

tion of thrombin did not change the pH value of the Toluidine Blue-fibrinogen mixture.

Visible absorption spectra were obtained at all dye concentrations. Absorption spectra were also obtained in the ultraviolet region, but the absorption contribution of fibrinogen was so great at the concentration used in the coagulation studies that accurate measurements were not possible. Toluidine Blue gave a peak at 285 m μ and fibrinogen one at 280 m μ ; the combination gave a peak at 282 m μ . The fibrinogen maximum is the same as was previously reported by Waugh and Livingstone (3).

The curves for the 1- and 2-min intervals have a similar shape but differ from those of the other time intervals at the 10- γ concentration, where the dye produced a definite acceleration in coagulation. This effect was lost, however, at the 15- γ concentration, where an increased coagulation time was observed. The accelerator effect seen at the 1- and 2-min intervals and at the 10- γ dye concentration cannot be explained on the basis of any effect on the thrombin because, although the dye-fibrinogen and dye-thrombin curves at the 2-min interval and at the 10- γ dye concentration were identical, the thrombin was still active in causing fibrin formations at the other dye concentrations and time intervals. If the thrombin had been inactivated, no fibrin formation would have been detected beyond the 10- γ dye concentration, but such interference with the reaction did not occur except at the 100- γ dye concentration or when the time interval of interaction between the dye and the thrombin was increased to 10 min.

On the other hand, it may have been possible that Toluidine Blue and fibrinogen reacted to form a complex similar to those observed by Michaelis (4) for the Toluidine Blue-nucleic acid system. If such were the case, however, definite differences in the absorption spectra of the dye-fibrinogen system would have been apparent in the visible region, and there might have been a definite downward shift in the curve, indicating the presence of the dimeric and polymeric forms of Toluidine Blue. There were, however, no obvious changes in the absorption curve in either the presence or absence of fibrinogen. Furthermore, the curves were identical with the Michaelis (4) curves for the monomeric form of Toluidine Blue. These data indicate an additive effect rather than a combination of the two substances. Both the visible and ultraviolet absorption data indicate that the dye and fibrinogen do not form a complex, or, if such a complex is formed, it is a very loose one which does not prevent thrombin from converting fibrinogen into fibrin. Moreover, the coagulation-accelerating action of the dye at the higher dye concentrations might be considered further evidence of no rigid complex formation between the dye and the fibrinogen. Furthermore, the endpoint, fibrin thread formation, cannot be confused with isoelectric precipitation of the fibrinogen because the isoelectric point of fibrinogen is pH 5.4 (5), whereas our evaluations were made at pH 7.0. The

great differences in the molecular weights of Toluidine Blue (305) and fibrinogen (350,000) (6) make it doubtful that additional work using electrophoretic or ultracentrifuge techniques would increase our total knowledge of the reaction taking place. Further investigation must be centered on the other purified components of the blood coagulation system; work on these factors will be reported in detail at a later date.

References

1. HALEY, T. J., and STOLARSKY, F. *Proc. Soc. Exptl. Biol. Med.*, **73**, 103 (1950).
2. WARE, A. G., and SEEGER, W. H. *Am. J. Clin. Pathol.*, **19**, 471 (1949).
3. WAUGH, D. F., and LIVINGSTONE, B. J. *J. Phys. & Colloid Chem.*, **55**, 1206 (1951).
4. MICHAELIS, L. *Cold Spring Harbor Symposia Quant. Biol.*, Vol. XII. Menasha, Wis.: Banta, 131-142 (1947).
5. STENHAGEN, E. *Biochem. J.*, **32**, 714 (1938).
6. FERRY, J. D. *Proc. Natl. Acad. Sci. U. S.*, **38**, 566 (1952).

Manuscript received October 13, 1952.

The Part Played by Chlorophyll in Plant Transpiration Studied by a New Method: Hygrophotography

Joseph Sivadjian

Institut Pasteur, Paris, France

It is known that the transpiration of leaves increases under the effect of light, the first action of which is to cause an increase of the stomatal aperture. The effects of the various spectrum radiations on green leaves are not the same; blue and red radiations, which are precisely those absorbed by chlorophyll, prove to be the most efficient for increasing transpiration. The conclusion arrived at was therefore that light acting upon chlorophyll would thus play an important part in plant transpiration. Van Tieghem (1) was even led to assume that in addition to the transpiration function proper of the leaf, as shown by its cells not containing chlorophyll, there also occurred a release of water vapor due to chlorophyll activity, to which he gave the name "chlorovaporization."

This notion of chlorovaporization was not accepted by all botanists, some of whom considered that the phenomenon of chlorovaporization does not occur or if it does occur, causes the vaporization of a very small amount of water which cannot be demonstrated experimentally (2).

