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Quantitative Analysis of the Microtubule System in Isolated Fish Melanophores

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Isolated melanophores of the angelfish, Pterophyllum scalare, have been used in a morphometric analysis and a quantitative study of their microtubule system. Using transverse sections spaced at regular intervals, the changes associated with the process of pigment aggregation have been determined. Upon the concentration of pigment granules in the central cell region, almost half of the cytoplasmic portion is also withdrawn from the peripheral cell regions. Counts of microtubules within a cell sector in cells with pigment aggregated and dispersed, respectively, reveal a) a constancy of the number of microtubules in this sector regardless of the distance from the cell center, and b) a reduction of microtubule number in cells with pigment aggregated by about 58%. On the basis of these counts, the total number of microtubules has been calculated. In the dispersed state, about 2,400 microtubules extend between the center and the periphery of the cell, while their number is about 1,000 in the aggregated state.

Using a 13-protofilament model of a microtubule and relevant data on size and molecular weight of microtubule subunits, the amount of tubulin present as microtubules is calculated. In the average, the cells contain $1.95 \cdot 10^8$ monomers corresponding to $1.78 \cdot 10^{-8}$ mg tubulin. A tentative estimation of the concentration of tubulin inside a melanophore yields values of 6.1 mg/ml for the whole cell and 16.5 mg/ml for the cytoplasm alone (excluding membrane-bound organelles). Based on this estimation, a comparison with microtubule assembly in vitro is made.

Key words: fish melanophores, electron microscopy, microtubules, tubulin, quantitative analysis

The movement of pigment granules in vertebrate chromatophores has early been the subject of scientific interest [2]. Among the cell types studied so far, pigment cells of fish have always received particular attention. The ease with which granule movements can be observed even in an ordinary light microscope, and the impressive velocities of granule movements in some types of chromatophores, made them suitable as a model system for the study of vesicle transport within cells. Knowledge of the mechanism of pigment movements will certainly help to understand intracellular movement in general.

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A broad spectrum of both morphological and physiological evidence leaves little doubt that microtubules play an essential role in pigment granule translocation [1, 7, 15, 29]. Particularly their behavior during pigment aggregation and dispersion has recently been a major point of interest. Porter [14] concluded from his studies on erythrophores of Holocentrus ascensionis that microtubules disassemble, at least in part, during aggregation and reassemble with dispersion. A more quantitative approach has been undertaken in two other studies where microtubules have been counted in melanophores in the dispersed and aggregated state, respectively [10, 21]. Unfortunately, the results of these counts are contradictory: While Schliwa and Bereiter-Hahn [21] observed, in concurrence with the view of Porter [14], a substantial reduction in the number of microtubules upon pigment aggregation, Murphy and Tilney [10] stated that their number stays unchanged.

In an attempt to clarify this point we have examined the distribution of microtubules in a pigment cell system slightly different from those used so far. Melanophores were isolated from the dermal tissue and allowed to settle on a glass coverslip. This procedure, while preserving the physiological activities of the cells, allows circumvention of some of the difficulties involved with quantitative electron microscopy of tissue melanophores. The use of this system not only facilitates oriented sectioning for a morphometric analysis but also offers other advantages in comparison with cells in situ: Due to a missing barrier of epidermal cells, ion, hormone or drug treatment immediately reaches the cell without diminution or delay of its effect. The same is true for fixatives, so that the image of the fixed cell can be expected to resemble more closely that of the living cell immediately before fixation.

This report describes morphological and physiological characteristics of isolated melanophores with particular reference to the microtubule system. The morphometric data presented here tempted us to determine the content of microtubules and to estimate the concentration of tubulin in this cell.

MATERIALS AND METHODS

Isolation of Cells

Single scales of adult specimens of the cichlid Pterophyllum scalare are removed with tweezers and immediately transferred to Ringer's solution. For one experiment, 2–4 scales are treated with 0.5–2 mg/ml collagenase for periods of 20 min up to 1 h in calcium-free Ringer's solution. The duration of treatment depends on the type of collagenase used and even on its lot number. Good results are achieved with type II collagenase from Serva (Heidelberg). Hyaluronidase used initially proved not to be necessary and has been omitted in later experiments. During the last step of enzyme treatment melanophores are freed from the remainder of the scale with a gentle water stream generated with a pipette. The floating cells are carefully collected with a micropipette under a dissecting microscope and, together with a small drop of calcium-Ringer's, are placed on a coverslip. Cells settle and attach within a few minutes. In order to allow easy observation and rapid change of media, coverslips with melanophores are mounted on a microscopic slide with two glass stripes as socles. The cells are kept in a 1:3 mixture of amphibian culture medium (Gibco Biocult, New York) and Ringer's solution. If the last steps of isolation are performed under a hood, isolated cells can be kept viable under sterile conditions for up to four days. Usually, experiments are performed within 3–6 h after isolation under semi-sterile conditions.

Light Microscopy

For light microscopic observations, a Leitz Orthoplan microscope equipped with Nomarski interference contrast optics is used. Ilford Pan F film served as photographic material.

