The Response of Stomata of Etiolated Onion Leaves to Light and Transpiration During the Greening

VIRGIN[1,2] showed that the presence of chlorophyll is a necessary prerequisite for the response of stomata to illumination. These results have been obtained from indirect experiments by the measurement of transpiration rate using a corona-hygrometer technique. Since these studies are of an indirect nature, they have the limitations inherent in indirect experimentation, and they should, if possible, be tested by a direct method. Such a method has been developed by ZELITCH[3], which permits rapid and accurate measurements of stomatal aperture by means of impression cast in silicone rubber. As one aspect of a study of stomatal movement in the starch-free[4] onion leaves, we have been interested in the possible role of chlorophyll synthesis in etiolated onion leaves in the response of stomata to light. The leaves of onion lack the mechanism for the conversion of sugar to starch; therefore, in this plant, the stomatal movement cannot be considered in terms of the classical osmotic effect controlled by the reversible reaction sugar $\rightleftarrows$ starch.

Material and methods. Commercial onions (*Allium cepa* L.) were placed in beakers with water and kept in darkness in the laboratory for a period of 30 days. Some of the plants were kept in light at the laboratory window to obtain the green leaves. The course of the experiment was as follows. Etiolated and green leaves taken from darkness were illuminated from 2 sides. Plants were placed between 2 screens with continuously circulating water for removing IR-irradiation. The light source was four 200 Watt photoflood lamps, which gives a light intensity of 20,000 lux. After 2 h of illumination, plants were darkened for 2 h and then the light was turned on again for 3 h. For the determination of transpiration, beakers with plants were accurately weighed at 09.00 and 16.00. The determination of total chlorophyll content in leaves was made spectrophotometrically according to the method of BRUINSMA[5]. Stomatal apertures were measured by means of silicone rubber impressions as described by ZELITCH[3]. The replicas were taken from the central portion of the lamina on its lower surface, every 20 min during the 120 min of light and the following 120 min of dark periods. The control plants were the green seedlings treated by the same procedure. These observations continued for 4 days.

Results. The data presented below are an average of 4 replications. Figure 1 shows the response of stomata of etiolated and green leaves to light. Each curve represents the widening of stomatal apertures as a function of time of illumination and closing in darkness. From Figure 1 it is clear that stomata of etiolated leaves do not respond to light on the first day of illumination.

[1] H. J. VIRGIN, Physiologia Pl. *9*, 482 (1956).
[2] H. J. VIRGIN, Physiologia Pl. *10*, 170 (1957).
[3] I. ZELITCH, Proc. natn. Acad. Sci. USA *47*, 1423 (1961).
[4] O. V. S. HEATH, in *Plant Physiology. A Treatise* (Ed. F. C. STEWARD 1959), vol. 2, p. 193.
[5] J. BRUINSMA, Photochem. Photobiol. *2*, 241 (1963).

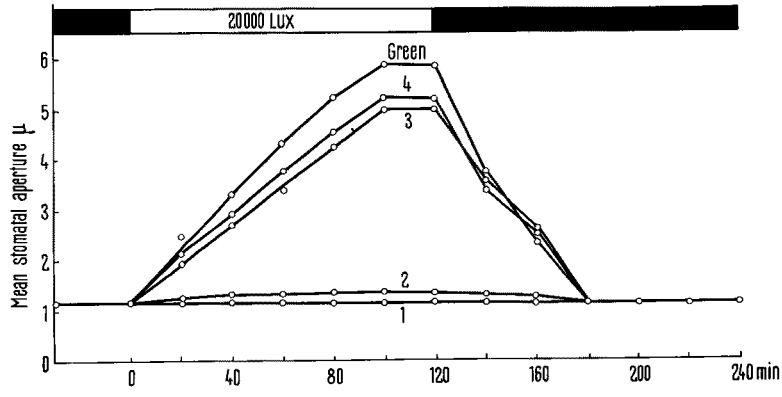

Fig. 1. Time course of opening of stomata in light and closing in darkness of etiolated onion leaves during the first, second, third and fourth day of greening. The stomata of green leaves is a control.

