. Engaged in an investigation on the part played by the adrenal cortex in biological oxidation with the ultimate aim of elucidating its function in the renal system, Szent-Györgyi⁵⁰ isolated from the cortex a highly reducing hexose derivative—hexuronic acid. This compound he found also in vegetables and fruits where it appeared to function in connection with peroxidase systems, thus associating the adrenal cortex with this oxidizing mechanism. Amongst other observations he recorded that hexuronic acid decolorized indophenol—a fact which prompted him to suggest that the acid was probably identical with the reducing substance postulated by the writer (Zilva⁶⁸). Professor Szent-Györgyi kindly offered to supply the writer with hexuronic acid in order to test this suggestion experimentally, but unfortunately owing to the scarcity of the material at the time and his subsequent departure from England the matter was left in abeyance. However, another substance isolated from orange juice by Szent-Györgyi, which he thought might be the vitamin (private communication by letter) was in the meantime (June-July, 1928) tested by the writer at Professor Szent-Györgyi's request and was found to be antiscorbutically inactive in daily doses of 3 mgm. and 5 mgm. (cf. Svirebely and Szent-Györgyi⁴⁸).

Not many years elapsed before the chemical identity of vitamin C was established and, as in the case of its isolation, in a manner not expected. Tillmans*, in trying to devise a method for discriminating between natural lemon juice and the artificial product employed 2,6-dichloroindophenol, it being known that this compound was decolorized by orange juice, with the result that he found that only the fresh natural lemon juice reduced the indicator. In communicating this observation at a meeting in Nuremberg the point was raised whether this reducing capacity had any connection with vitamin C. On investigating this matter further he found⁵¹, as the writer had done before, a certain parallelism between the two properties, but unlike the writer, he was inclined to attribute the two properties to one substance. The writer's observation that decitrated lemon juice retained its antiscorbutic activity after oxidation, Tillmans maintained could be explained by assuming that the substance was reversibly oxidized whilst remaining antiscorbutically

* Whilst writing this review (February, 1935) news reached me of Professor Tillmans' death. I should like to take this opportunity of expressing my sincere admiration not only of his capacity but also of his truly scientific spirit,

active. In this first stage of oxidation, according to him, the vitamin was more prone than in the original reduced form to destruction by further oxidation. It is of interest to note that Szent-Györgyi⁵⁰ had found that hexuronic acid could also be reversibly oxidized but his work was not known to Tillmans in 1930. The similarity, however, between the reducing substance which Tillmans considered to be vitamin C and hexuronic acid was thus indicated then. Later (Tillmans et al.,^{52, 53, 54, 55, 56}) when he had worked out his thesis in greater detail and became acquainted with the work on hexuronic acid he actually suggested that vitamin C was probably identical with it. Very soon after the appearance of Tillmans' last series of publications, King and Waugh^{38, 57} claimed to have isolated a crystalline substance from lemon juice, by the decitration and lead acetate method mentioned previously, which, in their opinion, was hexuronic acid and which was antiscorbutically active. Simultaneously Szent-Györgyi^{47, 48} examined an authentic sample of crystalline hexuronic acid from the adrenal cortex and found it also potent.

The antiscorbutic activity of hexuronic acid could not, however, be taken as a final proof that this compound was identical with vitamin C (Zilva⁷²). Workers in the vitamin field were particularly reminiscent of precedents in which active crystalline substances owed the particular vitamin potency not to the crystalline compound but to adhering 'impurities.' A similar condition could be visualized in the case of hexuronic acid. Crucial evidence was nevertheless soon obtained which militated against this view. Szent-Györgyi⁴⁹ was able to show that the monoacetone derivative of ascorbic acid—under which name hexuronic acid became at this stage to be known—was antiscorbutically active but not to the full extent and that the ascorbic acid recovered from it was, on the other hand, fully active. Hirst and Zilva²⁸ found that when ascorbic acid was oxidized with iodine to its first oxidation product (dehydroascorbic acid) it was almost as active as it was before oxidation. This observation was similar to the one made by the writer⁶⁸ when the vitamin in decitrated lemon juice was oxidized with indophenol. By reducing the dehydroascorbic acid, ascorbic acid was re-obtained which was as active antiscorbutically as the original compound from which the oxidation product was prepared. These results strongly pointed to the fact that ascorbic acid was active per se. The alternative explanation that the vitamin was in association with ascorbic acid and could, like it, be reversibly oxidized and regenerated quantitatively was in the highest degree improbable. In view of these facts the writer's hypothesis that the reducing substance and the vitamin were not identical but in close association became untenable and Tillmans' theory was thus shown to be correct. The observation that synthetic ascorbic acid obtained from inactive material was fully active (Reichstein and Oppenhauer⁴⁵, Haworth, Hirst and Zilva²⁴) supplied the final and incontrovertible proof.

