CONDITION OF CHLOROPHYLL IN THE LEAF[1]

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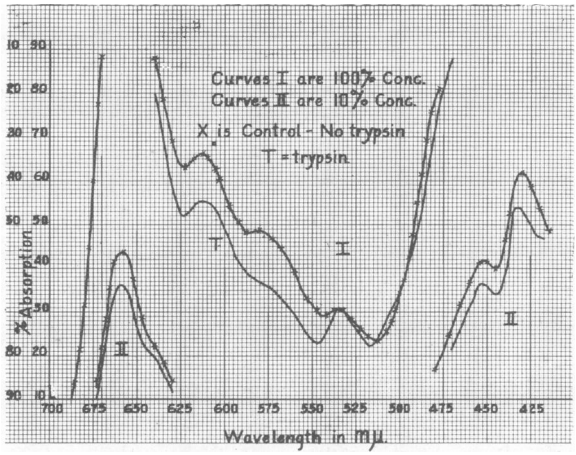
(WITH TWO FIGURES)

Extensive study of the microscopic structure of chloroplasts has been carried out by many workers. While this has given us considerable information about the probable nature of the condition of the chlorophyll pigments in the chloroplast there is still much to be explained.

The present experiments were undertaken for the purpose of investigating the physical and chemical state of the chlorophylls in the leaf.

Trifolium repens leaves were ground with sand, extracted with buffer solution, filtered, and centrifuged so that only the colloidal material was retained as the triturate. Using this triturate it is easy to recognize when the chlorophylls are losing the magnesium from the molecule: first, by the proportional increase of the intensity of the 534 mμ absorption band (ether as solvent) as compared with the other bands; and later, by the shift of the 662 mμ band toward the red end and appearance of a typical pheophytin spectrum.

Figure 1[2] shows the effect of adding 0.15 gm. of a crude trypsin (Eimer



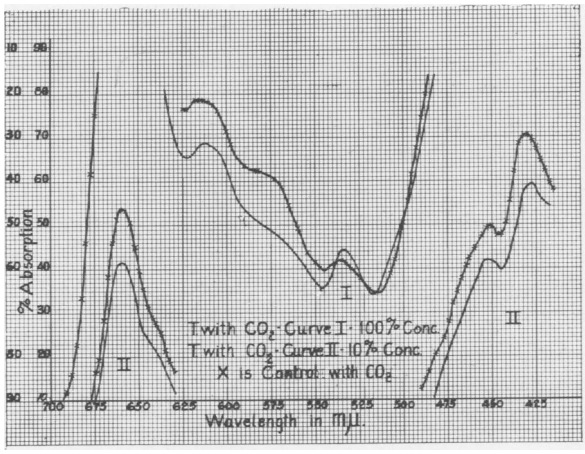
and Amend B 54) to 75 ml. of triturate and letting stand for 12 hours at 25° C. After the trypsin acted, the triturate was extracted with acetone and the chlorophylls transferred to ether by means of water. It is clear that the intensity of the 534 mμ band is proportionately greater with the use of trypsin.

[1] From the C. F. KETTERING Foundation for the Study of Chlorophyll and Photosynthesis.

[2] The curves for figures 1 and 2 were made by Dr. V. M. ALBERS (ALBERS, V. M. A recording photoelectric spectroradiometer. Jour. Opt. Soc. Am. 28: 121–123. 1938.).

The use of 0.0375 gm. and 0.075 gm. of trypsin clearly indicated that the degree of intensity of the 534 mμ absorption band was dependent upon the amount of trypsin added if the time, temperature, pH, and the amount of the triturate remained constant.

Figure 2 shows the same experiment except that carbon dioxide was



bubbled through the triturates one hour before the chlorophylls were extracted. It is evident that the 534 mμ absorption band in the triturate containing trypsin is proportionately more intense than the control after the passage of carbon dioxide into the triturates. The pH of the triturates at the beginning of the experiment was 5.6 (measured with a glass electrode) and remained about 5.6 in both the control and the one to which trypsin had been added. After bubbling carbon dioxide through the control and the trypsin-containing triturate the pH was 5.0. An attempt was made to bring about the same increase in intensity of the 534 mμ band in the control by bubbling carbon dioxide through the triturate three hours. This further addition of carbon dioxide from a tank did not produce detectable change in the intensity of the 534 mμ band or bring the pH lower than 5. These experiments were repeated with NORTHRUP'S crystalline trypsin and gave the same kind of results. Similar results were also obtained when triturates with pH 6.8 were used as starting material. In this case the pH after adding trypsin and without trypsin was 6.4, and yet the 534 mμ absorption band became more intense in the triturate where trypsin was used.

It seems fair to conclude from these experiments that trypsin causes the Mg to be freed from the chlorophyll molecules with the formation of pheophytin, and makes the chlorophyll molecules of the triturate more susceptible to the diplacement of the Mg by means of the addition of carbon dioxide. It is also clear that it is not just a matter of the change in the pH of the triturate

since that would naturally form pheophytin if kept too far on the acid side of neutrality for a period of time such as twelve hours at 25° C. It must also be clearly understood that no pheophytin formation has been observed when the triturate is definitely alkaline.

One might propose several explanations for these reactions. It appears that the action of trypsin on the substrate, to which the chlorophylls must be closely bound by adsorption or chemical combination, is acted on by trypsin, and the hydrogen-ion concentration of some allied substance is so altered that pheophytin begins to form; yet no lower pH is recorded. The most obvious substrate to be thought of is a protein. If this is true it may be a good assumption to consider the chlorophyll pigments as being bound to their substrate through a Mg-protein linkage. There is, however, another assumption which is being tested: will the trypsin not hasten the release of Mg from pure colloidal chlorophyll by acting directly on the magnesium-nitrogen linkages in the chlorophyll molecule? If this were true, then the assumption of a linkage between chlorophyll and a substrate native protein would not be so obvious.

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