Metabolic energy requirements during teleost melanophore adaptations[1]

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Summary. Inhibition of oxidative phosphorylation by 2,4-dinitrophenol or sodium cyanide promotes complete melanosome aggregation in teleost melanophores. This aggregation is not promoted via the α-sympathetic receptor sites because it occurs in the presence of tolazoline hydrochloride, an α-receptor blocking agent. Interpretation of these results suggests metabolic energy release is required in the centrifugal direction only.

Visual and microcinematographic observations of pigment granule migrations in dermal teleost melanophores have demonstrated differences in the movements of individual melanosomes during aggregation and dispersion[2]. Centripetal granule migration was continuous and smooth; centrifugal migration was discontinuous and erratic. It has been proposed that melanophores whose pigment granules are not migrating are metabolically inactive while those with migrating granules are in an active state[3,4]. Inhibitors of oxidative phosphorylation have been shown in *Fundulus* melanophores to diminish the extent of granule redispersion in a dispersing medium[5], while the presence of ATP has been found necessary for pigment dispersion[6]. In the experiments reported below, the action of 2 inhibitors of oxidative phosphorylation, 2,4-dinitrophenol (DNP) and sodium cyanide (NaCN) dissolved in a dispersing saline has been found to induce in teleost dermal melanophores a complete and reversible aggregation different in nature from that induced by the catecholamines noradrenalin (NAD) and adrenalin (AD).

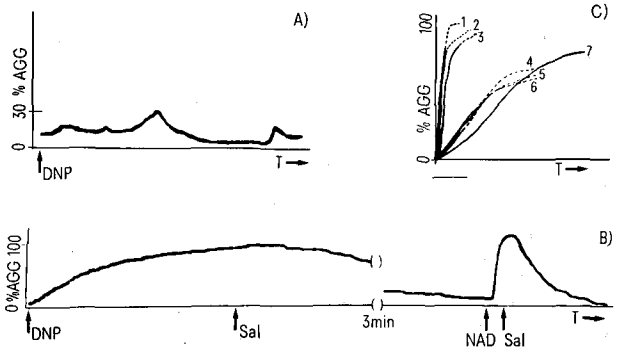
Methods. Chemicals tested on the melanophores were dissolved in plaice saline[7]. Melanophores immersed in the saline alone dispersed from an aggregated state and maintained dispersion. Skin from the teleost, *Pseudopleuronectes americanus*, a close relative of plaice, *Pleuronectes platessa*, was isolated in a perfusion chamber mounted on a Leitz binocular microscope. Both the interdigitating scales and the epidermis[8] were removed to facilitate diffusion. Changes in light transmission due to melanophore responses exposed to the various solutions were monitored by an RCA 931-A photomultiplier tube mounted via a light pipe to one eyepiece of the microscope. Simultaneous visual observations were made with the other eyepiece. The voltage between the anode and the photocathode varied between 1000 and 1250 V, depending upon the particular preparation. The output of the photomultiplier was recorded on an HP 680 strip chart recorder. A Roscolene No. 815 filter was interposed between the light source and the photomultiplier to eliminate light variations due to the concurrent erythrophore granule migrations.

Results and discussion. The action of 10^{-5} M DNP and 10^{-4} M NaCN has previously been reported to diminish the extent of dispersion[5]. In my experiments, above a threshold concentration of 10^{-4} M DNP and 10^{-4} M NaCN, every melanophore responded with a slow, nearly complete and reversible aggregation. Below that level, no visible effect was apparent. At 10^{-4} DNP, dispersed melanophores exhibited irregular pulsations (figure, A). Aggregated melanophores showed no response to either inhibitor except to extend the time needed for initiation of the subsequent saline-induced disperion.

The time characteristics of DNP- and NaCN-induced aggregation differed from catecholamine (CAT)-induced aggregation. The rate of aggregation induced by an oxidative phosphorylation inhibitor (OPI) was significantly slower than that for a catecholamine stimulant (figure, B, C and table). Onset of DNP-induced aggregation was delayed about 4–5 times compared to that of CAT-induced aggregation. Redispersion by saline was not

	Centripetal direction	Centrifugal direction
Ratio of time intervals between 20% and 80% maximal response: (OPI / CAT)	$6.1 \leqslant R_1 \leqslant 15.5$* n = 6	$1 \leqslant R_2 \leqslant 3.0$* n = 6
Ratio of onset time: delay (CAT / OPI)	0.23 ± 0.08 n = 6	0.41 ± 0.18 n = 6

*The wide variation represents the variations in the absolute rates recorded during different experiments.