Electron Microscopy

Isolated cells are fixed with 3% glutaraldehyde in either 0.1 M cacodylate or phosphate buffer (pH 7.2) for 15–30 min, followed by 2% osmium tetroxide made up with potassium bichromate and sodium chloride [21] for 30 min. After rapid dehydration in ethanol cells are embedded in an Epon-Araldite mixture. Following polymerization of the resin, coverslips are removed with a cold shock in liquid nitrogen. Single isolated cells are then either cut exactly parallel to the substrate surface or reembedded for sections perpendicular to the surface. For this purpose, the desired starting point for the transverse sections is marked, a small block of resin containing the cell is cut out and then enclosed in fresh embedding medium. Sections cut with a diamond knife on an LKB ultramicrotome are stained with lead citrate and examined on a Hitachi H 500 electron microscope.

Morphometry

For morphometric measurements and counts of microtubules, prints of electron micrographs with a final magnification of 40,000 are used. Area determinations are performed on a MOP AM 02 image analyzer (Kontron, Munich).

Solutions

Ringer's solution is of the same composition as described previously [21]. Fresh solutions of adrenalin (10^{-4} M), atropine (10^{-4} M), theophylline (10^{-3} M), cyclic adenosine-monophosphate (10^{-4} M), and dibutyryl-cyclic adenosine-monophosphate (10^{-4} M) are made up in culture medium before each experiment. Colchicine (Serva, Heidelberg) is used in a concentration of $5 \cdot 10^{-5}$ M.

RESULTS

Light Microscopy

Under the isolation conditions described in Materials and Methods melanophores remain in the dispersed state after separation from the scale and they retain most of the cell processes. Attachment on a glass surface is enhanced by the presence of calcium ions in the Ringer's solution; within 2–3 min the cells are firmly attached so that a medium change does not detach or distort them.

Under proper circumstances, the appearance of a freshly isolated cell closely resembles that of a cell in the scale. In many instances, however, they are more or less asymmetric. Within a few hours of culture the cells lose their dendritic outline by fusion of lateral cell process membranes and spread on the substratum. Apart from the spreading process, no other modifications of the cell outline are observed; in particular, the cells remain completely immobile. Within 10 h of culture many cells assume a more or less discoidal shape (Fig 1). Spreading is completely inhibited by $5 \cdot 10^{-5}$ M colchicine, indicating an involvement of microtubules in this process.

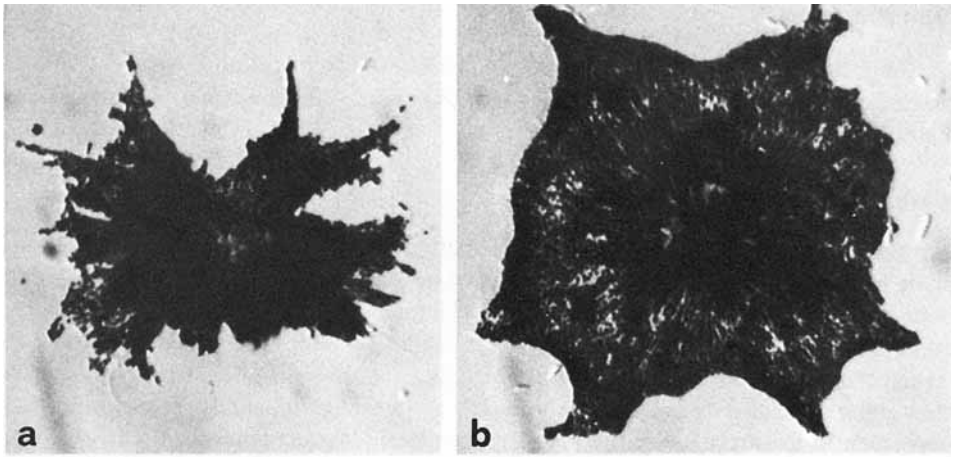

Fig 1. The effect of culture on the outline of isolated melanophores: a) cell photographed immediately after isolation from the scale; b) same cell after 16 h of culture (× 740).

The behavior of melanosomes in isolated melanophores is quite similar to that of cells in situ and need not to be described in detail here. Briefly, melanosomes are in constant centripetal and centrifugal motion with only short distances being traversed. Mass movements, the complete aggregation of all melanosomes in the cell center and their redispersion, occur at slightly lower velocities as in cells in the scale (1.5–2 μ/sec for aggregation, 0.3–0.6 μ/sec for dispersion).

One important difference between isolated and tissue melanophores deserves to be mentioned. Cells in the scale can be induced to aggregate their pigment by elevating the concentration of potassium ions in the medium, and redispersion is initiated by lowering the concentration of this ion. Variations in the concentration of potassium and even a repacement of most of the other cations by this ion have no effect on the movement of melanosomes in isolated melanophores. The cells remain in the dispersed state as if in Ringer's solution.

Like melanophores in situ, mass aggregation can be induced by adrenalin. As a potent dispersing agent, atropine has been used predominantly, but the cells also disperse their pigment granules in response to cyclic adenosine-monophosphate and its dibutyryl derivative.

