ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLV, nr 4
1976

Effects of water soluble oncostatic fraction from *Rheum officinale* Baill. rhizomes on *Allium cepa* root meristem

III. Morphological changes in cytoplasm

A. DAWIDOWICZ-GRZEGORZEWSKA

Institute of Botany, Warsaw University, Warsaw

(Received: May 28, 1976)

Abstract

Spiral concentric and linear configurations of membraneous structures were observed in the cytoplasm of fixed meristematic cells from the root apex of *Allium cepa* under the light microscope. They appeared after incubation in sublethal and lethal conditions in *Rheum officinale* rhizome extracts. During postincubation these structures disappeared. They were interpreted as ER membranes. On the basis of literature data the physiological significance of these structures is discussed. It would seem that they are an indication of enhanced metabolic activity evoked either exogenously by various injuries as a form of defence reaction of the protoplast or endogenously during cytodifferentiation. Prolonged treatment in noxious conditions causes the ER membranes to transform into inactive myelin structures.

INTRODUCTION

ER identification in the light microscope arouses no doubts since the publication of Porter and Machado (1960). The basic feature of ER — variability of cistern shape has been documented by the cinematographic method in vivo on animal material by Buckley (1964) and by intravital observations in phase contrast on plant material (epidermal cells from onion scales *Allium cepa*) (Url, 1964; Url and Bolkar-Nordenkampf, 1965). There are numerous modifications of the morphological ER pattern after injury by various noxious factors. Goldblatt (1969) enumerates various possible reaction of ER and among them fragmentation and hypertrophy. A specific manifestation of ER reaction to the stimuli applied is the formation of complicated spirals visible in the light microscope which have been given various synonymous

names: "fingerprints" (Verbin et al., 1969), myelin forms (Buckley, 1962) concentric lamellar formations (Stenger, 1966), concentric spirals (Nickerson and Curtis, 1969). Membraneous structures form in the cytoplasm owing to various circumstances. An exhaustive list of the latter is given by David (1970). In meristematic onion cells they have been observed after treatment with a growth retardant — Fosfon and low temperature (Podbielkowska and Kacperska-Palacz, 1971) and under conditions of hypoxia (Podbielkowska et al., 1975). Numerous papers dealing with animal material described various factors inducing these formations, and among them X-rays (Volk et al., 1966), ultrasounds (Curtis, 1966), low temperature (Trump et al., 1964), sodium fluoride and cyanide (Buckley, 1962), carcinogenic compounds (Herdson et al., 1964; Stenger, 1966) vinblastin sulphate (Krishan et al., 1968). In all the above mentioned cases these structures are considered to be ER. Accumulation of smooth ER membranes has been most frequently described (Stenger, 1966; Burger and Herdson, 1966; Verbin et al., 1969), of rough ER less frequently (Krishan et al., 1968; Podbielkowska et al., 1975). The morphological features of the structures observed by me in the light microscope also seems to constitute a system of ER membranes. In connection with the nonuniform interpretation of the significance of these structures (see Discussion) observations were undertaken in order to establish the experimental conditions inducing their appearance and histological localization.

MATERIAL AND METHODS

The object studied were adventitious roots of 3-cm initial length of the onion (*Allium cepa*) growing in darkness at room temperature. The experimental conditions have been described in detail in Part I of the present study (Dawidowicz-Grzegorzewska, 1976). Material was collected after 6, 12 and 24 hours of incubation in extract diluted within the range of 1—50 per cent. The root apexes were fixed in CrAF (percentual composition 0.5—0.5—20) and in AF (percentual composition 0.5—20). Longitudinal microtome sections 6.6 μm thick were stained with iron hematoxylin and counterstained with fast green (after CrAF), 0.25 per cent toluidin blue at pH 3.4 and 7.4 (after AF) and with 0.1 per cent fast green pH 8 (after AF). Microphotographs were taken with a Reichert Zetopan type Exacta-Varex camera.

AUTHOR'S OBSERVATIONS AND DISCUSSION

Various configurations of the membraneous structures were observed in the cytoplasm of meristematic cells from fixed root apexes of *Allium cepa*. They were noted exclusively during incubation under sublethal

(6 h in 25% and 12 h in 12.5% extract) and lethal conditions (12 h in 50% extract). During incubation in weak solutions (1 and 5%) the structures did not appear except in the degenerating marginal cells of the root cap. During postincubation these structures disappeared as early as after 24 h.

