

PHYSIOLOGICAL AND PSYCHOLOGICAL ASPECTS OF
FLATFISH CAMOUFLAGE

by

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ABSTRACT

1. Two flatfish, Paralichthys lethostigma and Pseudopleuronectes americanus, responded to changes in the substrate texture by changing two general features of their skin appearance: average body reflectance and average contrast between spots and the background. (These controls resemble the brightness and contrast controls of a television set.)
2. Body reflectance is related to the dermal melanophore-iridophore complex. Variations in average reflectance are due to changes in the adapted state of dermal melanophores. Detailed reflectances from different parts of the skin vary because the relations in the melanophore-iridophore complex depend upon position within the skin.
3. The fixed spots contain a high density of epidermal melanophores. The density of epidermal melanophores elsewhere varies from 0 to less than 50% of the number in the fixed spot regions.
4. Density of dermal iridophores varies inversely with the density of epidermal melanophores. The density of dermal melanophores is approximately uniform throughout the entire skin surface.
5. The junction between the nerve and dermal melanophore seen in electron microscopy appears to be a typical sympathetic junction. Nerve profiles within 600Å of a dermal melanophore always contained small vesicles with dense cores. The dense cores were enhanced with 5-hydroxydopamine; 6-

hydroxydopamine eliminated nerve profiles close to the melanophores. These observations are consistent with an exclusively catecholamine innervation to dermal melanophores.

6. The cellular physiology of dermal melanophores was investigated in detail. Melanosomes aggregated in response to catecholamines. Alpha-receptor site blocking agents prevented catecholamine-induced aggregation. Adrenalin reversal was observed indicating the presence of beta-receptor sites.

7. Melanosomes aggregated automatically when oxidative phosphorylation was blocked by DNP and NaCN. Aggregation induced in this manner was not inhibited by Tolazoline hydrochloride, an alpha-site blocking agent. Pigment granule dispersion required a constant supply of metabolic energy.

8. The function of the nerve innervation to dermal melanophores is to control the degree of melanophore aggregation. Parker's notion of dual nerve innervation controlling melanophore aggregation and dispersion is incorrect in P. americanus.

9. Flatfish exhibit both camouflage and predatory behaviors which seem predicated on their physical appearance. Their appearance appears to conform to the known rules of texture matching. The success of their camouflage and predatory behaviors appears to be based on the inability of other visual animals to discriminate flatfish from their backgrounds.

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INTRODUCTION

Fishes of the order Heterosomata, the flatfishes, have been attributed in the zoological literature a unique and remarkable ability: active control of visible texture adaptation (Sumner, 1911; Mast, 1916). Changes in appearances relating to the reflectance, color and texture of flatfish skin were described. The first systematic studies of the variations available to these fish were conducted early this century, although certain comments interspersed earlier research (Cunningham, 1890). In more modern terminology, the flatfish was attributed in regards to a textural response the ability to actively mimic various aspects of a spatial frequency representation of the bottom upon which the fish lay. A claim was made (Pitkin, 1912, cited in Mast, 1916) that the fish directly transformed its retinal image into an observable pattern painted on the fish's skin. Sumner (1911), Mast (1916) and Osborn (1939) have shown that the transformation could not possibly have been exact. The variations in appearance available to the fish were restricted by the morphology of the skin and the distributions of chromatophores within it. More recently, Chinarina (1960) demonstrated that flatfishes in the Soviet Union behave like all others; and de Groot et al. (1969) showed that the pattern must lie below the fish, not along the walls of the holding tank.

Since the original reports, the phenomenon of flatfish texture adaptation has assumed legendary proportions.¹

This thesis reexamines the basic phenomenon and examines the physiological system controlling aspects of the adaptations. It concludes that the facts of camouflage change are less dramatic than reported, and