Electron Microscopy

Transverse sections demonstrate that isolated melanophores in the dispersed state are of more or less constant thickness with a slight elevation at the cell center where the nucleus is located (Fig 2a). In the aggregated state, the nucleus may be displaced to the upper periphery of the melanosome mass (Fig 2b). The pigment granules form a spherical body whose diameter depends on the size and melanosome content of the cell and varies between 8 and 10 μ. The centrioles maintain a middle position within the pigment mass (Fig 2b). The transition between the spherical mass of pigment granules and the flat melanosome-free remainder of the cell shows a slight constriction.

Using sections taken at 10, 15, and 20 μ distance from the cell center (the centriolar region), some morphological parameters of cells with pigment dispersed and aggregated, respectively, have been determined (Table I). Upon the aggregation of pigment

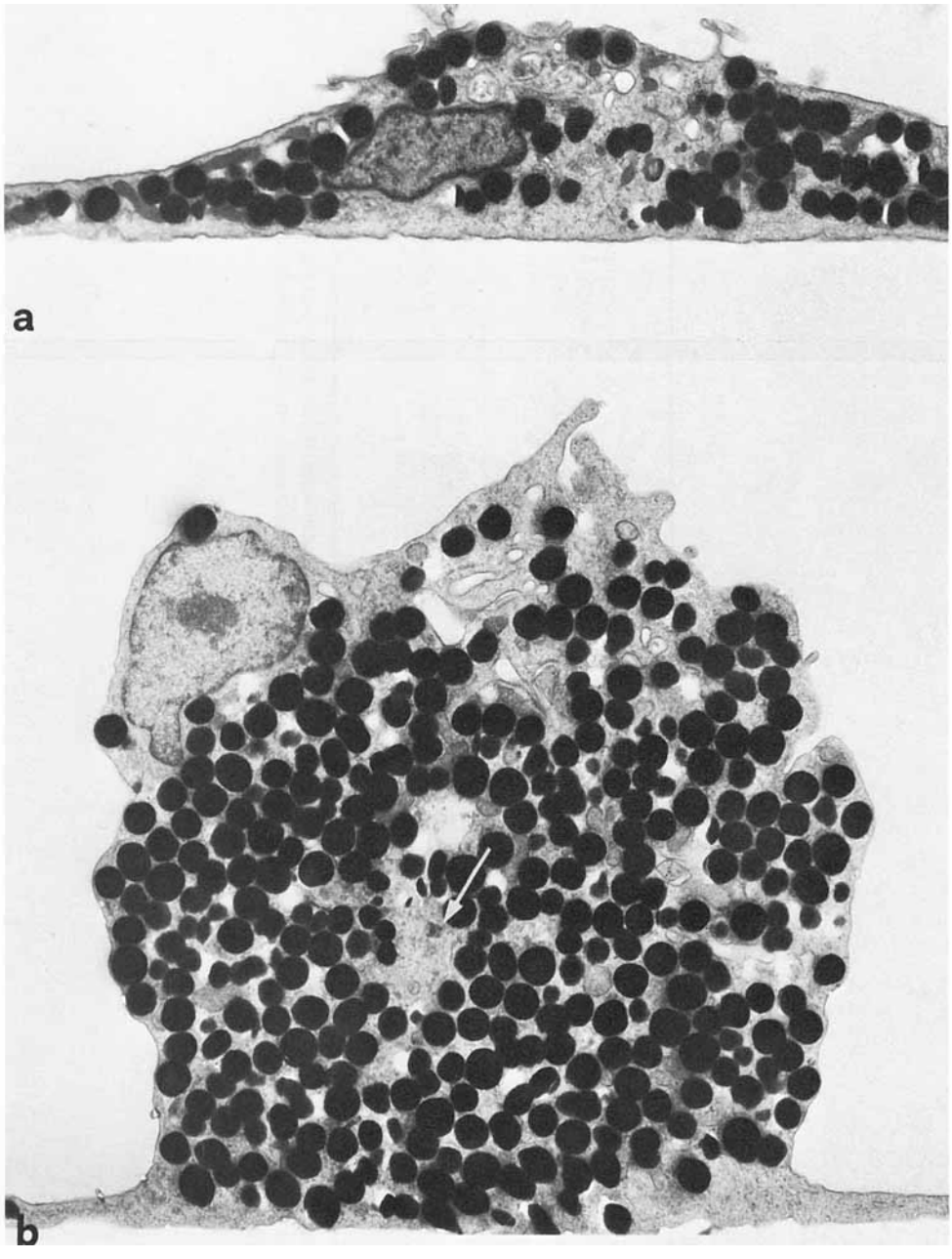

Fig 2. Transverse section at the level of the centriolar region: a) melanophore in the dispersed state; b) melanophore in the aggregated state. The nucleus is displaced to the periphery of the sperhical mass of pigment granules. Arrow indicates the centriole (× 10,200).

granules in the cell center, the thickness of the cell periphery is greatly reduced, measuring only 25% of the value of cells with pigment dispersed (Table I, columns 1–3; see also Fig 4). The reduction in thickness is not only due to the elimination of pigment granules and other membrane-bound organelles (mitochondria, vesicles) which make up about 63%