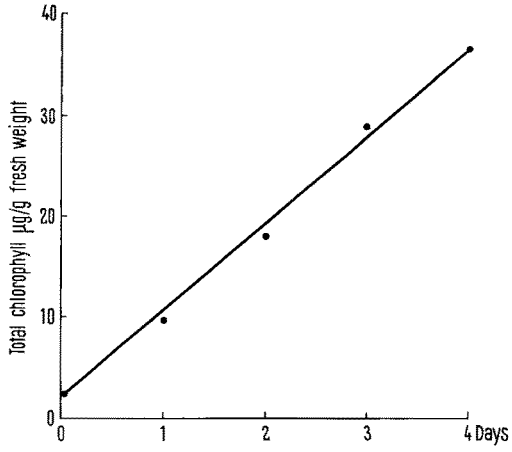

Fig. 2. Total chlorophyll content in etiolated onion leaves during the greening. Chlorophyll content in green leaves: 144 $\mu g/g$ fresh weight.

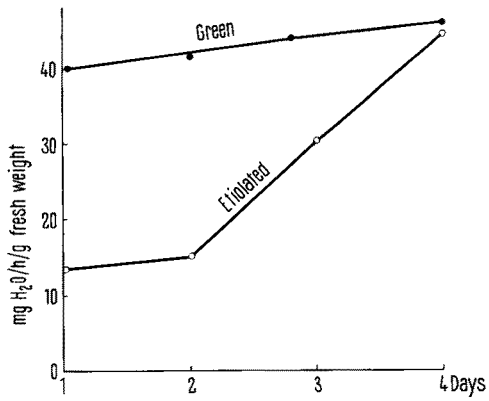

Fig. 3. Rate of transpiration either etiolated or green onion leaves.

On the second day of illumination there was a very little widening of stomata. Only on the third and particularly fourth day of illumination, the opening reaction of stomata was similar to that of the green leaves. The time required for the maximum widening of stomata in light was 100 min, after which the stomata did not open wider. The closing reaction was faster and stomata narrowed to the initial value within 60 min. As is seen from Figure 1, stomata both of etiolated and green leaves do not close completely in darkness. On both etiolated and green leaves, there were about 28,000 of stomata/cm^2, and their sizes were similar. The average length of stomata was 19.5 μ.

Figure 2 shows that the total chlorophyll content in leaves increased linearly within 4 days of greening (5 h of light/day) and reached about $^1/_4$ of concentration in green leaves. The rate of transpiration of etiolated and green leaves is presented in Figure 3. From this Figure it can be read that transpiration of etiolated leaves on the first and second day of greening was by about 3 times less than that of green leaves. On the third day of light period, transpiration raised by about 2 times, and finally on fourth day of greening it was similar to that of green leaves. The data of the Figures 1, 2 and 3 suggest a close relationship between the response of stomata to light, the transpiration and the synthesis of chlorophyll in leaves. The opening reaction of stomata starts to work well when the concentration of chlorophyll in leaves reached a certain level (28.1 μg/g fresh weight) below which stomata do not respond to light. The rising in the transpiration rate is correlated in time with the opening reaction of stomata. It is doubtful whether such a response of stomata of onion leaves is due merely to the production of osmotically active substances in guard stomatal cells, because it has been shown[6] that photosynthesis was about 50 times too low to account for the maximum rates of osmotic-pressure change observed during the opening.

Recently RASCHKE[7] has showed that the movements of stomata of maize leaves were a function of light intensity and were similar either in the atmosphere of air, CO_2-free air or in pure nitrogen. In other words, the reactions of stomata in light were the same during photosynthetic absorption of CO_2 and after removal of CO_2 from the atmosphere. It is a possibility that, in light in etiolated leaves, the developing mechanism of photophosphorylation in chloroplasts[8] provides the energy, may be involved in the operation of a 'pump' that increases the turgidity of guard cells as postulated by ZELITCH[9]. The fact that the stomata of both etiolated and green leaves of onion were similar, suggests that light has no visible effect on the formation and development of stomata, and that they are presumably under genetic control. The observation that the stomata of green and albino mutant barley leaves were similar has been reported by SHOW[10,11].

