

“THE LAW OF CONSTANTS AND THE LAW OF PRODUCT IN PHYSIOLOGY”—A CRITICAL NOTE.

BY R. S. INAMDAR.

Professor J. C. Bose has just produced another new book entitled “The Physiology of Photosynthesis” (By Sir Jagadish Chunder Bose, Longmans, Green & Co., 1924) which is a good study in methods. So far as the results are concerned, there are many things which one would like to say something about. But it seems hardly worthwhile since Professor Bose himself does not discuss the significance of his results from the point of view of the actual mechanism of the Physiological process nor has he cared to correlate his results with those obtained by other Physiologists working in the field for more than a quarter of a century.

But one cannot refrain from saying a few words about certain “laws” which he has formulated and which appear to be the main theme of his book. Professor Bose, being a “Physical Physiologist”, apparently wishes to see all the Physiological processes through the perspective of the well-established Gas Laws, for he says not only that the total rate of change with reference to any one external or internal factor influencing the rate, is proportional in a simple linear way to the intensity of that particular factor, but also that the total result obtained under the influence of many variables is equal to the product of the influence of all (chapters 26 and 27). While agreeing that the simple Gas Laws have been applied with success to various physical or quasi-chemical phenomena, such as solution, ionisation, osmotic pressure, etc., where the phenomena are connected with the Physical impacts of molecules or particles analogous to molecules, one cannot help remarking that the statement, made in this form without any discussion of the nature of the Physiological processes, completely ignores the Complexity of the Physiological reactions, *viz.*, the complex equilibria set up by various physico-chemical reactions taking place in heterogeneous media in the cell. So far as the assimilation of carbon is concerned, it is now well recognised that there are at least two phases, the photo-chemical and the chemical and it would indeed be surprising if the totality of the result of all these phenomena under the influence of a given factor or a combination of factors conformed to the simple laws of the kinetics of a gaseous

molecule. It may be that he is right in thinking so, but before carrying conviction he will have to apply his laws to the cell-physiology, taking into consideration the various reactions concerned in the process, the many reactants and their complex equilibria. He has also to discuss the significance of his results from the point of view of the law of limiting factors which has now been established for the influence of various factors on many Physiological processes. The book does not contain any discussion on these fundamental questions.

First his method of obtaining constant coefficient of activity for any one particular factor may be questioned. He obtains them in two ways, *viz.*, (1) by the differential method (chapter 5) and (2) by the direct "Physiological" method as he calls it (chapter 26). By the differential method he obtains the constant, K, by the formula

$$K = \frac{Aa - Ab}{a - b}, \text{ where } A \text{ is the Physiological activity at the given intensities and } a \text{ \& } b \text{ the different intensities of the factor.}$$

This "constant" varies, according to his own figures, from the lowest to the highest intensity of every factor he has used, CO₂ concentration, light intensity and temperature. Thus, for example, taking his results of the assimilatory activity under varying concentrations of CO₂ obtained for specimen 1 on p. 116, his K will be found to vary from 37.6 for the first two values to 40.2 for the 2nd and the 3rd, 45.25 for the 3rd and the 4th and 2.03 in the last pair of values on a decreasing scale later on. Yet he takes K as a "constant", 39.27, calculating the result from the 1st and the 3rd values. This "constant" he compares with similar "constants" obtained for other specimens in the same way. Nor does he give any adequate reason for this careful choice of the values except that he wants to take the middle values when the curve is nearly in a straight line (p. 37.) It is true that Physicists are in the habit of choosing the middle values as they are least likely to be influenced by deviations, but then one asks for the probable Physiological explanation for the deviations from the normal. So long as this explanation is not forthcoming, the law cannot be accepted as having even a limited application.

His second method is very ingenuous. He produces the middle part of the curve relating to the assimilatory activity with reference to any one factor, back to the abscissa line, and the point where it strikes the line is termed by him the "Physiological Zero". He marks the further intensities for the factor from this point as zero and he calls the scale thus obtained the "Physiological Scale" of measurement, which he compares with the absolute scale of measurement of temperature. From the values of CO₂ concentration, temperature and light intensity thus obtained on the "Physiological Scale", he

determines the constant of coefficients by the simple formula, $K = \frac{A}{a}$, where K is the coefficient, A the assimilatory activity and a the intensity of the factor on the "Physiological scale". The coefficients thus obtained of course agree again only in the middle portion of the curve so that it is an argument in a circle. All the other results are rejected by him. Further, one cannot help questioning his way of obtaining the absolute "Physiological Zero" also. Why produce only the middle portion of the curve back? Is there any satisfactory Physiological explanation to it at all? To this he would probably reply that he has independently found that, at that particular intensity of the factor which is represented by the 'Physiological Zero', the photosynthetic activity becomes arrested, (cf. p. 244, last sentence). We shall revert to this question again below.