TABLE I. Some Morphometric Parameters of Isolated Melanophores

Distance from cell center (μ)	1 Thickness pigment dispersed (μ)	2 Thickness pigment aggregated (μ)	3 % thickness (aggregated)	4 Membrane-bound organelles	5 Cytoplasm (dispersed)	6 Cytoplasm (aggregated)	7 % reduction of cytoplasm upon aggregation
				volume density			
10	2.0 ± 0.3	0.6 ± 0.2	30	65 ± 7	35 ± 7	21 ± 8	40
15	1.4 ± 0.3	0.3 ± 0.1	21	61 ± 8	39 ± 8	20 ± 8	49 ($P < 0.01$)
20	1.3 ± 0.2	0.3 ± 0.1	23	62 ± 6	38 ± 6	21 ± 7	45
average	1.6	0.4	24.7	62.7	37.3	20.7	44.7

Measurements were made in six cells in the dispersed state and five cells in the aggregated state. Determinations of the fraction of the volume occupied by various organelles and cytoplasm are based on stereological measurements using methods outlined by Weibel [28].

of the total cell volume (column 4). A considerable portion of the cytoplasm is also withdrawn (columns 5 and 6); about 45% apparently moves with the other cell constituents to the central cell region (column 7).

With respect to microtubules, even a single thin section made parallel to the substrate reveals some of their characteristics in isolated melanophores (Fig 3): their precise radial arrangement relative to the cell center, their straightness, and also their relative length. Transverse sections of cells with pigment dispersed show microtubules to be more or less evenly distributed throughout the cross section area with a slight tendency to associate with the upper and lower membrane (Fig 4a). The number of microtubules is apparently reduced in cells with pigment aggregated (Fig 4b).

In order to quantitatively follow changes in microtubule number in isolated melanophores, microtubules have been counted in transverse sections of cells in the dispersed and aggregated state, respectively. The sections in which counts were made are spaced at intervals of 5 μ with a section at the level of the centrioles as starting point. The results of counts made in a cell sector (see Fig 5) are summarized in Table II. A remarkable difference in the number of microtubules between the two states is revealed. Despite the relatively low number of cells in which counts were made, this difference is highly significant ($P < 0.01$ according to Student's t test).

Using the figures listed in Table II, the total number of microtubules present at distances of 5, 10, 15, and 20 μ from the cell center can be determined if it is assumed that the microtubules counted in the respective transsections also pass the corresponding segment of a circle (Fig 5).

The total number of microtubules (N) then is

$$N = n \cdot \frac{360^\circ}{\alpha}$$

where n is the number of microtubules counted and α the sector angle (in that case 22.6°). From Table III it can be inferred that the total number of microtubules at 5, 10, 15, and 20 μ from the cell center varies slightly -- equaling approximately 2,400 in the dispersed state and approximately 1,000 in the aggregated state. The reduction in number of microtubules in the aggregated state is about 58% regardless of the distance from the cell center.

Estimation of the Content of Tubulin

Given the average dimensions of isolated melanophores, we will be able to estimate the content of tubulin in this cell type.

Number and length of microtubules. The calculations shown in Table III suggest, but do not prove, a continuity of the microtubules present at various distances from the cell center. However, according to results of microtubule tracings in scale melanophores [20] a continuity of microtubules between their presumptive site of origin in the cell center and the cell process base has been demonstrated. In addition, the overwhelming majority of microtubules within a 6-μ-long segment of a cell process extends its entire length, suggesting a continuity from the base to the tip of the cell process. This impression also arises from immunofluorescence microscopy with tubulin antibodies [23], although in that case individual microtubules are difficult to resolve.

If these and the present findings are combined, we can conclude that an isolated melanophore in the dispersed state has about 2,400 unfragmented microtubules running from the center to the periphery of the cell. The slightly lower number of microtubules