The observed structures constituted systems of parallel membranes (2—8) situated close to the walls (Plate II, Photos 2, 3) or else they formed configurations of spirally convoluted concentric membranes with diameter 3—13 μm (Plate I, Photos 1, 2; Plate II, Photos 1, 2). The most numerous and best developed cytoplasmic membranes were found in the peripheral meristem cells: in the root cap, dermatogen and in the marginal periblem (Plate I, Photos 1, 2; Plate II, Photos 1, 2) and they were least numerous in the plerome, appearing as single circles, ovals or some few linear structures (Plate II, Photo 3). On the basis of cytochemical staining the presence of alkaline and acid proteins and of RNA in them was revealed.

Various functions are attributed to the membraneous structures in the cytoplasm. One interpretation is that they are a morphological manifestation of cytoplasm degeneration (Buckley, 1962; action of respiratory inhibitors). Buckley considers the membraneous structures as myelin forms developing from ER after a long period of exposure to noxious factors extracting lipids from the ER membranes.

Another concept ascribes the presence of concentric membranes to the regeneration of the cytoplasm (Stenger, 1966). The latter author observed these structures in cells of parenchymatous tissue agglomerations considered as a manifestation of liver regeneration phenomenon after injury due to tetra (CCl_4). Damaged cells contained no membraneous structures.

It would seem from my own observations that these structures arise both in sublethal and lethal conditions. Their pronounced development in the marginal cells of root tissue and occurrence under lethal conditions would rather support the supposition of their degenerative pathological character. On the other hand, the presence of these structures does not necessarily indicate lethal changes since they disappear, when after a sublethal dose of the inhibitor, normal environmental conditions are restored. The evaluation and distinction of effects caused by toxic factors and of cytological regeneration are no doubt difficult and speculative. One thing is certain, that one of the first reactions of protoplast to damage is drastic hypertrophy of rough and smooth ER. Fawcet (1964) proved, by acting with phenobarbital on liver cells, that there occurs an increase in the number of new smooth ER membranes in a compact concentric arrangement. This is associated with an increased accumulation of enzymes synthesized earlier on rough ER (acid phosphatase, Burger and Herdson, 1966) in the ER cisterns. Data have been

reported concerning the detoxifying role of smooth ER, as indicated by the presence of enzymes indispensable for metabolic degradation of toxins (Remmer and Merker, 1963). These authors found that, as the consequence of administration of a number of chemical compounds (among them phenobarbitol, chloramphenicol), enzymes appear in ER cisterns, directly associated with metabolic degradation of these compounds. The spherical compact arrangement of the concentric membranes points to the localized character of this degradation (Hruban et al., 1963).

Cytoplasmic membraneous structures were also observed under normal physiological conditions, among others by Jensen (1965) in *Gossypium* synergids, by Rodkiewicz and Mikulska (1966) in *Lilium candidum* megasporocytes, by Beck and Greenawalt (1968) in germinating *Neurospora crassa* conidia, by Marciniak (1975) in *Lilium regale* microsporocytes. All the above mentioned cases concern cells with enhanced metabolic activity. It would seem that the concentric arrangement of the membranes in the cytoplasm indicates an increased metabolic activity evoked either endogenously in the differentiation process or exogenously by varius injuries, as a form of defence reaction of the protoplast. Prolonged action of the noxious factor causes transformation of the ER membranes into myelin forms. These are the last developmental stage in the same reaction of the cytoplasm (Stenger, 1966). The myelin structures, in contrast to smooth ER, exhibit no enzymatic activity (absence of acid phosphatase, Herdson and Kaltenbach, 1965).

The author is deeply indebted to Professor Henryk Teleżyński for critical reading of the manuscript and for his helpful remarks during preparation of the paper for publication.

REFERENCES

Beck D. and Greenawalt J., 1968. Factors affecting the formation of membranous structures in the cytoplasm and mitochondria of *Neurospora crassa*. J. Cell. Biol. 39: 11a.

Buckley J. K., 1962. Cellular injury in vitro: phase contrast studies on injured cytoplasm. J. Cell Biol. 14: 401—420.

Buckley J. K., 1964. Phase contrast observations on the endoplasmic reticulum of living cells in culture. Protoplasma 59: 569—588.

Burger P. and Herdson P., 1966. Phenobarbital — induced fine structural changes in rat liver. Amer. J. Path. 48: 793—811.

Curtis J. C., 1966. Early changes in cell fine structure of mouse liver exposed to pulsed ultrasound. J. Cell Biol. 41: 24a.

David H., 1970. Zellshädigung und dysfunktion. Protoplasmatologia 10: 59—62.