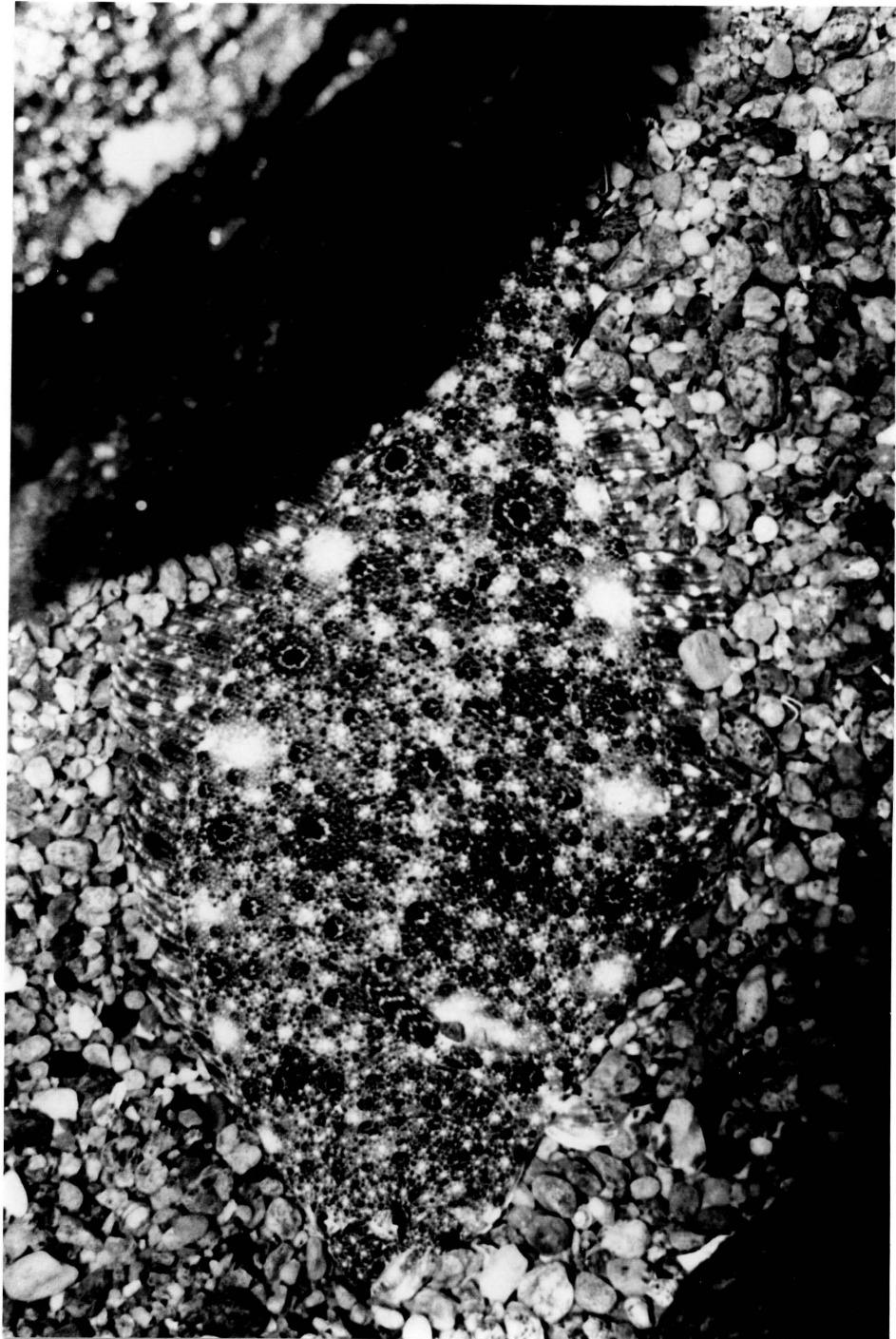
attributes the previous reports to certain psychological effects in the observers' vision not known to the early researchers. Further, it proposes that most, if not all, seen changes in textural appearance of flatfishes can be accounted for by simple and general variations in skin reflectance and contrast of patches within the skin much like the controls of brightness and contrast of television.

Of the 30,000 species of fish, flatfish appearance is singular. The first section examines the natural history of flatfish as it relates to camouflage.

Next I ask, how do flatfish perform on different environments? In the second section, the behavior of Paralichthys lethostigma and Pseudopleuronectes americanus is examined. Changes in their textural appearances were restricted to two dimensions: skin reflectance and spot contrast. When a fish rested upon a background suitable to its structure (Fig. 1), it camouflaged far better than when the sizes of the background elements differed from the sizes of its scales and from the sizes of its skin markings (see Fig. 2-5).