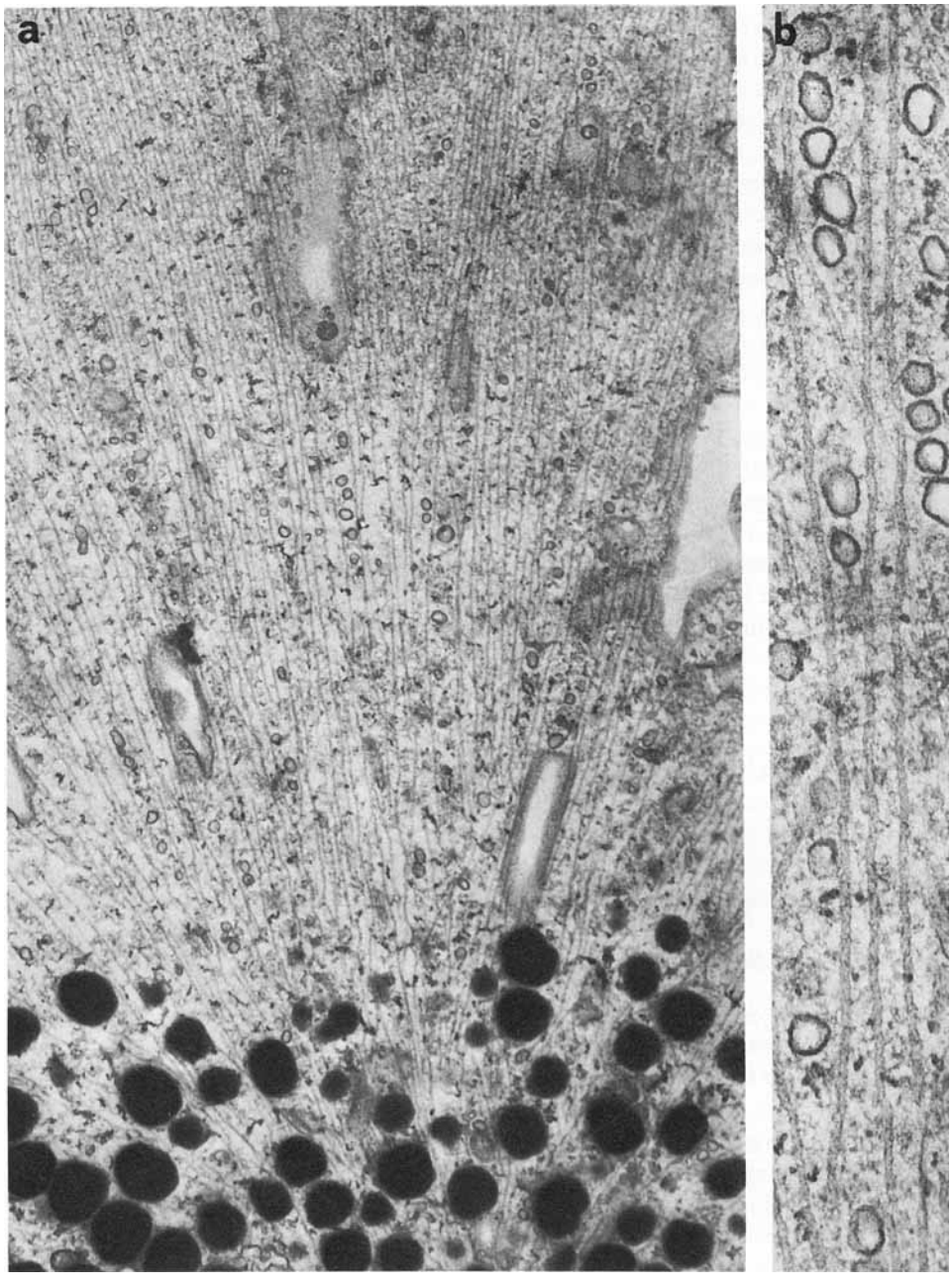

Fig 3. Horizontal section of an isolated melanophore in the aggregated state: a) section from the bottom side of the cell. Note the precise radial arrangement of microtubules relative to the cell center and their straight course (× 22,000); b) higher magnification of microtubules extending almost the entire length of the micrograph (× 46,000).