Dawidowicz-Grzegorzewska A., 1976. Effects of water extracts from rhisomes of *Rheum officinale* Baill. on *Allium cepa* root meristem. I. The mitotic activity. Acta Soc. Bot. Pol. (in press).

Fawcett D. W., 1964. Hypertrophy of the agranular reticulum in the liver associated with administration of barbiturates. J. Cell Biol. 23: 30a.

Goldblatt P., 1969. The endoplasmic reticulum. Handbook of molecular cytology, ed. A. Lima-de-Faria. North Holland, 1101—1130.

Herdson P. B., Garvin P. and Jennings R., 1964. Reversible biological and fine structural changes produced in rat liver by a thiodantoin compound. Lab. Invest. 13: 1014—1031.

Herdson P. B. and Kaltenbach J. P., 1965. Electron microscope studies on enzyme activity and the isolation of thiohydantoin-induced myeline figures in rat liver. J. Cell Biol. 25: 485—493.

Hruban Z., Swift H., Spargo B., Wisser R. and Kleinfeld R. G., 1963. Focal cytoplasmic degradation. Amer. J. Path. 42: 657—683.

Jensen W. A., 1965. The ultrastructure and histochemistry of the synergids of cotton. Amer. J. Bot. 52: 238—256.

Krishan A., Hsu D. and Hutchis P., 1968. Hypertrophy of granular ER and annulatae lamellae in Earles L cells exposed to vinblastine sulphate. J. Cell Biol. 39: 211—216.

Marciniak K., 1975. Cytochemical investigations on the megasporocyte and embryo sac in *Lilium regale* at various stages of development. Acta Soc. Bot. Pol. 44: 335—349.

Nickerson P. A. and Curtis J. C., 1969. Concentric whorls of rough ER in adrenocortical cells of the *Mongolian Gebril*. J. Cell Biol. 40: 859—862.

Podbielkowska M. and Kacperska-Palacz A., 1971. Effects of Phosfon-D and low temperature on the morphology of cell protoplasts. Protoplasma 73: 469—473

Podbielkowska M., Maciejewska B. and Kacperska-Palacz A., 1975. Morphology of protoplast as affected by an inhibition of respiration. Protoplasma 83: 201—208.

Porter K. R. and Machado E. A., 1960. Studies on ER. IV. Its form and distribution during mitosis in the cell of onion root tip. J. Biophys. Biochem. Cytol. 7: 167—180.

Remmer H. and Merker H. J., 1963. Drug-induced changes in the liver ER; association with drug-metabolizing enzymes. Science 142: 1657—1658.

Rodkiewicz B. i Mikulska E., 1966. Struktury w cytoplazmie rozwijającego się woreczka zalążkowego obserwowanego w ME. Acta Soc. Bot. Pol. 35: 239—256.

Stenger R. J., 1966. Concentric lamellar formations in hepatic parenchymal cells of carbon tetrachloride treated cells. J. Ultrastr. Research, 14: 240—253.

Url W., 1964. Phasenoptische Untersuchungen an Innenepidermen der Zwiebelschuppe von *Allium cepa* L. Protoplasma 58: 294—311.

Url W. und H. Bolhár-Nordenkampf, 1965. Beitrage zur Frage der lichtmikroskopischen Sichtbarkeit des endoplasmatischen Retikulums in Pflanzenzellen. Osterr. Bot. Zeitsch. 112: 586—603.

Verbin R. S., Goldblatt P. J. and Farber E., 1969. The biochemical pathology of inhibition of protein synthesis in vivo. The effects of cycloheximide on hepatic parenchymal cells ultrastructure. Laborat. Invest. 20: 529—537.

Volk B. W., Wellmann F. and Lewitan A., 1966. Thet effect of irradiation on the fine structure and enzymes on the dog pancreas. Amer. J. Path. 48: 721—753.

Author's address:

Dr Alina Dawidowicz-Grzegorzewska
Institute of Botany, Warsaw University,
ul. Krakowskie Przedmieście 26/28
00-927 Warszawa, Poland

Wpływ rozpuszczalnej w wodzie onkostatycznej frakcji z kłączy Rheum officinale Baill. na merystem wierzchołkowy korzeni Allium cepa L.