Presentation of the results, both past and present, depended upon techniques which could be shown to exaggerate the changes in fish appearance. A photograph involves the compression of the dynamic range of a scene onto the dynamic range of a negative image. A print from the negative involves a second compression because print material has an inherently restricted dynamic range. Consequently, the dynamic distribution of the final image may vary markedly from the original scene when photographic technique is uncontrolled. Moreover, the dynamic range of the human eye varies depending upon its adaptation state (Craik, 1938). Since a visual impression varies depending upon several factors, including physiological ones like retinal adaptation state and

Figure 1: Cryptic camouflage of Paralichthys lethostigma



psychological ones such as attention, a good match between impression and photographic image may be engineered. This phenomenon is most apparent in the famous 'checkerboard' adaptations displayed by flatfish.

Part Three is concerned with the morphology of the visible skin. Within the skin are the chromatophores, the pigment-containing cells. There are three types in the flatfish I investigated: Iridophores, containing crystalline platelets that reflect and scatter light; Melanophores, star-shaped cells containing melanin granules (melanosomes) that migrate into and out of the cellular processes; and bright-colored cells, stellate cells (reddish Erythrophores and yellowish Xanthophores) containing carotenoid- and pteridine-filled vesicles that migrate within the cell processes. Behavioral adaptations of the fish are reflected by a pigment distribution change within the chromatophores. This section identifies the number and distribution of chromatophore types relating to textural appearance and identifies a structural basis for the difference in contrast between adjacent areas leading to the appearance of patches.

Epidermal melanophores were found to be associated with the contrast relationship. Dark skin regions that stood out from the rest of the skin were found to be densely packed with epidermal melanophores. The number of epidermal melanophores in regions not as distinct was found to be significantly less. In regions consistently lighter, none was found.

The iridophore distribution was found to be the inverse to that of the epidermal melanophores. Dermal melanophores were uniformly (within 20%) distributed everywhere in the skin. During melanophore adaptations these cells act to modulate the light reflecting from iridophores. When the processes were filled with melanosomes, little light reflected from iridophores and the fish appeared dark. When the processes were

mostly empty, much light reflected from iridophores and the fish appeared light. Hence, the dermal melanophores acted to set the background reflectance upon which the epidermal melanophores 'set' the contrast relationship. In other words, changes in skin reflectance, observed behaviorally, correspond to the physiological adaptations of dermal melanophores.

Lastly, the mechanism of physiological control over the dermal melanophores has been examined in Parts Four and Five. I show that dermal melanophores are innervated by one class of catecholamine-containing fibers; and that the pigmentomotor fibers act to modify melanophore adaptations from a position of dispersed pigment granules to some degree of aggregation. This physiology agrees with the behavioral notion of an automatic system without feedback that changes the fish's appearance.

The phenomenon of flatfish camouflage raises questions with respect to the perceptual mechanisms used in detecting camouflaging animals. Detection of a camouflaged animal has certain curious aspects. The predominant requirement for detection of a form is its edge. Animals prevent the visualization of a continuous body edge through multiple devices. A body edge reflects a sharp reflectance discontinuity between body and background. By diminishing the extent of the discontinuity, the edge becomes less distinct. Flatfish do not present a sharp edge because their fins are transparent and carry certain chromatophore patterns. The fins act to grade smoothly the texture of the bottom to the texture of the skin, thereby preventing a sharp discontinuity. Flatfish also throw sand on their fins by flexing the dorsal and ventral fins a number of times (the Bedding Reaction, Breder, 1955). Though

the relations presented in the last section are speculative, they present certain properties belonging to form-perceiving animals that camouflaging animals must deceive to remain camouflaged.

PART ONE: ECOLOGY OF FLATFISH CAMOUFLAGE

The adaptive properties of flatfish correlate well with its unique way of life. Flatfish are members of the only fish order exhibiting an asymmetric structure subsequent to larval metamorphosis. Unlike other bottom dwellers whose dorsoventral axis is compressed and mediolateral expanded (for example, the skates and rays, *Raiiformes* and *Torpediniformes*) or the local New England goosefish (*Lophius americanus*), the flatfish's mediolateral axis is compressed and its dorsoventral expanded. During metamorphosis, members of the order *Heterosomata* rotate 90° on their rostrocaudal axis, clockwise for the family *Bothidae* and counterclockwise for members of the families *Pleuronectidae*, *Soleidae*, *Cynoglossidae* and *Psettodidae* (Norman, 1934). This rotation is accompanied by various structural adaptations. The underside eye migrates to the upper side (Williams, 1902); the underside nasal pore is shifted or diminished in size as is the related olfactory lobe and the underside skeletal muscle structure develops less than the upper side (Cole and Johnstone, 1901). As any fisherman knows, the underside skeletal muscle is white and thinner than the darker upper side musculature. Platt (1976) has demonstrated central nervous system reorganization between the vestibular and eye muscle nuclei relating to the change in body orientation.

