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] $p < 0.05$.

Statistical evaluation was made by Student's *t*-test.

Results. As is shown in the Table, whole blood serotonin of normal blood amounted to 110–142 ng/ml. Negative ionization reduced these values to 84–89 ng/ml, whereas positive ionization raised them to 194–197 ng/ml. Blood fractionation into plasma, erythrocytes and thrombocytes showed a similar trend in all 3 blood constituents. Blood plasma contained 40–47 ng/ml serotonin; negative ionization reduced these values to 21–23 ng/ml, while positive ionization raised them to 70–83 ng/ml. The erythrocyte suspension contained 56–69 ng/ml serotonin; negative ionization reduced these values to 29–37 ng/ml, while positive ionization increased them to 103–110 ng/ml. The thrombocyte suspension contained only 10–20 ng/ml serotonin; negative ionization reduced these values to 5 ng/ml, whereas positive ionization increased them to 28–34 ng/ml.

Discussion. The experiments demonstrate the propensity of positive ions to cause serotonin release from the blood and that of negative ions to suppress such release in vitro. Thus the theory of the 'Krueger Effect' has been well proved. Krueger himself showed in vivo that big concentrations of positive ions raised serotonin blood levels in mice, while high concentrations of negative ions lowered them[7].

These results also indicate that during the separation of the thrombocytes from the erythrocytes part of them decayed and released their serotonin. In order to study the disposal of this serotonin, we carried out the experiments listed in the Table with siliconized blood fractions of plasma, erythrocytes and thrombocytes; furthermore with non-siliconized syringes and Petri dishes on full blood. In the former set-up, positive air ionization increased serotonin in the thrombocyte fraction only. In the latter set-up, microscopic inspection showed complete decay of the thrombocytes which had released their serotonin. This was taken up by the plasma (20–30%) and the erythrocytes (70–80%); in other words, in the absence of thrombocytes, serotonin transport is taken over by the erythrocytes, a phenomenon not duly appreciated until now. The decrease in serotonin levels after negative ionization in vitro is probably due to its normal breakdown by monoamine oxydase[12]. However, in vivo serotonin can also be converted to 5-hydroxyindole acetic acid (5-HIAA) by the cytochrome oxidase system, as shown by KRUEGER and SMITH[3]. The mechanisms of this breakdown are now studied by us in vivo and in vitro by applying special enzyme inhibitors.

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Initial Transdetermination in the First Leg Discs of Different *Drosophila* Species[1]

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Summary. Initial transdetermination leads from lateral and medial halves of male first leg imaginal discs almost exclusively to structures of the base and the spread (blade) of the wing. Mesonotum never appeared. The frequency of transdetermination is species-specific and most probably cell-autonomous. Medial halves transdetermine more frequently than lateral halves. Under the influence of an equivalent amount of blastema growth *D. nigromelanica* transdetermine with a much higher frequency than *D. virilis*.

Transdetermination can be defined as a change of the determined state of cells to a different state from which they will initiate a pathway of differentiation that leads to structures that no longer correspond to the initial state of determination.

Transdetermination was discovered in blastemas of larval imaginal discs that were first cultured in the abdomen of adult females[4]. In this medium no differentiation but an extensive proliferation occurs. In order to find out whether the determined state remained stable, test pieces of the proliferated blastemas were transplanted back into metamorphosing larval hosts. With this method we found that there exists for each disc a given probability for the event and the direction of transdetermination[5–9]. After an initial transdetermination event, which would for example lead in *Drosophila melanogaster* from genital blastemas to leg structures, changes of second and third order could lead to wing or head and then to the notum of the mesothorax. Transdetermination of higher orders seems to occur only in cultures which have been transferred twice or many times from one adult host to another.

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