26 per cent., av. 15; crude protein 5 to 26, av. 10; uronic anhydride 9 to 37, av. 23; pentosan 6 to 19, av. 12; "true" starch 27 to 62, av. 39; total determinations 89 to 150,[3] av. 100. The individual values have been computed to percentages of their respective samples of wood and the "true" starch compared with the usual expression for this factor. These results appear in table I, wherein successive pairs of samples represent differences in either cultural or developmental conditions which might be expected to entail a lesser starch content in the second sample. It may be seen that the "true" starch content exhibits wider ratios than the conventional starch values, in relation to tissue conditions, while the other carbohydrate constituents vary irregularly.

A similar examination of the hemicellulose extract resulted as follows: ash 9 to 17 per cent., av. 13; crude protein 1 to 4, av. 3; uronic anhydride 12 to 27, av. 21; pentosan 19 to 38, av. 25; "true" glucosan 1 to 34, av. 15; total determinations 65 to 92, av. 77. As computed to percentage of the tissue, the results are presented in table II. From these it appears that no consistent correlation exists between any or all of the carbohydrate constituents and the cultural history of the tissue. Furthermore, the "true" glucosan content varied independently of the "true" starch content. In these respects the hemicellulose fraction seems to be less definitely and directly related to plant performance than is the starch fraction.

It is recognized that the element of assumption is retained in the present treatment. Moreover, the results with one sample indicate that the compensations here applied are not generally applicable without either modification or reservation. Nevertheless the data appear to be more informative than the conventional values. From the quantitative aspect, starch was decidedly more prominent than the uronic acid constituents extracted with it; and the accompanying quantities of pentoses were rather insignificant. In the hemicellulose extract, on the other hand, the lead in proportion of extractives rotated among the constituents in question.—HENRY OTTERSON AND W. E. TOTTINGHAM, *Department of Agricultural Chemistry, University of Wisconsin.*

DETECTION AND ESTIMATION OF FORMALDEHYDE WITHIN THE CELL OF A GREEN PLANT BY THE ALLISON APPARATUS

(WITH ONE FIGURE)

Most theories consider formaldehyde the first or at least an early step in the formation of sugars from carbon dioxide and water by the green

[3] Only one sample approached this departure, the next highest recovery being 114 per cent.

plant. The demonstration and estimation of this compound within the plant cell is therefore of great importance.

The inadequacy of the usual chemical methods is well presented by SPOEHR[1] and until now no satisfactory method has been found. The work of KLEIN and WERNER[2] and of POLLACCI and BERGAMASCHI[3] with dimedon appeared to give proof of the presence of formaldehyde in photosynthesizing plant cells. BARTON-WRIGHT and PRATT[4] have shown, however, that formaldomedon is formed when a solution of sodium bicarbonate is exposed to light in the absence of green plants.

Important factors that must be considered in a test to detect formaldehyde in photosynthesizing plant cells are: 1. Is the test specific? 2. Is it sufficiently sensitive? 3. Is the substance detected present in the normal cell and not, as may happen in macerated tissue, a decomposition product?

The ALLISON[5, 6] apparatus furnishes such a test. It gives readings specific for each compound in solution regardless of others that may be present. Compounds are detected without change. The requirements are that the solution must be sufficiently clear and uncolored to transmit adequate light to make the necessary observations, and that the concentration of the substance being detected is greater than three parts in 10^{12}. Detailed descriptions of the method[7] may be found in the literature, so that only enough will be given here to explain the present experiment.

The solution to be investigated is placed in a glass tube through which light passes, and if minima are obtained at scale readings characteristic of a specific compound, this compound is known to be present. If solutions are of sufficient dilution, quantitative determinations may be made with the aid of a circle so adjusted as to give the number of degrees through which the Nicol prism of the apparatus must be turned just to allow or just to prevent the appearance of the desired minima.

Since considerable light can pass through small unicellular algae, such as *Chlorella,* this type of organism appeared to be a means whereby the

[1] SPOEHR, H. A. Photosynthesis. pp. 289–291. Chemical Catalog Co. New York. 1926.

[2] KLEIN, G., and WERNER, O. Formaldehyde as an intermediate product in carbon dioxide assimilation. Biochem. Zeitschr. **168**: 361–386. 1926.

[3] POLLACCI, G., and BERGAMASCHI, M. Demonstration with dimethylhydroresorcinol of formaldehyde in living plants during chlorophyll photosynthesis. Atti Accad. Lincei **10**: 687–689. 1929.

[4] BARTON-WRIGHT, E. C., and PRATT, M. C. Studies in photosynthesis. I. The formaldehyde hypothesis. Biochem. Jour. **24**: 1210–1216. 1930.

[5] ALLISON, FRED. Magneto-optic method of analysis as a new research tool. Ind. & Eng. Chem. Analytical Ed. **4**: 9–12. 1932.

[6] ————————, and MURPHY, EDGAR. A magneto-optic method of analysis. Jour. Amer. Chem. Soc. **52**: 3796–3806. 1930.