The asymmetric pigment cell distribution is most striking. The side in view from above contains a full complement of chromatophore types while the side in contact with the substrate (except for abnormally pigmented individuals) has only a vast plexus of iridocytes. During metamorphosis, flatfish lose the pigment cells in

the skin of the side upon which the fish will rest (Agassiz, 1879; Norman, 1934). The loss of pigment on the underside is a phylogenetic development correlated with the demersal niche these fish occupy. Illumination of a flatfish from below has been found to induce ventral skin melanogenesis (Cunningham, 1891, 1893, 1895; Osborn, 1940, 1941), but not to diminish melanophore loss during or just after metamorphosis (Agassiz, 1879). The Greenland halibut (Reinhardtus hippoglossoides) has been observed to occupy the epipelagic zone. Correspondingly, it is normally pigmented on both sides (de Groot, 1970).

It is most apparent to any viewer that the pigmentation of the visible side serves to camouflage an individual flatfish against its natural background. Camouflage is a relational property between an observer and the observed. A camouflaged animal is not easily distinguished from its background even though it may clearly be identified as different from the background when isolated from it as in a photographic cutout or placed in the center of a photograph, as is commonly presented. As Cott (1957) pointed out, animal forms are recognized by an observer because they differ from one another in various observational aspects: color, hue, shading, or texture. From the observer's point of view, elimination of these differences in a natural setting is sufficient to obscure the camouflager. That is not the same as saying a camouflager must look like its setting as has been claimed for flatfish. Thayer (1909) described the pigment side pattern as an "exquisite imitation of the surface of the sand bed; or marked with variegated pebble patterns. . . or with seaweed colors and patterns." Sumner (1911) noted that the spots of a flatfish "convey the impression

of depressions among the pebbles which seem to surround them." It is saying that a camouflaged animal in its setting must not be perceptible in the Helmholtz sense (Treatise on Physiological Optics, V. III, p. 1, Dover 1962 reprint of 1925 edition), of having a visual existence, form and position.

Cott argued that the shapes and forms of animals that actively or passively camouflage themselves necessarily contained specific adaptations that aid their obscurity. He identified four general aspects of appearance around which most variations in structure occurred. These features are color resemblance to the background, countershading, disruptive coloration (a superimposed pattern that does not follow the outline of the animal) and elimination of a shadow. These aspects of visualization may be condensed into one condition: the elimination of the effect of light upon a three-dimensional object against differently textured backgrounds. Those four aspects mentioned by Cott provide a description of the classes of structural modifications that have evolved successfully to fulfill the required condition.

It is interesting to note that the modifications in appearance evolved due to the interactions between the structure modifying and its natural predators or observers. Predators preyed on those animals whose appearances were distinguishable from their background. The genes of those animals whose shape or appearance thwarted predation would occupy an increasingly larger fraction of that species' gene pool. Hence, those shapes or appearances would predominate after a time. This process, image shaping by predation pressure, appears in a style of camouflage called Batesian mimicry. In this case prey evolved a resemblance to a third species which the predators avoided. (For example, see McCosker, J.E., Sci.

(1977), 197, pp. 400-401, 'Fright Posture of the Plesiopid Fish Calloplesiops altivelis: An Example of Batesian Mimicry'.)

