with TBV, at a multiplicity of 10 $TCID_{50}$ per cell, no shortening of a latent period of IF-positive cell appearance or increase of IF-positive cell number was observed.

Finally, the effect of cell synchronization on the proportion of IF-positive cells in HEp-2-Sof culture was investigated. Preliminary experiments revealed that HEp-2-Sof doubling time did not exceed 48 h. Proliferating pool of the culture was almost the same as previously reported[5]: nearly 95% of cells became labeled after 48 h incubation with 1 μCi/ml ^{3}H-thymidine. These data suggest that HEp-2-Sof cell life cycle has not markedly changed, and enable us to synchronize the cells on the basis of previously reported estimation of HEp-2-Sof cell cycle parameters[5].

Synchronous cultures were obtained by the double thymidine block technique[6]. HEp-2-Sof cells were seeded in tubes containing coverslips at a concentration 1×10^5 cells/ml. 24 h later exponentially growing cultures were treated with 2 mmol of thymidine for 21 h ($G_2 + m + G_1$), then washed 3 times with warm medium, allowed to grow for 10 h (S) and again treated with 2 mmol of thymidine for 21 h. After release from the second block cells were pulse-labelled every 2 h with ^{3}H-thymidine and prepared for autoradiography. Percentage of labelled cells and mitotic index were determined in the same samples stained with haematoxyline. Proportion of IF-positive cells was scored in parallel coverslip cultures. Figure 2 illustrates the pattern of synchronous growth of HEp-2-Sof cell culture. A good correlation has been observed between the entrance of cells into S period and the increase of IF-positive cell number in culture. The proportion of IF-positive cells gradually decreased as the majority of cells left the S period and proceeded to mitosis. Infection of S-phase synchronized HEp-2 cells by TBV caused only a slight increase in number of IF-positive cells as compared to infected asynchronous HEp-2 cells[7]. The alternative results obtained for HEp-2 cells primarily and chronically infected with TBV may reflect the particular type of cell-virus relations at persistent TBV infection in HEp-2-Sof system.

The reason for S-phase dependence of viral antigen production in HEp-2-Sof culture is so far obscure. It seems feasible that the incorporated state of TBV genome in HEp-2-Sof cells[3] is responsible for the observed phenomenon. Expression of several integrative viruses is known to be increased during cellular DNA replication phase[8,9]. Whether S-phase dependent enhancement of TBV antigen production is of identical origin remains to be determined. Nevertheless it is now apparent that cell cycle activities may be operative in TBV antigen production during growth of persistently infected HEp-2-Sof cells.

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Distribution of Cadmium, Zinc and Copper in the Mussel *Mytilus edulis*. Existence of Cadmium-Binding Proteins Similar to Metallothioneins[1]

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Summary. The study of the distribution of Cd, Zn and Cu in homogenates of mussels has shown that Zn and Cu are principally associated with high mol. wt. proteins. The same distribution is observed for Cd in untreated mussels, but in Cd chronically intoxicated animals, the metal is principally bound to low mol. wt. proteins synthesized in response to the uptake of the cation and similar to metallothioneins of vertebrates.

The existence of metallothioneins, specific Zn-, Cd-, Hg- and Cu-binding proteins, is now quite established in various organs of mammals, birds and recently of fishes[3,4]. These proteins, present in the cytoplasmic soluble fraction, are characterized by their extraordinary metal-binding capacity, their high content in cystein (approximately $^1/_3$ of the total amino acid residues), their low molecular weight (about 10,000) and their inducible nature on administration of heavy metals[4–7]. The role of metallothioneins in the detoxification of Cd and Hg has frequently been implied[8–10]. The very important acccumulation of these two metals in the organisms, as well as their very slow rate of elimination, is likely to be explained by their binding to metallothioneins.

To our knowledge, there is no report on the finding of such proteins in invertebrates. Owing to the enormous cadmium accumulation observed in molluscs[11], the possible existence of metallothioneins in one species of the phylum has been investigated.

