

A Double Polarity in Dictyosomes in Root Hairs of Cress (*Lepidium sativum* L.) after Treatment with Potassium Cyanide

Dictyosomes of plant cells have a well defined structure. They are composed of a stack of flattened cisternae or saccules bounded by smooth surfaced membranes. Most often cisternae consist of a plate-like region continuous with a peripheral system of interconnected tubules and of vesicles. Successive cisternae of a dictyosome stack are different, thus reflecting a polarity across the dictyosome. This polarity is evidenced morphologically by the height of cavities within dictyosome cisternae, by the appearance of cisternal membranes and in some cases in the content of the cisternae or the adjacent vesicles. One can separate a proximal pole^{1,2} or forming face³ ('Regenerationsseite'⁴) with a high cisternal cavity from a distal pole^{1,2} or maturing face³ ('Sekretionsseite'⁴) with less high cisternal cavity. After treating root hairs of cress with potassium cyanide, some dictyosomes showed a double polarity, an observation which to the author's knowledge has not yet been published.

Material and methods. Seeds of cress (*Lepidium sativum* L.) were grown in agar. About 36 h after planting seeds, root hair bearing zones were excised and cut to slices with a sharp razor blade. These slices were incubated for 2 h in sealed vessels containing a modified nutrient medium after WHITE⁵ with 10⁻³ molar KCN added. Following incubation, the whole specimens were fixed in phosphate buffered 2% glutaraldehyde and postfixed in 2% osmium tetroxyde. Blocks were embedded in an epon araldite mixture according to MOLLENHAUER⁶. Sections were double stained with uranyl acetate and lead citrate and viewed in an Elmiskop I A.

Observations. Following the treatment mentioned above root hairs cease to grow. The noticeable secretory activity of dictyosomes connected with wall formation in root hair tips⁷ ceased. This is morphologically indicated by a significant decrease in the number of secretory vesicles in the vicinity of the maturing faces of dictyosomes and by a reduction of the peripheral tubular system of dictyosomes. Approximately 1% of all dictyosomes within a treated root hair consist of an unusual number of cisternae (8–12 compared to 1–7 in other dictyosomes under equal condi-

tions and 3–6 in untreated root hairs) (Figure 1) and revealed a double polarity (Figure 2). The cisternae with the smallest cavity height which are usually characteristic for the maturing face of normally functioning dictyosome are located in the middle of these extended stacks. These cisternae in the middle of the dictyosomes frequently show a wavy structure. This might be some kind of artefact introduced by fixation reflecting a certain alteration in membrane composition. Both faces of such enlarged stacks show cisternae with cavities the height of which corresponds to that of a forming face of a normally functioning dictyosome. Cisternae in this area often have a dish-like shape. At both poles there is an accumulation of electron lucid vesicles. Arrangement and number of intercisternal fibrillar elements seem not to be altered by cyanide treatment.

Comments. Cyanide affects the energetic cell metabolism. The morphologically noticeable alterations concerning the secretory activities of dictyosomes have repeatedly been described⁸. It is reasonable to consider that the double polarity of some dictyosomes in root hairs of cress treated with cyanide as mentioned above is caused by a face to face apposition of two originally solitary dictyosomes. Initiated possibly by protoplasmic streaming or cell organelle movement, the maturing faces of two dictyosomes stick together thus giving rise to the unusual polarity. This apposition probably is made possible

¹ P. P. GRASSÉ, C. r. Acad. Sci., Paris 245, 1278 (1957).

² G. W. WHALEY, in *Probleme der biologischen Reduplikation*. (Ed. P. SITTE; Springer, Berlin-Heidelberg-New York 1966), p. 340.

³ H. H. MOLLENHAUER and W. G. WHALEY, J. Cell Biol. 17, 222 (1963).

⁴ E. SCHNEFF, in *Protoplasmatologia* (Springer, Wien, New York 1969), vol. VIII, 8, p. 15.

⁵ P. R. WHITE, A. Rev. Biochem. 11, 615 (1942).

⁶ H. H. MOLLENHAUER, Stain Technol. 39, 111 (1964).