Because the four aspects of visual identification identified by Cott are general, we may suppose that a limited repertoire of variations in appearance throughout the animal kingdom are necessary to fulfill the condition of imperceptibility. Each animal will reflect structural changes relating to its particular niche. Does this inference mean the strategy of form perception, i. e., the detection of a particular form (an animal) against a background, is a general process among all image-resolving creatures? That is, the different styles of form perception may be homologous. I think it does if one further condition is considered. If the appearance of a camouflaged animal does not objectively (as measured by a device) resemble the natural surround, but only vaguely resembles it, then the inference can be made with validity. In this case, the principle of indiscernability of identicals cannot apply. Individual techniques for form vision may then map onto a general strategy for all image-resolving creatures. Camouflage, successful against one visual observer, will be successful against others with the same range of sensory receptor sensitivities. Flatfish camouflage evolved against its natural predators, for instance the bluefish Pomatomus saltatrix (Lux and Mahoney, 1972). It works to a large extent against my vision both above and below water. Hence, to some degree, my form abstracting mechanism must map onto that of its natural predators. This last statement might be construed as of anthropomorphic origin. It should not be. Compare it with the following statement by H. E. Hinton, Professor of Zoology, University of Bristol, regarding insect camouflage:

" . . . When an animal with some kind of protective resemblance is seen from near enough so that the question of visual acuity is not involved, it seems reasonable enough to believe that anything that will deceive the naturalist will also deceive a lizard or bird. For instance, I have studied insects for some forty years, and I am of the unshakeable opinion that if a beetle can deceive me into thinking that it is a wasp it will certainly also deceive a lizard or bird. (1973)"

Although our conclusions are similar (cryptic camouflage that works against human vision works against animal vision), the rationales differ. The origin of Hinton's statement is anthropomorphic; that is, man is the measure upon which a task is judged. I postulate only that measuring devices resemble each other when the principle of measuring is similar. Consequently, conclusions I reach regarding the effectiveness of various camouflaging tactics in flatfish probably apply to its natural predators.

A question can be raised as to whether changes in adaptation by fish are adaptive in a functional sense. The fact that an observer cannot see a single individual does not mean that adaptive changes serve to preserve entire populations. This point has been examined in the past. Mast (1916) adapted flatfish to a particular color and when allowed to settle on one of two colored backgrounds, the fish overwhelmingly picked the one to which they had most recently been adapted. Sumner (1934, 1935a-b) tested directly whether or not adaptive changes led to individual survival. A Galapagos penguin was allowed to prey upon black adapted mosquito fish (Gambusia patruelis) placed into a pale gray tank containing an equal number of preadapted pale mosquito fish. The penguin chose the unadapted fish almost twice as often as the fish adapted to that tank; the penguin chose more than twice as many pale

fish in a black tank as black-tank adapted fish. Likewise, a Night Heron and sunfish (Apomotis cyaneus) preying upon mosquito fish in similarly adaptive and non-adaptive situations produced similar catch proportions. There can be little doubt that adaptive changes by mosquito fish in these experiments facilitated survival of the 'correctly adapted' population.

Fish have been attributed an ability to modulate their shading or tone as a function of the amount of incident light and of light reflected from below (the albedo) as measured by the fish's eyes (Sumner, 1929). Illumination in the pelagic zone is always directional because sunlight necessarily enters the water through a cone whose vertical angle is 97.2° . In order to create the illusion of a flat patch indiscernible from the general tone of the midwater, fish have evolved an intrinsic graded shading that is constructed by a non-uniform distribution of melanophores, denser at the dorsal pole and thinning towards the ventral pole. The appearance due to melanophores distributed in this fashion is called countershading. Shading produced by directional illumination is opposed by the countershaded pattern. The interaction between the two leads to the appearance of a flat surface, uniform in tone, that is difficult to discern. Elimination of the curved appearance due to the indirect illumination eliminates one cue for recognition. Countershading is a general property of many land animals as well as fish.

A unique feature of Piscine physiology is the ability to change the state of pigment adaptation in the melanophores thereby changing the absolute value of the countershading gradient to compensate for the change in illumination of it and its background as the day progresses.

However, flatfish rarely present themselves to view above the ocean floor. Their mode of swimming differs from midwater fishes. They spend much time fixed at one site. When they move, they swim from site to site and rarely more than a few feet above the substrate (personal observation). Consequently, they are not differentially illuminated by light and so are not countershaded in the same sense as midwater fishes. But Sumner (1929) has shown that flatfish change their reflectance in accordance with a change in illumination. Thus, the illumination-induced reflectance change of a differentially countershaded fish is analogous to a uniform reflectance change in flatfish because dermal melanophores are evenly distributed in the visible skin.