III. *Zmiany morfologiczne w cytoplazmie*

Streszczenie

W cytoplazmie utrwalonych komórek merystematycznych z wierzchołków korzeni cebul *Allium cepa* obserwowano w MŚ spiralne, koncentryczne i liniowe konfiguracje struktur błoniastych. Występowały one po 6 h i 12 h inkubacji w 25% i 50% ekstrakcie z *Rheum officinale*. Podczas postinkubacji następował zanik tych struktur. Na podstawie obserwacji własnych oraz danych z literatury o analogicznych strukturach w materiale roślinnym i zwierzęcym, struktury te zinterpretowano jako błony ER. Przedyskutowano na tle danych z literatury fizjologiczne znaczenie tych struktur. Wydaje się, że stanowią one przejaw stanu wzmożonej aktywności metabolicznej, wyzwalanej bądź egzogenicznie poprzez różne uszkodzenia jako forma obronnej reakcji protoplastu, bądź endogenicznie — podczas cytodyferencjacji. Przedłużające się trwanie warunków szkodliwych powoduje przekształcenie błon ER w nieaktywne struktury myelinowe (MF).

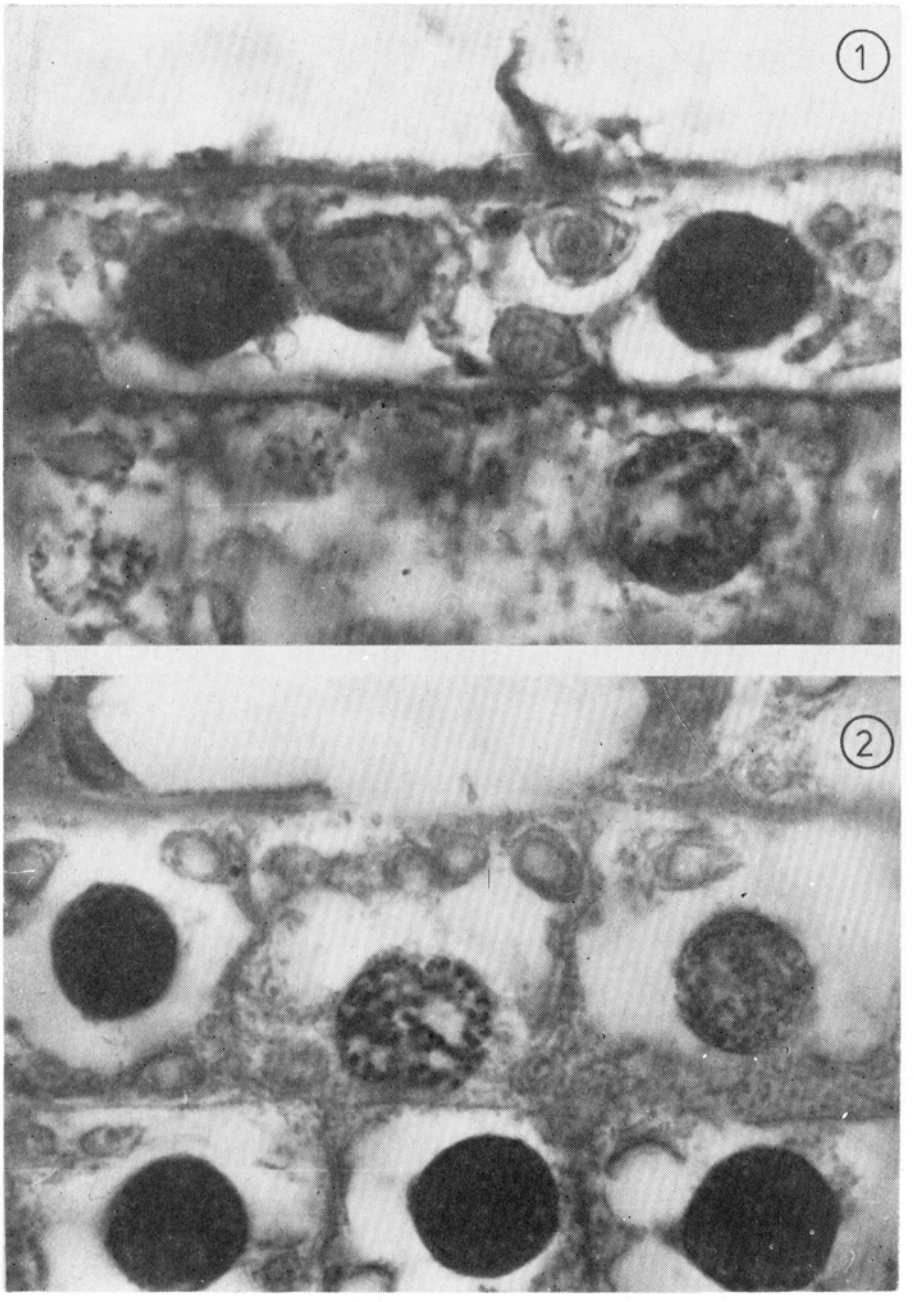

Photo 1. Concentric membraneous structures in the calyptra cells. 12-h incubation of roots in 50 per cent extract. Photo 2. Concentric membraneous structures in the dermatogen cells. 12-h incubation of roots in 12.5 per cent extract

