Materials and methods. Animals were collected, held, and staged as previously reported[4,7]. Only crabs having melanophores at stage 1 were used in an experiment. The illumination exposure rate varied from 11,600 $\mu w/cm^2$ to 140 $\mu w/cm^2$, as measured with a Gossen 'Luna-Pro' light meter. In all cases the entire animal was above a white background within the cone of illumination coming from the lamp (GE 1630 – 6.5 volts). Exposure times varied from 5 to 15 sec. Melanophores were staged every 5 min after irradiation for a total of 30 min except in the cytochalasin B experiments where they were staged every 15 min for 1 h. Cytochalasin B (Aldrich) at a concentration of 10 $\mu g/ml$ in a 0.1% DMSO crustacean saline[9] solution was perfused into isolated legs[10]. After 15 min in the drug, the legs were irradiated in a white pan for 6 min (GE F15T8-15 watt blacklight lamp near UV) for a total exposure of 400,000 $\mu J/cm^2$ as measured by a Black-Ray UV-meter using a J-221 cell.

Results and discussion. The melanin dispersing responses of intact and eyestalkless crabs to illumination is shown in Figure 1. Intact animals exhibited an integrated response (IR)[11] of 6 at an exposure of 1.9×10^4 $\mu J/cm^2$, while eyestalkless animals required an exposure of 6×10^5 $\mu J/cm^2$ to respond. The additional increase in IR of the eyed crabs at exposures in excess of 6×10^5 $\mu J/cm^2$ could be explained as a primary response. Figure 2 shows clearly that cytochalasin B does indeed inhibit the primary pigment-dispersing response of these melanophores.

These results suggest that in eyed fiddler crabs bright illumination causes the release of some melanin-dispersing hormone in addition to the amount of this hormone that is normally released in response to the shade of background and as a consequence of the circadian and circatidal rhythms of color change of these crabs. This response of the eyed crabs to the increased illumination was probably a response to the brightness of the illumination incident on the eyes directly from the source of illumination. An exposure of approximately 100 times more illumination is required to produce the same IR in eyestalkless crabs than in intact crabs (Figure 1). Even with an unchanging albedo, melanin dispersion increased in eyed crabs with increasing illumination when the exposure of illumination was too weak to evoke a primary response in the melanophores of eyestalkless crabs and presumably also, therefore, in the melanophores of the eyed crabs. These experiments reveal clearly that there is an intensity component that does not depend on the albedo, and that the primary response of the melanophores of *Uca pugilator* has a much higher threshold than does the secondary response. The fact that these intact crabs still exhibit melanin dispersion when exposed to illumination while being held against a white background which fosters melanin concentration[6] is further support for concluding there is a brightness component which is not part of the response to the albedo. Small changes in either the length of the staging period or the amount of illumination used can have a profound effect on the results obtained. As shown in Figure 2, cytochalasin B inhibits pigment dispersion in the melanophores of isolated legs regardless of whether the legs are perfused with melanin-dispersing hormone[8] or exposed to bright illumination (primary response). These observations suggest that the same pigment-dispersing mechanism [perhaps involving microfilaments[12,13]] is responsible for pigment dispersion in these melanophores regardless of whether the dispersion is induced by a blood-borne factor or by direct irradiation of the pigment cells.

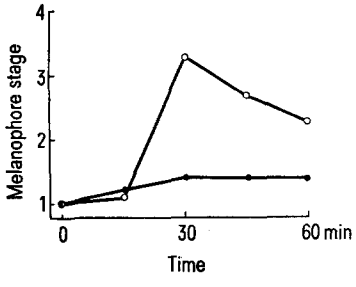

Fig. 2. Effect of cytochalasin B on melanin dispersion in irradiated isolated legs. Closed circles, legs that received cytochalasin B; open circles, control legs. The dark bar shows the period of irradiation of both groups of legs. Each point represents the mean for 20 legs.