⁷ K. ZAAR and E. SCHNEFF, Planta 88, 224 (1969).

⁸ E. SCHNEFF, Flora 153, 23 (1963).



Fig. 1. Dictyosome from a normally growing root hair. The polarity of the dictyosome is clearly evidenced by the height of cisternal cavities. In addition, the maturing face of the dictyosome is indicated by the presence of secretory vesicles. $\times 68,000$.

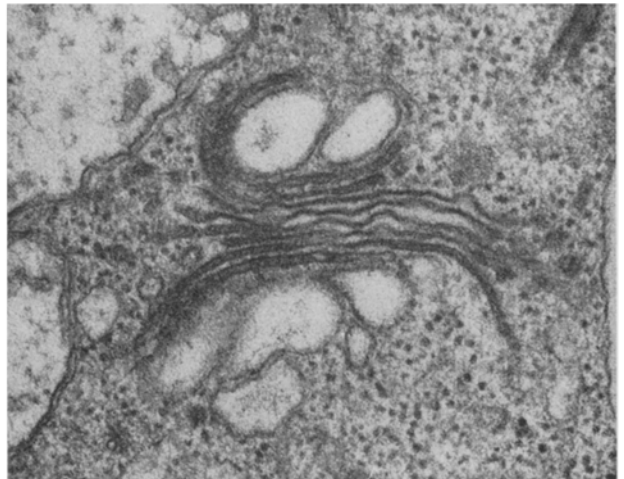


Fig. 2. Dictyosome with a double polarity after treatment with cyanide. Cisternae with the smallest height of cisternal cavities are located in the middle of the stack. These cisternae show a wavy structure. At both faces of the dictyosome there is an accumulation of electron lucid vesicles. $\times 69,000$.

by the configuration of maturing face cisternae and the lack of secretory vesicles. The shape of the cisternae at the forming face and the presence of vesicles in this area presumably prevent an involvement of these cisternae in dictyosome association. The occurrence of dictyosomes with a double polarity requires special intercisternal adhesive mechanisms. It is difficult to imagine that the association of the dictyosomes in this form is mediated by a particular affinity of similarly composed or functionally adequate membranes. It is more probable that intercisternal materials are involved in the adhesion of two dictyosomes. These intercisternal materials are often represented by fibrils. These materials are expected to occur also at the surface of the outermost cisterna of the maturing face.

After the cyanide treatment, approximately 1% of all dictyosomes within a root hair show a double polarity. The overwhelming portion of single dictyosomes is characterized by similar morphological alterations⁹ of which some are specified above. For that reason it is questionable whether the assemblage of two dictyosomes is a direct consequence of cyanide action. It is more likely that cyanide (1 mM, 2 h) brings about the precondition(s) for the adhesion of the dictyosomes by modifying their maturing faces. The formation of 'twin-dictyosomes' is probably dependent on a sufficiently close approach of two altered dictyosomes with their paralleled maturing faces.

It is uncertain for how long this dictyosome configurations exist. One has to take into consideration a limited duration of these structures. These facts probably

account for the rare occurrence of dictyosomes with a double polarity in cyanide treated root hairs of cress.

The 'twin-dictyosomes' described above are in no way related to dictyosome associations observed in diatoms ('Doppelpfättchen'^{10,11}) which consist of two single dictyosomes separated by elements of the endoplasmic reticulum¹².

Zusammenfassung. Nach der Behandlung von Wurzelhaaren der krausen Gartenkresse *Lepidium sativum* mit 10^{-3} M KCN treten Dictyosomen mit verdoppelter Polarität auf. Diese ungewöhnlichen Dictyosomenformen entstehen möglicherweise durch die Zusammenlagerung von zwei in ihrer sekretorischen Aktivität gestörten Dictyosomen. Die Zusammenlagerung erfolgt wahrscheinlich an den Sekretionsseiten der Dictyosomen.

K. ZAAR

Lehrstuhl für Zellenlehre der Universität Heidelberg, Berlinerstrasse 15, D-69 Heidelberg (Germany), 5 May 1971.