The hygrophotographic method, which has been described elsewhere (3), offers a very sensitive and extremely simple means for definitely solving this problem. This method is based on the use of mercury and silver iodide gelatin photograph films or plates whose preparation has been described and whose properties have been shown (4). These plates, which are sensitive to light and normally yellow, blacken rapidly when exposed to light. They are also extremely sensitive to moisture and to water, which instantaneously discolors the plate blackened through exposure. This

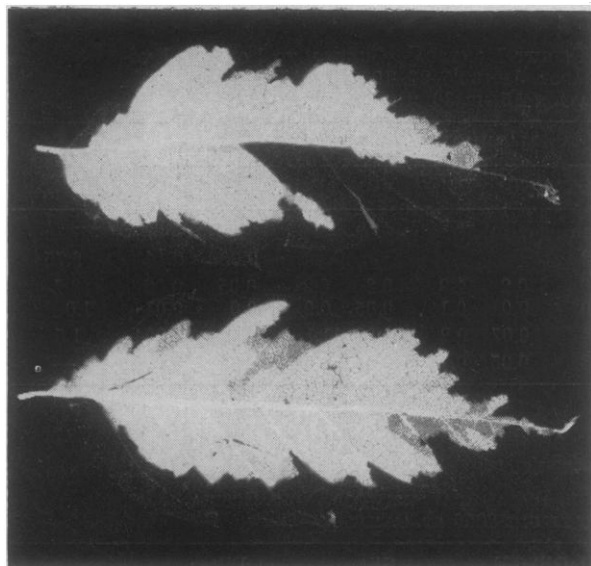


FIG. 1.

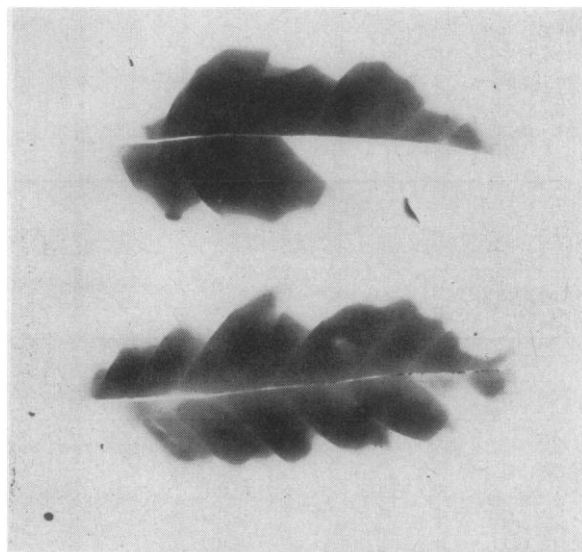


FIG. 2.

sensitiveness to moisture is such that it could serve for the study of the *perspiratio insensibilis* of the skin in human physiology and in pharmacology (5) and of leaf transpiration in plant physiology (6), or to record rains or dew and mist (the last two on spider webs) in meteorology (7).

When, in a printing frame a variegated leaf is applied on a hygrophotographic plate preliminarily blackened by exposure, the hygrophotographic image appearing on the plate exactly reproduces, under the effect of transpiration which is more active in the green parts than in the etiolated parts, the contours of the green parts, the yellow areas not yielding any impression on the plate. This phenomenon is quite striking with leaves having an active transpiration

such as those of *Acer negundo* (Figs. 1 and 2), *Abutilon savitzii*, *Tradescantia zebrina*, and margined leaf of *Pelargonium*. It is not appreciable with coriaceous and evergreen leaves such as those of *Aucuba japonica* or *Evonymus japonicus* the transpiration of which is extremely low.

Figure 1 shows two leaflets of variegated *Acer negundo* photographed by direct lighting of leaflets placed on the sensitive paper; green parts therefore show up in white and etiolated parts in black. Figure 2 represents the hygrophotography of these same leaflets; the green parts, which transpire actively, show up this time in black.

Examination of these images shows that chlorophyll plays an active part in transpiration by promoting the release of water vapor by the leaves.

References

1. VAN TIEGHEM, P. *Bull. soc. botan. France*, **33**, 152 (1886).
2. BONNIER, G., and LECLERC DU SABLON. *Cours de botanique*, **2**, 2448.
3. SIVADJIAN, J. *Compt. rend.*, **223**, 1296 (1949); **230**, 390 (1950).
4. ———. *Bull. soc. chim.*, **17**, 483 (1950).
5. ———. *Compt. rend.*, **234**, 1911 (1952).
6. ———. *Ibid.*, **232**, 1956 (1951); *Bull. soc. botan. France*, **99**, 138 (1952).
7. ———. *Ibid.*, **234**, 647 (1952).

Manuscript received October 10, 1952.

Numbers of Fungi and Bacteria in Transatlantic Air¹

S. M. Pady² and C. D. Kelly³

Department of Botany and Department of Bacteriology, McGill University, Montreal, Quebec, Canada

Aerobiological studies of fungi and bacteria were commenced in 1947 with samples from the Canadian Arctic (1, 2). Early qualitative work was supplemented by techniques and apparatus designed for quantitative sampling from rapidly moving aircraft (3). Much variation was found in numbers of microorganisms in both temperate and arctic regions which appeared to be correlated with specific air masses (4, 5). To obtain further data two flights were made in June and August 1951 from Montreal, Quebec, Canada, to London, England, and return, with RCAF squadron 426 in a North Star (DC-6) aircraft. This is a preliminary report on the numbers of fungi and bacteria obtained from continuous sampling on these two trips, and, as far as the authors are aware, it is the first quantitative study of fungi and bacteria over the Atlantic Ocean.

The samplers were mounted as in previous flights (3), and the following samples were taken: 16 filters, 121 sets of plates in the McGill-GE sampler, and 19

¹ This work was supported by grants from the Defence Research Board, Ottawa, Canada.

² Present address: Department of Botany, Kansas State College, Manhattan.

³ The invaluable technical assistance of Sharon Layne and Lucia Kapica is gratefully acknowledged.