[9] C. F. A. PANTIN, J. exp. Biol. *11*, 11 (1973).
[10] M. FINGERMAN, Science *123*, 585 (1956).
[11] C. K. BARTELL and D. J. FLUKE, Radiat. Res. *30*, 811 (1967).
[12] B. S. SPONNER, K. M. YAMADA and N. K. WESSELLS, J. Cell Biol. *49*, 595 (1971).
[13] N. K. WESSELLS, B. S. SPONNER, J. F. ASH, M. D. BRADLEY, M. A. LUDENA, E. L. TAYLOR, J. T. WREEN and K. M. YAMADA, Science *171*, 135 (1971).

The Influence of Cobalt on the Endoplasmatic Reticulum of the Horse Bean (*Vicia faba* L.)

R. HERICH and M. BOBÁK

Department of Plant Physiology, Comenius University, Odborarske nam. 12, CSSR-886 04 Bratislava (Czechoslovakia), 14 November 1975.

Summary. The exogeneous application of cobalt induces the differentiation of membrane complexes of the endoplasmic reticulum. After longer acting of cobalt these membranes are dilated and later destroyed. This fact can manifest itself also in some disturbances of the cell division.

Cobalt is a very interesting element from the physiological point of view. Its application can induce the differentiation of malignant tumors on the one hand[1,2]; it can however also act as a cytostaticum, as a preprophase poison[3]. There is no data on the question, in what way exogeneously added cobalt influences the process of transformation of normal cells into cancerous ones. The ability of cobalt to influence karyokinesis and cytokinesis indicates that heterogeneous cell organelles are influenced by cobalt application.

The horse bean (*Vicia faba* L.) was used as experimental material. The experimental solution of $Co(NO_3)_2$ in distilled water had 0.1 and 0.2% concentrations. The influence of $Co(NO_3)_2$ was investigated after 6, 12, 24 and 48 h of treatment of cobalt solution. As a material for our studies, meristematic cells of root tips were used. These were

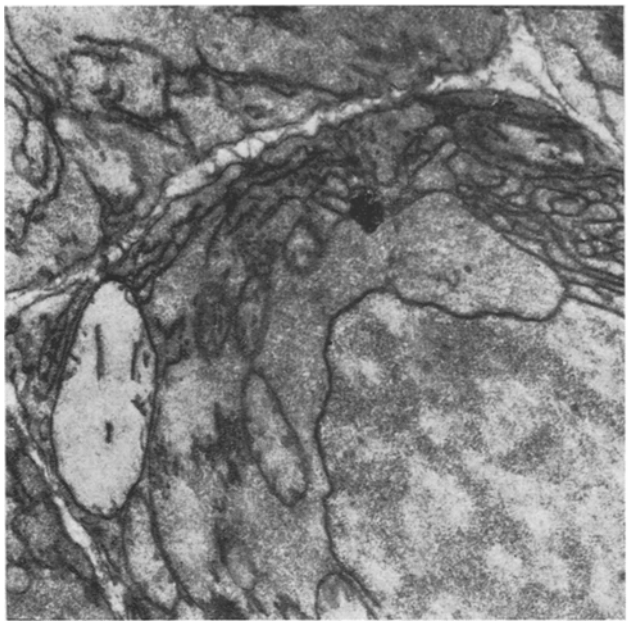

Fig. 1. Differentiation of the membrane complexes of endoplasmic reticulum after the application of the 0.1% solution of Co $(NO_3)_2$ for 6 h. × 12,500.

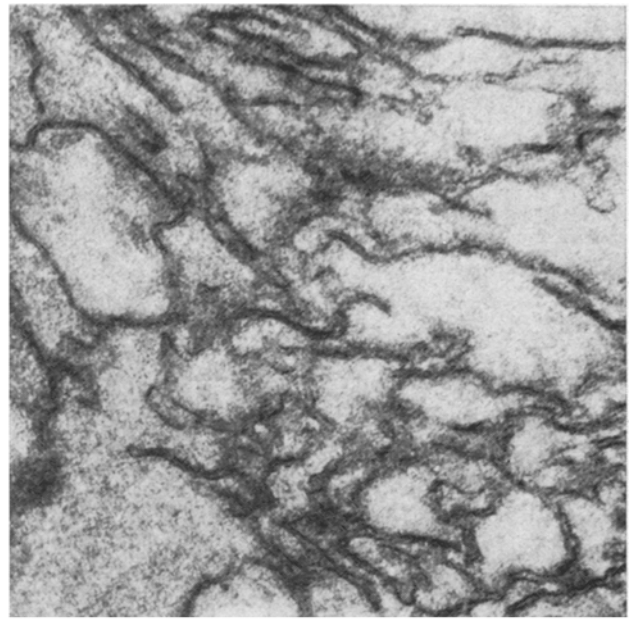

Fig. 3. Dilatation and step by step destruction of the endoplasmic reticulum membranes after application of a 0.2% solution of $CO(NO_3)_2$ for 12 h. × 28,000.