Methods. For each essay, the soft parts of 2 mussels are pooled and homogenized in 3 volumes of 0.5 *M* sucrose by means of a Polytron Homogenizer. The supernatants obtained by centrifugation at 100,000 *g* are chromatographied on Sephadex G-75 or LKB Ultrogel AcA 54 columns equilibrated in NH_4HCO_3 0.05 *M*. The fractions are analyzed for Cd, Zn and Cu by atomic absorption spectrophotometry.

Results and discussion. The results presented in Figure 1 have been confirmed by many other chromatographies. Graphs A and B show that the Cd accumulated by the mussels during the chronic intoxications is associated with 3 fractions. Fraction I is situated at the level of high molecular weight (mol. wt.) proteins. Fraction II, when large columns are used, corresponds to 2 unresolved peaks, the second one (IIb) having an elution volume corresponding exactly to that observed for metallothionein in different eel tissues[4]; the position of this peak is characteristic of substances having a mol. wt. close to 10,000. Fraction III has the same elution volume as free Cd. It must be pointed out that a number of authors have already observed heterogeneity at the level of the Cd-binding peak of low mol. wt.[3,9,12,13]. Note that fraction II does not contain high amounts of Zn and Cu, in contrast to metallothionein from eel liver[14].

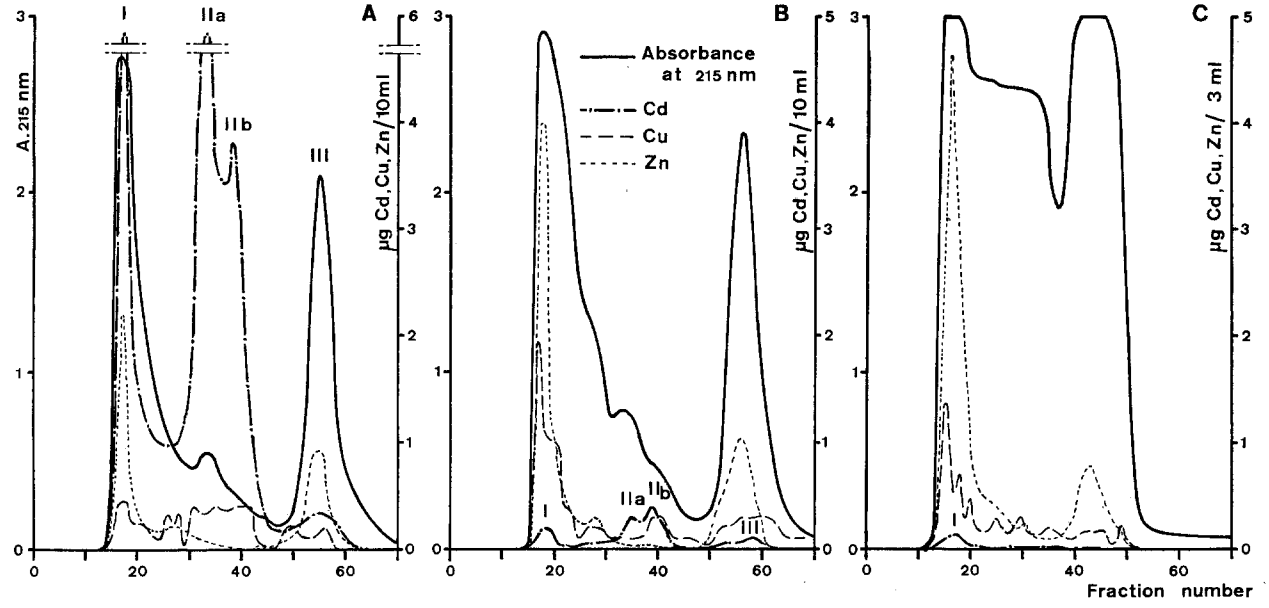

Fig. 1. Elution profiles on Sephadex G-75 column 76 × 3 cm (graphs A and B) or on Ultrogel column 40 × 2 cm (graph C) of supernatants of mussels.
A) Intoxication of 36 days in sea water containing 0.13 ppm Cd (as $CdCl_2$). Concentration of Cd in animals: 80 ppm wet wt. B) Intoxication of 90 days in sea water containing 0.005 ppm Cd. Concentration of Cd in animals: 2.8 ppm wet wt. C) Untreated mussels from the Belgian coast of the North Sea (concentration of Cd in water: 0.0001 à 0.001 ppm). Concentration of Cd in animals: about 1 ppm wet wt. In the 3 cases, the supernatant accounted for a little more than 50% of Cd. Fractions volume: 10 ml (*A* and *B*) or 3 ml (*C*).