Epipelagic fish adapt to a formless visual field that is graded in tone from top to bottom. They must appear as a blank patch to be invisible. These fish mostly are differentially countershaded as mentioned above. Fish living near the bottom, ledges, peaks, etc. contend with physical structures rising vertically from below. Kelp, grasses, corals, etc. emerge from a fixed position on the bottom to a free position above the bottom. Fish inhabiting these areas contend with a predominantly anisotropic background. That is, texture in one direction is not similar to texture in an orthogonal direction. The skin pattern on fish inhabiting this zone is overwhelmingly a repetitive one, such as stripes. (See Townsend, 1930 or Frank, 1969 for examples.) The stripes may be complete or incomplete and they extend from the dorsal to the ventral edge. The trumpetfish (*Aulostomus maculatus*), a predatory long-snouted reef fish, is striped in the rostrocaudal direction. Its normal predatory pose is vertical, snout down (Kaufman, 1976). In this position the skin texture does not contradict the anisotropic texture of its

environment.

Flatfish contend with isotropic backgrounds. That is, the texture of a bottom seen by a flatfish is independent of the particular direction of the fish's path over the bottom. Likewise, the markings on the fish's skin appear the same regardless of the direction from which it is viewed (Sumner, 1911; Mast, 1916; Hewer, 1927, 1931; Osborn, 1939; de Groot, 1969). These observations imply that a similarity between fish skin texture and background texture is required for cryptic camouflage. (One is immediately reminded of certain psychophysical color and texture adaptations in which the perceptibility of one pattern changes due to prior adaptation to a second. See Schiffman, 1976, pp. 249-253 for examples.)

Based on Sumner's and Mast's written descriptions, I had hoped that understanding the variations in flatfish texture adaptations would lead to an understanding of a biologically derived texture measure as computed by the fish. Winston (1977) phrased this idea in the following manner: "Evidently the fish measure and match a number of primal sketch parameters by adjusting the spots on their backs." That idea must be incorrect, however, because the fish does not see itself as it changes its contrast and reflectivity. The fish automatically assumes an environmentally regulated pattern much like a fixed action pattern (Eible-Eibesfeldt, 1970). The illumination-induced changes in flatfish appearance belong within the domain of a generalized and non-specific response rather than as a computed and exact measure.

PART TWO: TEXTURE BEHAVIOR

Early this century two papers explored the range of variations in appearances available to individual flatfishes. Sumner (1911) experimented with two Bothids, Rhomboidichthys and Lophopsetta (or Scopthalmus). Variability in their appearances had already been the subject of local folk tales. (For instance, Scopthalmus was commonly known as the 'windowpane' flounder.) Mast (1916) experimented with individuals of the migratory genus Parlichthys. Both researchers reported that the fish responded by changing the appearance of their skin to variations in the bottom they saw. Individuals could adapt to uniform backgrounds, varying from light to dark, by changing their skin reflectance. They showed some ability to adapt to a variety of partitioned backgrounds, small stones, large stones, shells, etc., but the variations were limited by the constraints inherent in the distributions of skin chromatophores.

A fish would 'mimic' the relative degree of patchiness from one background to a second. It was in this sense that a fish texture adapted. Some individuals responded to repetitions of the same surface by quickening their adaptive response. Others showed little or no response. Blinded fish assumed a uniform appearance (Pouchet, 1876; Osborn, 1939) proving that texture adaptations were visually initiated.

These adaptations led Sumner and later Mast to adapt an individual to four different checkerboards whose check size varied. Within some range of check sizes, a fish appeared to respond in a predictable fashion. When the size lay on either side of the responsive range, the fish tended to act as if it were on a uniform background. Mast was most emphatic about recognizing that the spot positions were identical regardless of the back-

-ground which evoked a particular display.

The methods used to convey the experimenter's visual impressions were curious. Quoting from Sumner (1911, p. 185)

no changes, by retouching or otherwise, were made in any of the figures... careful observations were made and recorded in each case at the time the photograph was taken, and in printing these descriptions served as a guide in attempting faithfully to reproduce the various shades and patterns assumed by the fish.

Mast (1916, p. 419 ff.) too, made notes on the fishes' appearances. Sometimes he included in his pictures a white-gray-black image, "but its application was rather limited." In other words, neither used a consistent and reproducible control measure. They used their impressions as a guiding measure. Impressions, however, are not suitable because they are the result of visual interpretation. I do not doubt that the researchers observed changes in the fish, but a description of those changes based on visual impressions could not accurately represent the changes because the details of the description evolved to convey a false impression.