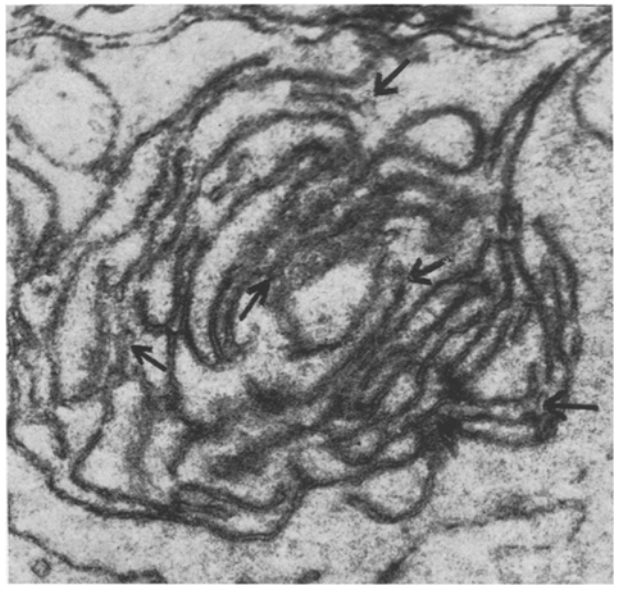

Fig. 2. A more detailed picture of the endoplasmic reticulum membrane complex induced by cobalt application. × 22,000.

fixed by a 1.2% solution of $KMnO_4$ and by glutaraldehyde combined with OsO_4. The ultrathin slices were contrasted according to the method of Reynolds[4].

Starting from previous cytological studies, we were interested in the question of the influence of exogeneously added cobalt on the endoplasmic reticulum (ER) of interphase cells. We supposed that the cytostatical activity of exogeneously added cobalt[3] will manifest itself also on the ER. Our supposition was proved completely right. The influence of exogeneously added cobalt on the ER of interphase cells manifests itself in two directions: 1. in the differentiation and arrangement of the membranes of the ER and 2. by step destruction of the membranes of the ER. The influence of exogeneously added cobalt on the differentiation and on the arrangement of the ER membranes can be well observed on the electronogram No 1. Within the interphasic cells, atypical complexes of ER membranes are differentiated. These ER membrane complexes are irregularly arranged in the basic cytoplasm. The ER is concentrated in most cases into these complexes. Out of them ER can be found only in single cases. An irregular shape of nucleus can be observed on the electronogram. The nuclei not influenced by cobalt are of a more or less global shape.

After longer acting of cobalt, it can be observed especially at higher concentrations that the ER membranes are dilated and later destroyed. This fact can be seen on the electronogram Nos. 2 and 3. The mitochondria can be well observed. They have their typical structure even at that time, when the ER begins to be destroyed after the application of cobalt. We suppose that observed cytostatic effect of cobalt, as well as the inhibition of the transition of the cells from interphase to the prophase after the application of cobalt[3], is in connection with the destroying effect of cobalt on the endoplasmic reticulum. The destroying influence of cobalt upon the ER can manifest itself also in some other disturbance of cell division[5,6].

[1] J. C. Heath, Br. J. Cancer *10*, 668 (1956).
[2] J. C. Heath, Br. J. Cancer *14*, 478 (1960).
[3] R. Herich, Chromosoma *17*, 194 (1965).
[4] E. S. Reynolds, J. Cell Biol. *17*, 208 (1963).
[5] M. Bobák, Acta Fac. Rerum nat. Univ. comen., Bratislava *8*, 17 (1974).
[6] A. Herichová, Acta Fac. Rerum nat. Univ. comen., Bratislava *9*, 73 (1974).