⁹ K. ZAAR, Thesis. Fakultät für Biologie. Universität Heidelberg (1972).

¹⁰ R. JAROSCH, *Protoplasma* 55, 406 (1962).

¹¹ R. W. DRUM, *J. Ultrastruct. Res.* 15, 100 (1966).

¹² The author is indebted to Prof. E. SCHNEFF, M. MAIWALD and D. DEICHGRÄBER for critically reading the manuscript.

Afferent Nerve Endings in the Avian Lung: Observations with the Light Microscope

Although a few observations have recently been made on possible afferent nerve endings in the vertebrate lung with the electron microscope¹⁻³, the evidence obtained with the light microscope for a sensory innervation appears to be very slight and in no class of vertebrate has the general structure and distribution of these afferent endings in the lungs been established. Unfortunately, in the few available accounts of possible afferent endings observed with the light microscope in the lungs of vertebrates most of the evidence for the endings is presented in the form of drawings rather than photographs; this evidence is therefore extremely difficult to interpret.

The recent experimental studies on the regulation of breathing in birds^{4,5} indicate that information on the precise location of the sensory nerve endings in the avian lung is urgently required. The distribution of elastic tissue⁶ and surfactant⁷ in the lungs of birds suggests that the atria, the small chambers connecting the lumen of each tertiary bronchus with its surrounding area of gaseous exchange, are extremely mobile and may therefore be the sites of sensory nerve endings monitoring movement of the lungs; moreover possible afferent axonal endings have been observed here with the electron microscope⁸. The presence of afferent nerve endings in the walls of the tertiary bronchi and atria was therefore investigated with the light microscope.

The lungs of 20 young and adult birds (*Gallus domesticus*) were examined with the light microscope using the methylene blue technique and modified Bielschowsky-Gros silver⁹ and Champy osmium tetroxide¹⁰ methods. The interpretation of the nervous tissue was based on

the discussions of CAUNA¹¹, MILLER and KASAHARA¹², MUNGER¹³, and POLÁČEK¹⁴ which indicate the existence of 3 types of afferent endings: free endings, encapsulated endings and neurite-receptor cell complexes.

¹ R. D. COOK and A. S. KING, *Experientia* 25, 1162 (1969).

² M. FILLENZ and R. I. WOODS, in *Breathing* (Ed. R. PORTER, Ciba Foundation Hering-Breuer Centenary Symposium; Churchill, London 1970).

³ J. M. LAUWERYS, J. C. PEUSKENS and M. COKELAERE, *Life Sci.* 9, 1417 (1970).

⁴ A. S. KING, V. MOLONY, J. McLELLAND, D. R. BOWSHER and M. F. MORTIMER, *Experientia* 24, 1017 (1968).

⁵ M. R. FEDDE and D. F. PETERSON, *J. Physiol., Lond.* 209, 609 (1970).

⁶ A. S. KING, R. N. W. ELLIS and S. M. S. WATTS, *J. Anat.* 101, 607 (1967).

⁷ A. S. KING and V. MOLONY, in *Physiology and Biochemistry of the Domestic Fowl* (Eds. D. J. BELL and B. M. FREEMAN; Academic Press, London 1971).

⁸ R. D. COOK, Ultrastructural Observations on the Bronchial Smooth Muscle, its Innervation, and the General Innervation of the Lung in *Gallus domesticus*. Ph. D. thesis, University of Liverpool (1971).

⁹ J. R. RINTOUL, The Comparative Morphology of the Enteric Nerve Plexuses. M. D. thesis, University of St. Andrews (1960).

¹⁰ S. D. SUTHERLAND, *J. Anat.* 97, 624 (1963).

¹¹ N. CAUNA, *J. comp. Neurol* 113, 169 (1959).

¹² M. R. MILLER and M. KASAHARA, *Am. J. Anat.* 115, 217 (1964).

¹³ B. L. MUNGER, in *Touch, Heat and Pain* (Eds. A. V. S. DE REUCK and J. KNIGHT, Ciba Foundation Symposium; Churchill, London 1966).

¹⁴ P. POLÁČEK, *Acta Fac. med. Univ. brn* 23, (1966).