Structure of the vitamin.—As was to be expected a compound of such physiological significance as ascorbic acid could not fail to engage the atten-

tion of carbohydrate chemists. An intensive quest led to the establishment of its chemical structure and the eventual realization of its synthesis. Only the outstanding achievements can be given here in the barest outline.

Of the several structural formulae proposed the one which has found general acceptance is that shown below. This was put forward by Hirst and his collaborators of Birmingham, whose proof of this structural formula was arrived at by the ingenious application of various methods employed in the study of the structure of the carbohydrates²⁵.

A little later the same formula was suggested independently by Euler and Martius¹⁵, their suggestions being based on indirect analogy with the substance reductone ($\text{CHOH}:\text{COH}:\text{CHO}$), which resembles ascorbic acid in its property of reducing indophenol. The synthesis of ascorbic acid was soon accomplished independently by Reichstein of Zurich⁴⁵ and by Haworth, Hirst and their school at Birmingham²⁴ from *l*-xylosone by way of the hydrogen cyanide addition compound. As xylosone is difficult to prepare this method of synthesis could not conveniently be applied in the preparation of large quantities of ascorbic acid. New methods have been developed in which the starting material is *l*-sorbitol, a sugar which is now readily available in quantity. In Reichstein's method⁴⁴ sorbitol is converted into its acetone compound and thence into 2-keto-*l*-gulonic acid, from which *l*-ascorbic acid is easily obtained (compare Maurer and Schiedt's transformation of methyl 2-keto-*d*-gluconate into *d*-arabo-ascorbic acid⁴¹). In Haworth's method^{22, 23} the requisite 2-keto-*l*-gulonic acid is produced directly by simple oxidation of *l*-sorbitol. By such methods *l*-ascorbic acid can now be produced at an economic price.

The schemes employed in the synthesis of *l*-ascorbic acid were eventually utilized in the preparation of a number of analogues and derivatives, of which some such as *d*-arabo-ascorbic acid (Maurer and Schiedt⁴¹, Dalmer and Moll¹⁰), *l*-rhamno-ascorbic acid (Reichstein⁴³, Reichstein, Schwarz and Grüssner⁴⁶) and *l*-gluco-ascorbic acid (Reichstein⁴³) have been found to be partly potent, whilst others had no antiscorbutic activity. As a striking example of these latter isomers one may mention *d*-ascorbic acid, the enantiomorph of the fully active *l*-ascorbic acid.

Chemical and physical properties.