Lastly, it is very surprising that, with all these variable "constants" for different factors, he should have been able to obtain, in chapter 27, rather a very close agreement between the observed values and the values calculated according to his law of product, which is a multiplication of the partial effects of each factor. Obviously he has again chosen only those intensities of different factors for his experiments on the effects of two or more variables, which conform to the views put forward by him. It is entirely on these results that he bases his "Law of Product". And he hopes that "The introduction of measurement on the Physiological scale and the establishment of the Law of Product will lead to as great an advance in Plant Physiology as the introduction of absolute measurement has accomplished in Physical Science"! (Concluding sentence at the end of chapter 28).

It is possible to prove almost anything by an ingenuous handling of figures but one asks for the probable scientific explanation of the truth of the view put forward. What is the fundamental significance of his "Physiological Zero"? Apparently he compares it with the zero on the absolute scale of measurement of Temperature. The real idea underlying the conception of the absolute zero is that, provided the matter remains in the perfect gaseous state at that temperature, the volume will have shrunk to nothing. Does Professor Bose suggest that there is a possibility of an absolute arrest of the assimilatory activity at the actual reacting surface, the chloroplast, even when CO_2 concentration, light intensity or temperature are supplied in certain quantities represented by his "Physiological Zeros", or as to a matter of that, in any quantities whatever, so long as the reacting surface remains unchanged? This view is of course untenable whatever the "tonic" condition of the chloroplast so long as it can react at all. Professor Bose might say that the absolute zero is a hypothetical

abstraction first because—273°C temperature has not been reached and secondly because no gas remains in the perfect gaseous state at that temperature—not even the permanent gases. But then there is the satisfactory explanation that a substance whose volume is to be measured at the absolute zero changes its properties meanwhile, either due to complications mentioned by Van der Waals and Dieterici or due to liquifaction. Applying this idea to the Physiological Zero, does Professor Bose suggest that the reacting complex in assimilation gets completely disorganised under lower intensities of external factors; or does he mean that it changes its fundamental properties, suddenly or gradually, when CO₂, light and temperature are supplied, separately or together, in certain quantities, with the result that we are never in a position to mark the absolute arrest of assimilatory activity at the actual reacting surface, which might have occurred had the assimilating complex remained unchanged? If these views are untenable, Professor Bose's "Physiological Zero" has no real physiological significance. It may merely mean that in his experiments and for the particular specimen he has chosen, the reacting chloroplast surface was not able to obtain CO₂, light intensity and heat, in sufficient quantities to give an appreciable evolution of O₂, when these factors were supplied externally in minimal quantities represented by "Physiological Zeros". His own results confirm this idea, for he says on p. 249 that there was incipient photosynthesis at a minimum intensity of light though there was hardly any collection of gas in the bubbler, indicating thereby that the rate of activity slows down gradually. Further, the differences which he has noted (in specimens in different 'Tonic conditions') in the "minimally effective intensity" (p. 37) of a factor to give an appreciable evolution of O₂, may, in all probability, relate merely to differences in the relative *rates* of reaction at the *reacting surface* and not to its absolute arrest when the intensity was below the given "minimum." One can also readily believe that, when no CO₂ and light reaches the chloroplast surface, there will be an absolute arrest of assimilatory activity, for then two of the requisites of the reaction are entirely wanting. But there is nothing startling or new about it and one fails to see how it can bring about as great a revolution in the Physiological Science as the introduction of the absolute scale of measurement has accomplished in Physical Science. One cannot, however, believe that theoretically there is any possibility of an absolute arrest of assimilatory activity with reference to temperature under any intensity, all other factors being equal. For we believe to-day that all Chemical reactions are going on at a slow pace at lower temperatures and that they assume significant proportions only when higher temperatures are reached. Is the Professor inclined to

deny this? Or does he suggest that the assimilatory activity has nothing to do with the chemical phenomena? One fails to see what else he means by his "hypothetical abstraction" of "Physiological Zero". As a matter of fact, the book produces an impression that he has fixed his attention entirely on the gross totality of the results obtained and not on the dynamics of the actual reacting complex.

When the fundamental significance of his "Physiological Zero" cannot be satisfactorily demonstrated, his statement that the coefficient of activity bears a simple linear ratio to the intensity of a given factor is also open to criticism. It may be that this statement holds true for the relation of assimilation to CO_2 concentration. But one cannot be so sure of the other two factors, light and temperature. Especially with regard to temperature, it has been repeatedly shown in Respiration not only that the coefficient does not follow a linear ratio but also that it goes on changing as the temperature rises, even within a small range of temperature which marks the limits of life. Van't Hoff's temperature coefficients also are found to decrease as the temperature rises but the range of temperature required for a reduction in the coefficient is comparatively large. So far, the Physiologists have not been able to offer a satisfactory explanation to this rapid reduction in the temperature coefficient. The only plausible explanation that suggests to oneself is that temperature influences not merely the rate of chemical activity in the cell but also brings about variation in the nature or quantity of the reacting complex itself, disturbing thus the complex equilibria and as a result, the total rate also. When the problems are so complex, Professor Bose's position that the coefficient of temperature bears a simple linear ratio to the intensity of temperature becomes hardly tenable.