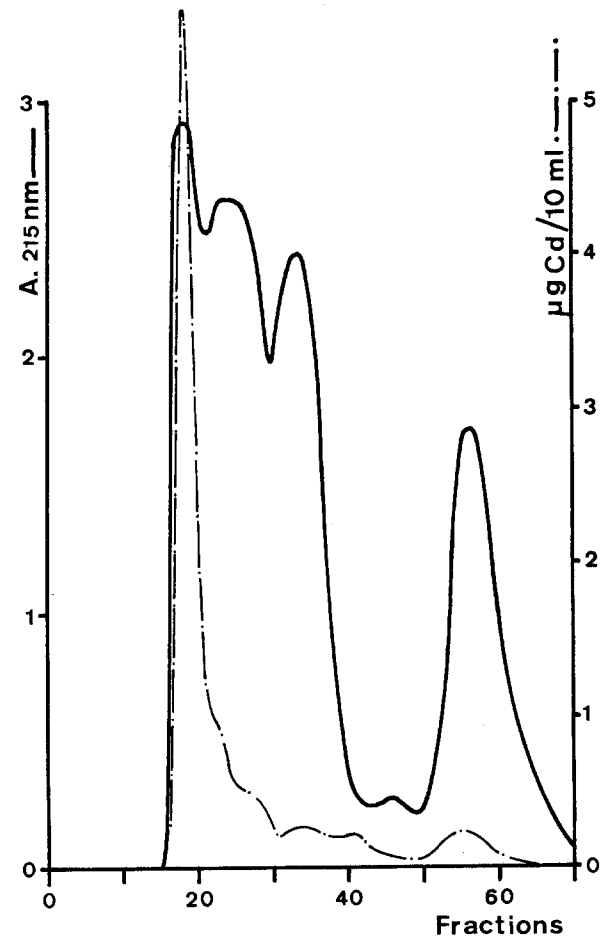

Fig. 2. Elution profile on Sephadex G-75 column (76 × 3 cm) of supernatant from mussels intoxicated during 3 days in sea water containing 13 ppm Cd (acute intoxication). Concentration of Cd in animals: 26 ppm wet wt. Fractions volume: 10 ml.

In the control mussels (graph C), Cd is almost exclusively bound to high mol. wt. proteins. This result and the fact that in the case of a short intoxication (Figure 2) the fraction II only contains very little Cd, seems to indicate that the metalloproteins contained in fraction II on chromatograms of animals submitted to a long Cd exposure does not exist in detectable amount in control mussels. It thus appears that Cd, even at very low doses, induces the synthesis of low mol. wt. Cd-binding proteins (Cd-BP) in mussel tissues.

According to preliminary results, this Cd-BP possesses a high percentage of cysteine and an ultraviolet absorption spectrum typical of metallothioneins. However, further isolation steps are now necessary for a better characterization.

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Thus, like various vertebrates, mussels are able to react in presence of Cd by producing Cd-BP of low mol. wt. that seem to belong to the group of metallothioneins. In mammals, the induction of metallothioneins is considered to be a protective mechanism against the toxic Cd^{++} ion. The existence of such proteins in molluscs would allow them to accumulate large amounts of Cd and to resist it, thus becoming a potential danger to man as food.

As OLAFSON and THOMPSON[3] have recently pointed out, in referring to the finding of a Cd-BP of low mol. wt. in blue green alga[15]: 'it thus appears that metallothioneins may be ubiquitous in the living world'.

