

tillon de vin par examen au microscope électronique après concentration des phages éventuellement présents au moyen d'une série de centrifugations (basse vitesse, haute vitesse et centrifugations zonales).

Les fractions susceptibles de contenir des phages ont été examinées à l'aide d'un microscope électronique Philips EM 300, après coloration négative à l'acide phosphotungstique à 2%, tamponné à pH 7,0.

Des bactériophages de 3 types morphologiques différents ont ainsi pu être mis en évidence: Type 1: a) phage à longue queue, non contractile et peu flexible, type B de BRADLEY<sup>6</sup> (Figure 2); b) petit phage à queue de longueur moyenne, non contractile, mais relativement flexible, type B de BRADLEY (Figure 3). Type 2: petit phage, enrobé dans une couche de mucopolysaccharides, capsomères non visibles, apparemment type E de BRADLEY (Figure 1). Type 3: petit phage, tête allongée, queue non contractile, relativement flexible, se rapprochant des phages du type ML de BRADLEY (Figure 4).

Notre hypothèse de départ a donc été vérifiée: le vin en fermentation malo-lactique est exposé, tout comme

les autres produits fermentés à l'aide de germes lactiques, aux attaques de bactériophages. Il est intéressant de relever que ces phages proviennent d'un produit dont le pH est inférieur à pH 3,5. En effet dans la plupart des autres cas, où des phages ont été mis en évidence, ils se trouvaient dans des milieux beaucoup moins acides.

L'existence de trois types morphologiques différents laisse en outre supposer, a priori, que l'ensemble des bactéries responsables de la fermentation malo-lactique est susceptible de subir une attaque de phages. Ce point ne devra donc pas être négligé si l'on veut un jour pouvoir maîtriser parfaitement cette fermentation.

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## Comparison of the Effects of Illumination on the Melanophores of Intact and Eyestalkless Fiddler Crabs, *Uca pugilator*, and Inhibition of the Primary Response by Cytochalasin B

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**Summary.** Approximately 100 times more illumination is required to produce pigment dispersion in the melanophores of eyestalkless fiddler crabs (*Uca pugilator*) than in the melanophores of intact crabs. The pigment in melanophores of isolated legs will normally disperse in response to irradiation, but this response is inhibited by cytochalasin B.

The melanophores of the fiddler crabs, *Uca pugilator* and *Uca pugnax*, exhibit two responses to illumination. The primary response is a direct response<sup>2</sup> of the melanophore to bright illumination, causing melanin dispersion, which darkens the animals' color<sup>3</sup>. Near UV-light is more effective than visible light in eliciting this response<sup>4</sup>. The secondary response involves color changes that are mediated through the eyes of the animal. Responses to the shade of background are secondary responses whereby the animal typically darkens when placed on a black background and blanches on a white one<sup>5,6</sup>. This response shows no preference to UV- or visible light<sup>7</sup>. In previous

experiments<sup>4,7</sup>, we found differences in pigment dispersion in intact and eyestalkless *Uca pugilator* due to the illumination required to stage the melanophores. We devised the experiments described below to test this difference in animals in which the pigment of both intact and eyestalkless individuals was maximally concentrated at the outset. There has been no similar previous investigation in crustaceans nor has there been any quantitative study of the exposures of illumination required to produce the same amount of pigment dispersion through primary and secondary responses in any animal. The final aim of this investigation was to determine whether the melanin dispersions that can be induced by direct illumination of the melanophores (the primary response) can be inhibited by cytochalasin B as is pigment dispersion that is mediated by the melanin-dispersing hormone<sup>8</sup>.

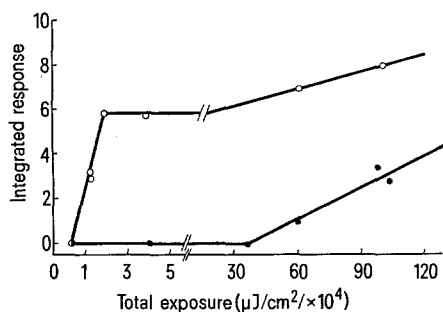


Fig. 1. Relationships between the integrated responses of melanophores of intact (open circles) and eyestalkless (closed circles) crabs as a function of exposure to illumination. Each point represents the average IR of 4 animals.

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**Materials and methods.** Animals were collected, held, and staged as previously reported<sup>4,7</sup>. Only crabs having melanophores at stage 1 were used in an experiment. The illumination exposure rate varied from  $11,600 \mu\text{w}/\text{cm}^2$  to  $140 \mu\text{w}/\text{cm}^2$ , 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  $10 \mu\text{g}/\text{ml}$  in a 0.1% DMSO crustacean saline<sup>9</sup> solution was perfused into isolated legs<sup>10</sup>. 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  $400,000 \mu\text{J}/\text{cm}^2$  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)<sup>11</sup> of 6 at an exposure of  $1.9 \times 10^4 \mu\text{J}/\text{cm}^2$ , while eyestalkless animals required an exposure of  $6 \times 10^5 \mu\text{J}/\text{cm}^2$  to respond. The additional increase in IR of the eyed crabs at exposures in excess of  $6 \times 10^5 \mu\text{J}/\text{cm}^2$  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<sup>6</sup> 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<sup>8</sup> or exposed to bright illumination (primary response). These observations suggest that the same pigment-dispersing mechanism [perhaps involving microfilaments<sup>12,13</sup>] 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.

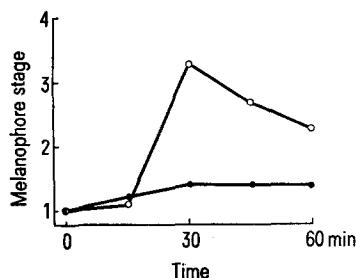


Fig. 2. Effect of cytochalasin B on melanin dispersion in irradiated isolated legs. Closed circles, legs that received cytochalasin B; open circles, control legs. The dark bar shows the period of irradiation of both groups of legs. Each point represents the mean for 20 legs.

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

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**Summary.** The exogenous 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<sup>1,2</sup>; it can however also act as a cytostaticum, as a preprophase poison<sup>3</sup>. There is no data on the question, in what way exogenously 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  $\text{Co}(\text{NO}_3)_2$  in distilled water had 0.1 and 0.2% concentrations. The influence of  $\text{Co}(\text{NO}_3)_2$  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