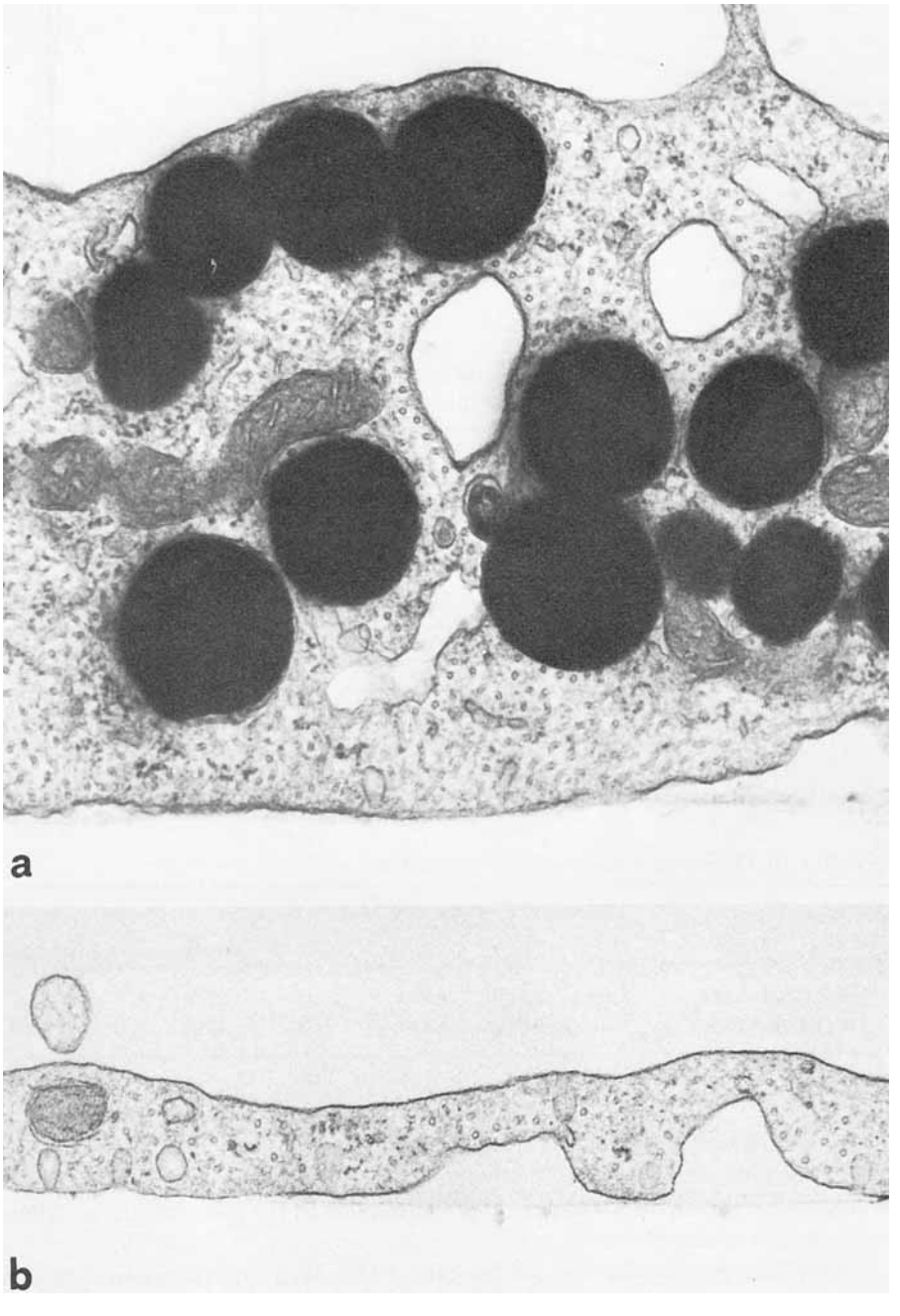

Fig 4. Transverse section at a distance of 5 μ from the centriole: a) dispersed state; b) aggregated state (× 34,000).

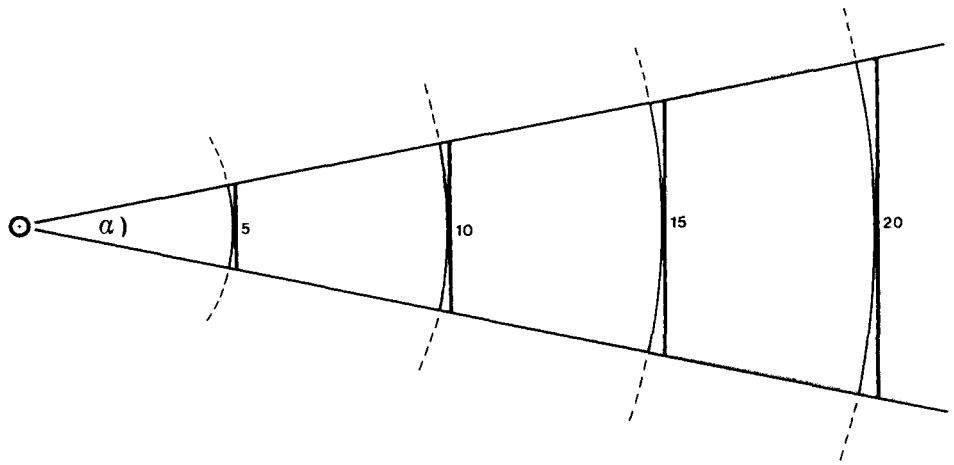

Fig 5. Scheme illustrating the size of the segment (α) and location of the transsections (5, 10, 15, 20 μ) in which microtubule counts were made. For the calculation of the total number of microtubules it is assumed that the microtubules also pass the respective segment of a circle indicated by a thin line.

TABLE II. Counts of Microtubules in a Cell Sector (sector angle 22.6°)

	Distance from cell center (μ)			
	5	10	15	20
Pigment dispersed	150 ± 22	160 ± 18	151 ± 24	138 ± 19
Pigment aggregated	58 ± 14	66 ± 10	70 ± 13	58 ± 18

Number of cells in which counts were made: pigment dispersed, five; pigment aggregated, four.

TABLE III. Calculated Total Number of Microtubules

	Distance from cell center (μ)					
	5	10	15	20	average	percentage
Pigment dispersed	2,390	2,550	2,405	2,200	2,386	100%
Pigment aggregated	925	1,050	1,115	925	1,004	42.1%

in the cell periphery (20 μ) could be attributable to the fact that in this place some of the microtubules have already ended. The total number of microtubules in the medium-sized cells used here seems to be remarkably constant because of the low standard deviations of the microtubule counts (Table II).

The melanophores used here can be envisaged in rough approximation as disks with an average diameter of 52 μ (range: 45–65 μ). The average length of a microtubule, then, should be slightly less than the radius, since not all microtubules reach the very cell center or the periphery. We therefore assume our "model cell" with a diameter of 52 μ to have 2,400 microtubules with an average length of 25 μ.