Zusammenfassung. In etiolierten Blättern der Zwiebel (*Alium cepa* L.) kann Licht erst nach Einsetzen der Chlorophyllsynthese die Öffnungsbewegung der Stomata induzieren.

J. POSKUTA and J. TOMCZYK

Department of Plant Physiology, University of Warszawa (Poland), 18 September 1967.

[6] M. SHOW and G. A. MACLACHLAN, Can. J. Bot. *32*, 784 (1954).
[7] K. RASCHKE, Planta *68*, 111 (1966).
[8] D. VON WETTSTEIN, Brookhaven Symp. Biol. *11*, 138 (1958).
[9] I. ZELITCH, Biol. Rev. *40*, 463 (1965).
[10] M. SHOW, Can. J. Bot. *36*, 575 (1958).
[11] Acknowledgments. The authors thank Prof. G. KROTKOV, Department of Biology, Queen's University, Kingston, Ontario, for his kindness in supplying a silicone rubber fluid. Thanks are due to Prof. P. STREBEYKO, Department of Plant Physiology, University of Warsaw, for his critical remarks during the preparation of the manuscript.

The Chromosomes of the Didelphiid Marsupial *Marmosa robinsoni* Bangs

The murine opposums (*Marmosa*) are the most diversified neotropical marsupials. CABRERA[1] recognized 37 living species, many of them further split into several subspecies. Such a diversified genus affords interesting material for studies on the causes of species diversification in extremely polytypic genera. The history of *Marmosa* as a whole has been revised by TATE[2]. Little is known about its evolutionary history, the genus being known in the fossil record only in the Pliocene and Pleistocene of Argentina[3].

Chromosome studies provide interesting information for investigating speciation. The available evidence about the chromosomes of *Marmosa* is almost nil. BIGGERS et al.[4] announced preliminary results in the study of chromosomes of *M. mexicana*, stating that it has a karyotype very similar to that of *Caluromys derbianus*, with $2N = 14$ chromosomes, but they have not yet reported the relevant evidences. It seems useful, therefore, to report the preliminary results reached in the study of *M. robinsoni*'s chromosomes even though they are only based on a few individuals.

Two male and 2 female individuals of *M. robinsoni* have been studied for chromosome analysis. They are the specimens MBUCV 1-1418, MBUCV 1-1429, MBUCV 1-1423 and MBUCV 1-1424 of the Collection of Mammals of the Institute of Tropical Biology, Central University of Venezuela. The first 2 were captured by the author in Los Llanos Biological Station, near Calabozo, Guárico, Venezuela. The last 2 were caught by C. J. NARANJO in 'hato Acapulco', about 25 km south of La Trinidad de Arauca, Apure, Venezuela. Although the localities are not typical for this species, this is one of the commonest mammals inhabiting the small clusters of trees in the savannas of Guárico and Apure. Series from the above localities closely agree with the description of *M. mitis casta* given by TATE[2], which, according to CABRERA[1], is to be named *M. robinsoni robinsoni* Bangs, 1898.

[1] A. CABRERA, Revta Mus. argent. Cienc. nat. Bernardino Rivadavia, Inst. nac. Invest. Cienc. nat. Zool. *4*, 12 (1957).
[2] G. H. H. TATE, Bull. Am. Mus. nat. Hist. *66*, 1 (1933).
[3] O. A. REIG, Acta geol. lilloana *2*, 255 (1958).
[4] J. D. BIGGERS, H. I. FRITZ, W. C. D. HARE and R. A. MCFEELY, Science *148*, 1602 (1965).