iron hematoxylin, 1500×

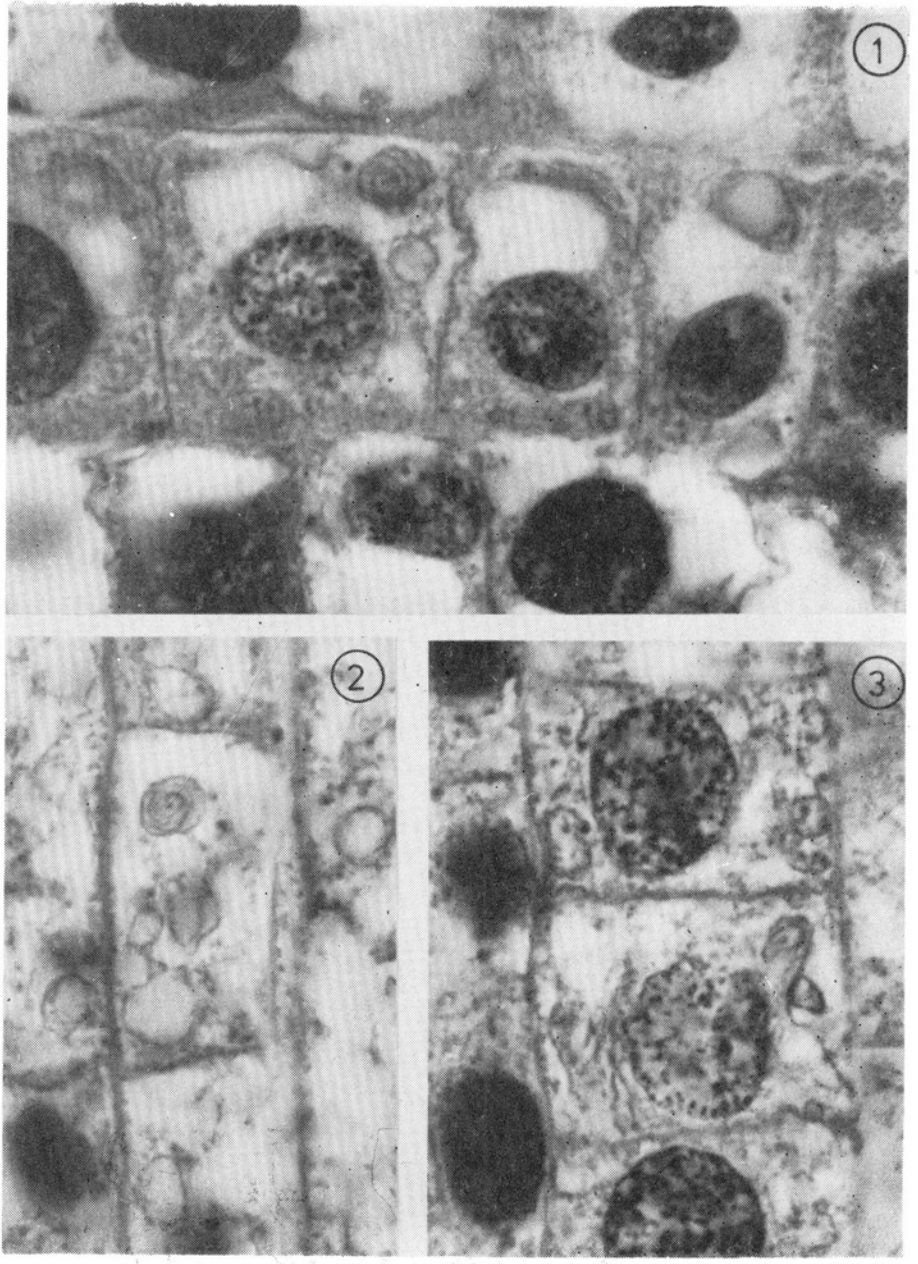

Photo 1. Concentric and linear membraneous structures in the dermatogen cells. 12-h incubation of roots in 12.5 per cent extract. Photo 2. Concentric membraneous structures in the periblem cells. 12-h incubation of roots in 12.5 per cent extract. Photo 3. Concentric, linear and curved membraneous structures in the plerome cells. 12-h incubation in 12.5 per cent extract

Iron hematoxylin, 1500×