Total amount of tubulin. For a calculation of the total amount of tubulin in polymerized state, additional data are needed.

a. A microtubule consists of linearly arranged protofilaments. Although their number seems to vary in different cell types [3], the most widespread protofilament number appears to be 13 [25]. We therefore assume the microtubules in melanophores also to be composed of 13 protofilaments.

b. Various studies show the protofilaments to be made up by subunits 4 × 5 nm in diameter with the 4-nm axis along the long axis of the protofilament (see review by Stephens and Edds [24]). This subunit most probably corresponds to the tubulin monomer with a molecular weight of 55,000 daltons [24].

Using these data, the total number of microtubule monomers present as microtubules in a cell in the dispersed state then is

$$\frac{2{,}400 \cdot 25 \cdot 1{,}000 \cdot 13}{4} = 1.95 \cdot 10^8$$

Since one mole of tubulin monomers with a molecular weight of 55,000 consists of $6.02 \cdot 10^{23}$ monomers, $1.95 \cdot 10^8$ monomers correspond to an amount of tubulin equal to

$$\frac{1.95 \cdot 10^8 \cdot 55{,}000}{6.02 \cdot 10^{23}} = 1.78 \cdot 10^{-8} \text{ mg}$$

Concentration of tubulin in Pterophyllum melanophores. With the morphometric data of isolated cells shown in Table I and additional measurements, a determination of the average volume of an isolated melanophore can be made. Given this volume, we will be able to tentatively calculate the concentration of tubulin within the cell.

As stated above, the diameters of the cells used here vary between 45 and 65 μ, the mean value being 52 μ. The thickness of individual cells, however, varies to a greater extent, being 3–4 μ in the central cell region and 1–1.5 μ in the periphery. A reasonable approach to a determination of cell volume can be made if our model cell is assumed to be composed of three stacked disks of cytoplasm with diameters of 52, 30, and 6 μ and thicknesses of 1.2, 0.5, and 1.5 μ, respectively (Fig 6; see also Fig 2a).
The total cell volume, then, is

$$\pi(26^2 \cdot 1.2 + 15^2 \cdot 0.5 + 3^2 \cdot 1.5)\,\mu^3 = 2{,}940\,\mu^3 \approx 2.94 \cdot 10^{-9} \text{ ml}$$

and the concentration of tubulin

$$\frac{1.78 \cdot 10^{-8} \text{ mg}}{2.94 \cdot 10^{-9} \text{ ml}} = 6.1 \text{ mg/ml}.$$

According to the morphometric data (Table I), the cytoplasmic phase of melanophores constitutes only about 37% of the total cell volume, while the rest is occupied by membrane-bound organelles. If this is taken into account, the concentration of tubulin in

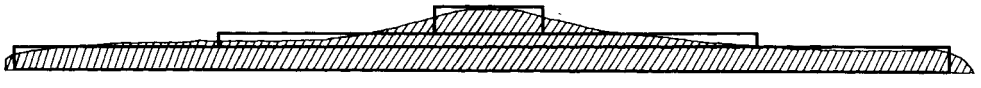

Fig 6. For a calculation of cell volume, a melanophore (hatched area) is split into three stacked disks. For further explanations see text.

the cytoplasm is 16.5 mg/ml. Calculations made for individual cells by using their specific data yield values between 14.2 and 19.5 mg/ml.

DISCUSSION

We made use of isolated melanophores in an investigation of the distribution of microtubules. The advantage of this system lies in the morphologic disposition of the flatly spread cells, while at the same time their physiologic properties are largely retained. As regards their response to hormones and the velocities of pigment movements, isolated melanophores are quite comparable to those in the scale. There seems to be only one difference with respect to the response to potassium ions. The pigment-concentrating action of potassium has been attributed to an effect on still intact nerve endings near the melanophores in scale preparations [7]. Since isolated melanophores have no nerve connections, this view is strongly supported. Interestingly, isolated erythrophores of the marine fish Holocentrus, which are also innervated in the scale [14], still are able to respond to potassium ions [4]. The difference between these two cell types seems difficult to explain at present. Electrophysiological experiments should show whether differences in the reaction of the membrane potential account for different responses.

The electron microscopic observations extend earlier studies made on cells in situ. Rounding up of the cell center and flattening of the cell periphery upon pigment aggregation are similar to the events described previously [19, 21], with the exception that the mass of aggregated pigment granules arches above the flat remainder of the cell. The process of pigment aggregation is not selective in the sense that only the melanosomes migrate to the cell center. Almost half of the cytoplasmic matrix (Table I) and most of the mitochondria obviously move together with the melanosomes. It is not established at present what cytoplasmic constituents, particularly structural components, are redistributed together with the pigment granules; most probably the material of the so-called microtrabeculae seen in whole-cell preparations under the high-voltage electron microscope [4] represents a prominent part of the constituents that move in association with the melanosomes*. According to Byers and Porter [4], the remaining cytoplasm is prevented from withdrawal by the persisting microtubules. However, two observations are not consistent with this view. First, melanophores devoid of microtubules (after cold plus colchicine treatment) remain completely unchanged in outline [22]. Second, if cells are not provided with a substrate but freely float in the culture medium, all cell processes will retract upon pigment aggregation [11], and of course all microtubules must disassemble. In that case, a situation similar to that commonly existing in sea urchin chromatophores is experimentally provoked, since these cells completely retract and extend their cell processes during pigment movements [26]. The recent demonstration of microtubules in these cells [27] further suggests that a disassembly-assembly cycle of all microtubules is associated with phases of complete aggregation and redispersion of pigment. In fish chromatophores, the persistence of part of the microtubules and the prevention of withdrawal of cell processes undoubtedly are interdependent phenomena, but it seems difficult to state cause and effect. The experiments with floating cells [11], however, clearly emphasize the importance of a substrate for the stabilization of cell processes.