[7] *Loc. cit.*

presence of formaldehyde could be detected within the plant cell. The low light requirement of such organisms for photosynthesis also makes them suitable, since the amount of light coming through the apparatus is so small that it is doubtful whether it would be sufficient for most plants to begin or to continue this process.

In testing the algal[8] culture for formaldehyde, a tube containing distilled water was first inserted and observations made to determine the presence of formaldehyde. The test gave negative results. A drop of the algal suspension was then introduced and the observations repeated. Minima previously determined to be those of formaldehyde appeared (scale readings 21.83 and 21.92). The contents of the tube were then filtered through hardened filter paper into another tube and the observations repeated. The minima characteristic of formaldehyde were absent, showing that the formaldehyde present when the previous readings were made was within the algal cell. To preclude the possibility that the formaldehyde was adsorbed by the filter paper from the external solution and therefore only appeared to have been in the cells, a formaldehyde solution of about the same concentration as that found in the tube with the algae was made. This solution was filtered through the same type of filter paper and was found to contain the same amount of formaldehyde before and after filtration.

A brief study was made of the rate of formaldehyde formation as affected by the time of exposure to light. The algal cells to be studied were kept in the dark over night and until placed in the apparatus for observations the next morning. Since a small amount of light must pass through the substance examined, and the amount of light necessary for photosynthesis for such algae is small, an initial negative reading could hardly be expected. The first circle reading for the appearance of formaldehyde was 3.5°, corresponding to a concentration, considering the contents of the tube as a whole, of about 3.6 parts in 10^{12}.[9] This is about as small an amount as can be detected by this method. The tube and contents were then placed 2 feet from a 60-watt Mazda lamp for four periods of 7 minutes each and a final exposure of 15 minutes. Determinations were made after each exposure to determine the amount of formaldehyde present. The tube and contents were again placed in the dark and observations made the following morning. Ten-minute intervals of exposure were made. The first exposure was made 1 foot from a 25-watt Mazda lamp. The other exposures were made 2 feet from the same lamp as was used the preceding day. The results are shown in figure 1. Circle readings (increased readings show an

[8] Probably a species of *Chlorella*.

[9] This assumes that the angle corresponds to the same concentration for formaldehyde as for calcium. Details for quantitative determinations with the circle will be published later.

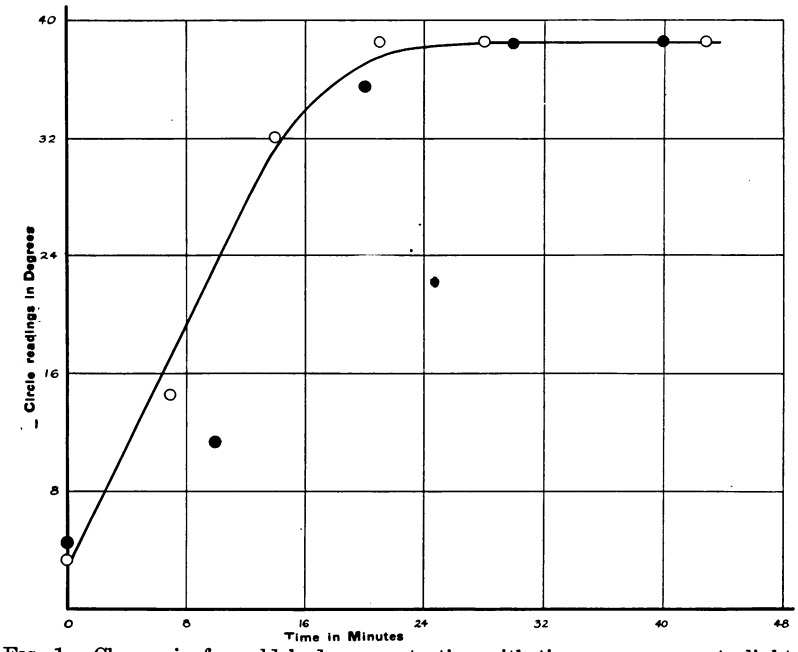

FIG. 1. Change in formaldehyde concentration with time on exposure to light: open circles, readings first day; solid circles, readings second day.

increase in concentration) were plotted against time of exposure. The final circle readings, 38.5°, correspond to a concentration of about 5 parts in 10^{10}. The algae were filtered out after the final observation and no formaldehyde was then found in the solution. The concentration within the algal cell could be determined only if the ratio of the amount of algae to the water in the tube were known and providing the effect were the same as if the formaldehyde were in the solution surrounding the algae. The fact remains, however, that up to a certain time the amount of formaldehyde in the cell increases on exposure to light of a certain intensity and after that remains the same, at least for the period observed.

This preliminary work suggests great possibilities, not only in further photosynthetic studies, but in determining various metabolic products of small organisms sufficiently transparent to allow enough light to pass through for work with the ALLISON apparatus. The minima for comparatively few organic compounds have been determined and the task of finding them may be long and tedious. Once these minima are found, the presence of compounds in solution in organisms of suitable types, such as bacteria and small, comparatively transparent fungi and algae, can easily be detected.—ANNA L. SOMMER, EDNA R. BISHOP, AND IRENE G. OTTO, *Alabama Agricultural Experiment Station, Auburn, Alabama.*