The availability of the pure compound made it possible to study its chemical and physical properties in greater detail. The characteristics of the vitamin previously established were fully confirmed. One feature, however, calls for comment. Pure crystalline *l*-ascorbic acid and the other active compounds are perfectly stable in solid condition, a fact which seems at first to be strikingly contradictory to the instability of vitamin C. This apparent paradox can nevertheless be explained. In studying the process of inactivation of the antiscorbutic factor it was found (Zilva^{69, 70, 71}, Johnson and Zilva³⁴) that certain phenolic substances, which are widespread in plant tissues, are subject to spontaneous oxidation giving rise to products capable of oxidizing in their turn the vitamin. In addition, Euler, Myrbäck and Larsson¹⁶ showed that traces of certain metals could catalyse the oxidation of ascorbic acid, an observation which explains why the pure compound becomes slowly inactivated in aqueous solution. Recent work throws further light on this subject. In water carefully distilled from and received in quartz apparatus, *l*-ascorbic acid is exceptionally stable even when this water is previously saturated with oxygen (Kellie and Zilva³⁶). This suggests that contact with oxygen in the absence of catalysts does not destroy the activity. The instability of the vitamin in natural juices or in concentrates when exposed to air is evidently due to the presence of various catalytic substances. That mere traces of these impurities may have a deleterious effect on the vitamin is seen from the fact mentioned above that concentrates were sometimes obtained by the writer which, weight for weight, were almost, if not as active, as crystalline ascorbic acid, but lacking the stability of the latter. The natural medium from which these preparations were made, namely, lemon juice, offered great technical difficulties in removing the adhering traces of these destructive impurities which imparted the instability to the vitamin in this condition.

It is significant that in the tissue of the living plant or animal biological conditions are such as to conduce to the stability of vitamin C. Only when the tissue is disintegrated is this balance upset. Since in the natural sequence of things the disintegration takes place through mastication as the food is consumed, no serious loss in antiscorbutic activity can take place before the ingested vitamin is utilized by the organism.

As in natural sources and in concentrates indophenol still remains the most specific reagent for pure *l*-ascorbic acid and its analogues. Nevertheless the specificity is far from being complete, so that this reagent is of doubtful value for detection and determination of these compounds. Since his introduction of this indicator in 1927 the writer had the opportunity of carrying out a critical study of this problem on a large scale, especially

between 1928 and 1933, during which time he and his colleagues were engaged in an extensive inquiry into the vitamin C of natural products. He arrived at the conviction that in spite of the excellent agreement between the biological and the indophenol titration values found in many instances, the latter procedure broke down in unexpected instances in a way which made the general application of the indicator in the assessment of antiscorbutic potency undesirable, particularly in the hands of the less cautious. For this reason he deliberately abstained from urging the utilization of this reaction only for the detection of the antiscorbutic factor. The same criticism may be levelled at the application of the selective absorption displayed by ascorbic acid in the ultraviolet region of the spectrum for the determination of vitamin C. The biological activity still remains the ultimate criterion for vitamin C. Among the biological methods the prophylactic method yields the most accurate results but is laborious. The curative method in which the test dose is administered to the animals from the tenth to fifteenth day on the basal diet until the thirtieth day when they are killed is almost as accurate and less laborious (cf. Johnson and Zilva³⁵). Another biological method—Höjer's method—is sometimes employed. This method is based on the fact that in guinea-pigs on a scorbutic diet there is an early change in the odontoblastic layer of the teeth (Zilva and Wells⁷⁴, Höjer^{29, 30}, Key and Elphick³⁷). The advantages claimed for this method, namely the small amount of material required, and the short duration of the test are possessed by the curative method which does not, on the other hand, share the disadvantages of the former. The chief of these are variability in the individual response of the experimental animals, the totally subjective interpretation of the results and the necessity of making histological sections.

In this review the chemical nature of vitamin C only has been briefly discussed. That this vital principle, the absence of which is responsible for the production of scurvy in animals incapable of synthesizing it, may play an important part in the metabolic functions of the animal organism needs little stressing. Nor is it likely that its synthesis and presence in the plant kingdom is without significance. A good deal of sound information bearing on these points which falls outside the aim of the present review has been already obtained in the course of study of the chemical nature of the antiscorbutic factor. The identification of the vitamin naturally quickened the pace of research in this domain, but it is not easy at the moment to assess the true value of many of these latter contributions in an excessively prolific field, especially as some results leave a debatable zone of possible error. Many gaps remain to be filled before a major hypothesis can be formulated. When the time, whether it be close or remote, is ripe for the integration of the essential and established facts in their true perspective, scientific workers might with advantage look back to Barlow's Bradshaw Lecture as a model for clarity of vision, logical exposition and, above all, intellectual probity.

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