The strictly radial arrangement of microtubules in combination with their straightness facilitated their quantitation in this particular cell type. The results of the micro-

*Schliwa M, Euteneuer U: High voltage electron microscopy of isolated fish melanophores. Manuscript submitted for publication.

tubule counts demonstrate a constancy of the number of microtubules at varying distances from the cell center which, at least in cells with pigment dispersed, reflect a continuity of microtubules from the center to the periphery of the cell [20]. Although tracings of microtubules in cells with pigment aggregated have not yet been carried out, a continuity of these microtubules may be inferred since the counts (Table III) vary only slightly around 1,000; our electron microscopic studies also support this contention because microtubules may be followed over long distances even in a single thin section. The results therefore suggest a complete disassembly of about 60% of the microtubules upon pigment aggregation. The factors that induce and control this process are presently not known. In principle, it could be either a passive or an active process. As a passive side phenomenon, the microtubule frame that is no longer needed in the aggregated state is eliminated. Microtubule disassembly could also be a necessary condition actively induced by the cell to somehow support ordered movements. The present status of our investigations seems to favour the latter possibility since pigment aggregation is also accompanied by profound changes in the organization of the initiating center of microtubules in the cell center (M. Schliwa and U. Euteneuer, manuscript in preparation).

Knowledge of total number and average length of microtubules in cells with pigment dispersed led us to estimate the amount of tubulin present as microtubules. Calculations of this nature may be of interest for a consideration of in vivo polymerization. Our value of $1.78 \cdot 10^{-8}$ mg tubulin for the "model cell" is of a similar order of magnitude as that estimated previously for the first-division spindle of Arbacia eggs ($2.7 \cdot 10^{-8}$ mg if the data of Cohen and Rebhun [5] are converted to a monomer diameter of 4 nm) or for spindles of crane fly spermatocytes ($0.57 \cdot 10^{-8}$ mg [6]). All these estimations are based on microtubule counts and therefore only the amount of tubulin in the polymerized state is taken into account. The amount of tubulin actually present in the cells may be higher; in Arbacia eggs, the total amount of tubulin is $12 \cdot 10^{-8}$ mg per egg according to a vinblastine precipitation method [16].

Despite the similarities of these calculations, the concentrations per cell may differ because the cells are of quite different size. For isolated melanophores, a value of about 6 mg/ml tubulin for the whole cell is obtained. Similar calculations for the Arbacia egg yield values of 0.18 mg/ml if the data of Cohen and Rebhun [5] are used and 0.52 mg/ml if the total microtubule protein pool is taken into account [16]. Even in that case a value about 10 times less than in melanophores is obtained.

An assessment of the concentration of tubulin is of particular importance for a tentative theoretical consideration of the rate of microtubule assembly in a living melanophore. The observations on these cells suggest that microtubule disassembly during pigment aggregation and reassembly during dispersion occur at similar velocities as the movements of pigment [19]. In case of redispersion, this means that microtubule polymerization takes place at a rate in the order of 0.4 μ/sec. According to in vitro studies of microtubule assembly, the rate of polymerization clearly depends on the concentration of tubulin [9, 18]. Rosenbaum et al [18] found a linear relation between protein concentration (up to 3 mg/ml) and rate of assembly. If their data are extrapolated to 15–20 mg/ml, the amount of assembled tubulin in the melanophore cytoplasm, an assembly rate of nearly 0.2 μ/sec results. Tubulin concentrations of more than 5 mg/ml yield an explosive assembly phase when shifted from 0° to 37°, which usually is completed within less than 1 min [8]. These characteristics correspond quite well to those of the pigment dispersion that takes place at velocities of 0.3–0.6 μ/sec and is completed within 40–60 sec.

Melanophores possibly also have a pool of unassembled subunits in excess of those present in the polymeric form. Such a pool has previously been shown to exist not only in Arbacia eggs [16] but also in rat pancreas cells [13] and mouse liver cells [12], where only 35% and 40%, respectively, of the total amount of tubulin seems to be in the form of microtubules. The "augmentation phenomenon" induced by glycols [17] also indicates the presence of a considerable subunit pool that normally does not participate in the formation of microtubules. Assuming a similar protein pool in melanophores, tubulin concentrations of 30–40 mg/ml in the cytoplasm result. However, the importance of such a subunit reservoir in the assembly process remains to be determined.

The assembly conditions in the living cell, particularly the factors that control the rate of assembly (or disassembly), are presently poorly understood. A comparison with in vitro assembly may be inadmissible at present. The cell probably operates with more effective mechanisms. Nevertheless, estimations of this kind may at least give hints for an understanding of differences and mutualities of microtubule behavior in different cell types.

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