His statement that the Limiting Maximum is relative and not absolute, (P. 43) requires also qualification. By this statement Professor Bose apparently means that two external factors might limit the rate of a Physiological process simultaneously which is not an impossibility even according to the law of limiting factors. If, for instance, light and temperature do not influence the photo-chemical and the chemical phases of the reaction separately but both together act upon the chemical phase alone in a similar way, as is postulated by the "Radiation Hypothesis" of chemical reactivity, an increase either in the light intensity or the temperature will bring about a simultaneous increase in the rate even when the other factor is limiting. It will then be analogous to an increase in the transpiratory activity by an increase in the wind velocity even when temperature or humidity is limiting the rate. Similarly taking the CO_2 and the temperature combination when light intensity is kept maximal, an in-

crease in the temperature even when CO_2 is limiting is likely to increase the rate of assimilation purely in a physical way by inducing greater molecular activity of CO_2 . Further, one has also to bear in mind that the external factors might induce variation in the nature or quantity of the reacting complex itself. But all such apparent "anomalies" have no real significance in the dynamics of metabolism, since they are all variations of one and the same law. The fundamental axiom of the law of limiting factors lays down that "when a rate of process is conditioned as to its rapidity by a number of separate factors, the rapidity of the process is limited by the pace of the 'slowest factor'". Anything that alters the *nature* or the *pace* of the slowest factor, will naturally alter the rapidity of the process.

One has therefore no objection in toto to the statement that the limiting maximum is relative and not absolute as judged by the gross totality of the rate. But his results are quite contradictory to those obtained by others in the field. When CO_2 concentration was limiting, an increase in the light intensity increased the rate of reaction and a similar result was obtained for the combinations of temperature and CO_2 , temperature and light, and for every other combination of more than two factors. Miss Matthaei (Phil. Trans. Roy. Soc. London., B., Vol. 197., 1904; pp 47—105.) changed the temperature and the light simultaneously keeping CO_2 at maximal value and found no increase in the assimilatory rate by an increase in the temperature when light was limiting. Experiments have also been carried out by the present reviewer keeping temperature at maximal value and varying light and CO_2 simultaneously (results unpublished). There is nothing to suggest that the results confirm Professor Bose's results in this respect. Under these conditions one begins to wonder whether the differences may not be due merely to physical causes. Professor Bose's experiments are open to criticism in this respect. In his experiments there is a unilateral light falling upon the leaves which do not face the light, the twig with its whorl of leaves hanging vertically downwards in the assimilatory chamber. Under these conditions there will not be a uniform illumination of all the Chlorophyll granules even with the glass mirror which he sometimes fixes behind the vessel. Secondly, he does not describe any stirring arrangement in his assimilatory chamber and he also uses a low concentration of CO_2 for his experiments on the effect of two or more variables, *viz.* from 3 to an increase of 7 mgrms of CO_2 per 100cc. It is well known that hydrodiffusion is very slow and there is a suspicion that, under the lower concentration of 3 mgms per 100cc, the CO_2 is not in a position to reach all the Chlorophyll granules, a fact which is borne out by his own results that there was no appreciable

assimilation when the concentration of CO_2 was 1.2 mgms per 100cc. This he calls his "Physiological Zero" which may be nothing but merely the inability of the chloroplast to obtain CO_2 due to the slow process of hydrodiffusion. Lastly, he changes his temperature comparatively rapidly, though in steps, and as his experiments last only for a short period of 3 to 5 minutes, one is not certain whether the thermal equilibrium is reached by all the parts of the leaves. He says that he has measured only the temperature of the water in the chamber but there is the leaf tissue itself which takes some time before attaining equilibrium with the temperature of the environment. With these experimental conditions, an increase in the intensity of any of the 3 factors will have the influence of throwing in, so to say, more chlorophyll granules into action and the increase of assimilatory activity thus obtained will have no physiological significance except that the reacting mass has increased. Here again Professor Bose seems to have fixed his attention on the gross totality of the results and not on the actual mechanism of the process; and one merely hopes that he will repeat his experiments to make sure that these defects have not crept in.

His laws and conclusions apart, some of his results do confirm what has been observed by others. For instance, it is well-known that the anaesthetics increase the Physiological activities in minute doses and later bring about a depression. Professor Bose has been able to confirm this phenomenon for carbon assimilation also. His results on the capacity of the leaves to assimilate organic acids are also interesting and confirmatory. In a few places an attempt is made to quote at least the results obtained by others, for instance, on p. 155, in connection with the relation of CO_2 concentration to assimilation. But in no place is an attempt made to discuss the significance of the results from the point of view of the mechanism of the assimilatory process.

The choice of the title of the book is most unhappy. For we do not know whether the process is photosynthesis or photolysis—more likely the latter—and it is better to adopt the non-committing term, "Assimilation of carbon".

The special value of the book lies, however, in the many delicate physical appliances which he has been able to devise with the aid of the huge workshop facilities he has at his disposal. But they are unsuitable for investigation of assimilation in land plants for which we have to fall back on the "Laborious methods" which he deplures.