A Pulsations due to application of 10^{-4} M DNP; time mark: 7.5 min. *B* Melanophore response to 5×10^{-4} M DNP followed by subsequent saline wash and 5×10^{-6} M NAD followed by saline wash; time mark: 30 sec. *C* Centrifugal response to various substances (numbered) normalized to T = 0: 5×10^{-4} M NAD (Nos 1–3); 5×10^{-4} M DNP (4–6); 5×10^{-4} M DNP and 10^{-3} M TOL (7): the dotted line indicates saline rinse; time mark: 30 sec.

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simply determined by the washout time, but depended upon the substance inducing aggregation. The rate of redispersion following DNP-induced aggregation was twice as slow as that for the catecholamine concentration inducing just-maximal aggregation. In addition, the rate of redispersion following DNP-induced aggregation slowed during the course of an experiment. Preparations were studied typically for 3–4 h. Experiments were terminated when the melanophores would not disperse following a 25-min perfusion with normal saline. The cells at that time were not completely unresponsive, because individual melanosomes at the edge of the aggregated melanosome mass displayed a degree of movement, although they never entered a cellular process.

In the presence of tolazoline hydrochloride (TOL), an α-sympathetic receptor blocking agent[9], the catecholamines no longer aggregated the melanophores[10,11]. TOL had no effect upon the aggregating properties of DNP or NaCN (figure, C), suggesting that these latter 2 agents are acting directly at the source of metabolic energy for the intracellular translocation mechanism.

The effects observed in these experiments suggest that a metabolic energy pool of limited size is available for melanosome migration in melanophores, and that it is specifically necessary for centrifugal migration. Once exhausted, centripetal migration necessarily follows. It has been suggested that microtubules are a permanent substrate upon which the melanosomes migrate, because their presence in the cellular processes does not correlate with the position of the melanosomes[12]. The observation of lucent areas surrounding microtubules in dispersed melanophores[13] suggests that a contractile element might be present in association with the microtubules. The motive force for melanosome migration might be provided through the action of that element acting against the fixed microtubular array. It is interesting to note that similar concentrations of DNP stopped protoplasmic streaming in the slime mold *Physarum polycephalum*[14] and particle extrusion by filipodia in 3T3 cells[15]. The intracellular components inactivated in both experiments were thought to be microfilaments. Interference with microfilament integrity in melanophores by cytochalasin B has been shown to inhibit centrifugal melanosome migration in *Rana pipiens*[16]. It is tempting to speculate that microfilaments[17] or filamentous-like materials are the likely candidates as the site of metabolic activity required for centrifugal melanosome migration in teleost melanophores.

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The effects of chronic peroxide intake on the peroxide metabolism enzyme activities of rat organs[1]

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Summary. In rats receiving a dilute aqueous solution of hydrogen peroxide for a prolonged period, the activity of the peroxide metabolising enzymes, i.e. superoxide dismutase, peroxidase and catalase, is significantly increased in most tissues.

The H_2O_2-producing superoxide dismutase (SOD; EC 1. 15.1.1), and the 2 most active H_2O_2-decomposing enzymes, peroxidase (P; EC 1.11.7) and catalase (C; EC 1.11.1.6) form the group of peroxide metabolizing enzymes (PME)[3,4]. Use of this collective term is justified, among others, by the fact that it is widely assumed that these 3 enzymes are the first appearance of forms of the protein-bound Cu and Fe in the course of evolution. All 3 enzymes are of great importance in aerobic life; they take part in the dismutation of the toxic radicale formed from molecular oxygen, the superoxide anion (O_2^-) formed by addition of 1 electron, and the peroxy radical (O_2^{2-}) formed by uptake of 2 electrons[5]. The aim of our experiments was to establish whether the levels of the PME in the various tissues are affected by chronic oral intake of reagent H_2O_2.

Materials and methods. Examinations were carried out on the CFY inbred rat strain, animals of the same age and sex being compared. The experimental animals were reared under identical conditions, with the exception that 1 group received 0.5% H_2O_2 added to the drinking water from the age of 1 month. Water-drinking rats were taken as controls. At the age of 3 months, rats from both groups were killed, and the activities of the PME in tissue homogenizates were determined[3,4]. The protein contents of the homogenizates were measured with the Folin phenol reagent by the method of Lowry et al.[6].

Results and discussion. The results are listed in the table. In the table, one column gives the results of the control measurements, with the $X \pm S$ values. The enzymatic activites have in all cases been calculated for 1 g wet tissue weight ($n = 10$).

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