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Effect of Air Ionization on Blood Serotonin in vitro[1]

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Summary. The effect of negative and positive air ionisation on siliconized blood serotonin was studied in vitro. The experiments showed that within 10 min positive ionisation increased serotonin levels in total blood (+40%), plasma (+90%), erythrocytes (+50%) and thrombocytes (+240%). On the other hand, negative ionization (10 min) lowered the serotonin content of total blood (−30%), plasma (−42.5%), erythrocytes (−41.7%) and thrombocytes (−72.3%), thus confirming the 'Krueger Effect' in vitro.

KRUEGER and SMITH[3] have shown that positive air ions cause accumulation of serotonin in the respiratory tract from where it may be transmitted to the entire body. They concluded that serotonin might be the major cause of the functional changes produced by positive air ions, as e.g. $(H_3O)^+ \cdot (H_2O)_n$. They further postulated that the reversal of positive ion effects through negative air ions, $O_2^- (H_2O)_n$ and $OH^- (H_2O)_n$, depends on accelerating the oxidation rate of free serotonin to 5-hydroxyindole acetic acid by the cytochrome-cytochrome oxidase system[4]. This working theory has been confirmed by many other reports on the effect of air ionization in vivo[5–8]. As it has recently been challenged by ANDERSEN[9], we decided to study the 'Krueger Effect' in vitro.

Materials and methods. Human blood was drawn in siliconized syringes from the cubital vein and immediately transferred to siliconized test tubes containing 1/10 volume of a mixture of 15% K_3EDTA and 0.02% potassium sorbate to prevent clotting[10]. The pH of the resultant blood was 7.6–7.8. 5 ml blood was spread on an open siliconized glass Petri dish 10 cm in diameter and exposed to either negative or positive ionisation. Negative ionization was provided by Ionotron-20 (Amcor-Amron, Herzliya, Israel) which has an output of $3.6 \times 10^5/cm^3/sec$ negative ions at a distance of 30 cm. For the production of positive ions, the wiring was reversed. After trying different distances and exposure periods yielding similar results, the distance chosen was 30 cm and exposure times set at 10 and 30 min, since no evaporation occurred during these periods and the pH remained constant. At 10 and 30 min, 1 ml aliquots were assayed for serotonin content[10] of whole blood, plasma, erythrocytes and thrombocytes, according to the separation method of the American Association of Blood Banks[11]. The plasma was separated from the cells by 10 min centrifugation in a Sorval RC-3 refrigerated centrifuge at $900 \times g$. The thrombocytes were separated from the erythrocytes by 10 min centrifugation at $4{,}500 \times g$ in the same centrifuge, resulting in a 90–95% separation, as evidence by microscopic inspection. A control not exposed to ionization was run simultaneously. All experiments were performed at room temperature (23 °C, at a relative humidity of 50 ± 5%).

Effect of negative and positive air ionization on blood serotonin in vitro

Experiments and Controls		Negative air ionization				Positive air ionization			
		10 min Serotonin (ng/ml)	Recovery (%)	30 min Serotonin (ng/ml)	Recovery (%)	10 min Serotonin (ng/ml)	Recovery (%)	30 min Serotonin (ng/ml)	Recovery (%)
Total Blood	Control	128 ± 12	100	110 ± 13	100	142 ± 18	100	136 ± 19	100
	Ionized	89 ± 11 [b]	69.5	84 ± 11 [b]	76.3	194 ± 20 [b]	140	197 ± 24 [b]	145
Plasma	Control	40 ± 4	100	42 ± 8	100	44 ± 12	100	47 ± 7	100
	Ionized	23 ± 5 [b]	57.5	21 ± 8	50	83 ± 16 [a]	190	70 ± 10 [b]	150
Erythrocytes	Control	63 ± 8	100	67 ± 10	100	69 ± 10	100	56 ± 12	100
	Ionized	37 ± 6 [a]	58.3	29 ± 6 [a]	43.3	103 ± 15 [a]	150	110 ± 20 [a]	198
Thrombocytes	Control	18 ± 4	100	20 ± 7	100	10 ± 8	100	11 ± 5	100
	Ionized	5 ± 1 [a]	27.7	5 ± 2 [a]	25	34 ± 7 [a]	340	28 ± 7 [a]	255

Each figure represents 6 experiments ± SEM. [a] $p < 0.001$, [b] $p < 0.05$.