On the basis of his impressions, Sumner postulated a physiological mechanism that he felt accounted for the adaptations:

It is probable that much of the diversity in the distribution of visible pigment in the skin of Rhomboidichthys is 'functional' rather than 'organic'... It may result not so much from diversity in the distribution of the chromatophores as from local differences in their tonus, under the influence of the nervous system...

This statement has led to the unique position flatfish occupy in the area of camouflage studies because it implied that an afferently measured visual function was transformed within the central nervous system and uttered efferently as a visible behavior. This type of behavior had not been attributed to another teleost.

Experiments were performed to investigate the appearance of flatfish

during adaptive changes. Variations in two parameters were found. They are, as mentioned previously, skin reflectance and contrast between a set of fixed spots and skin reflectance. The degree of adaptation in my experiments differed substantially from those previously reported.

METHODS: A chamber was constructed to restrain a fish upon a specific background. Within a 75 liter aquarium, an inner platform consisting of a 30 x 30 cm. base and two 9 x 30 cm. side pieces were mounted onto the inside of the tank with Dow Corning Silastic 732 RTV adhesive 10 cm. above the tank bottom. Holes were drilled through the three pieces to provide for water continuity between the inside and outside of the holding chamber. Aeration was provided until a photograph was to be taken. It was stopped for about 4 minutes every hour.

Various textures were photographed on Plus-X film, developed in D-76 normally and printed on Kodak resin coated paper. I found that this paper could withstand repeated immersions in salt water if, after each test, the paper was rinsed in fresh water and dried.

The pictured texture was folded so that it extended vertically about 2.5 cm. at each edge to make a tray. Two lights (Sylvania No. 2 EBV photoflood bulbs) were mounted at either end of the aquarium and projected into the holding platform at an angle of 45° . The camera (Honeywell Spotmatic with either a 35mm., a 50 mm., or a 105 mm. lens) was positioned directly over the restraining chamber. The shutter was depressed by remote release since an approach by the experimenter promoted a startle reaction by the fish which is often accompanied by a change in appearance. Small fish (less than 15cm. standard length) were restrained upon the textured paper by a glass ring.

Two species were studied: Paralichthys lethostigma, the

Southern flounder, provided by Gulf Specimen Co., Panama, Fla.; and Pseudopleuronectes americanus, the Winter flounder, obtained locally at the facilities of the Cohasset Marine Biological Station, Cohasset, Mass.

Adaptations to a specific pattern were allowed for thirty minutes to one hour. It has been shown that denervated melanophores of Phoxinus exhibited slow, background-dependent changes (Smith, 1931; Neill, 1940; Healey, 1951). These changes required hours or days. Neill (1940) divided the teleost chromatic response into a short-duration neural phase (less than thirty minutes) and a long-duration hormonal phase. I have only examined the neural phase because (1) motion pictures of fish adapting to different textures showed that the major contrast adaptation occurred within minutes, and (2) a long term chromatic response to changes in illumination and background has been observed in blinded Ameiurus and Fundulus (Wyman, 1924; Wykes, 1938). The long-duration phase may not originate in the retina.

For picture uniformity, an eight level gray scale was considered as an exposure standard. Shutter speed and f-stop needed to reproduce the gray scale determined the printing conditions for the fish pictures. To assure print uniformity when different adaptations by the same fish were compared, the negatives were taped and printed together.

Reflectance measures from the photographs were determined with a reflecting densitometer (courtesy of Bradford Howland). For Figs. 2, 3 and 5, the resulting reflectance graphs were integrated with a planimeter to arrive at an average reflectance value. For quantitative comparisons, the change in reflectance between the lightest area and another area was measured in terms of a Wratten Neutral Density filter which was necessary

to convert the reflectance of the lightest area to that of the other. These measures are consistent within each figure.

RESULTS: Figure 2 illustrates adaptations to backgrounds of different reflectances by an individual Paralichthys. The fish remained on a background for 25 to 30 minutes before each picture was exposed. Figure 2B is the reflectance curves measured between points A and B of figure 2A. The average reflectance of each picture and background reflectance are also indicated in fig. 2B. These figures show that the average skin reflectance follows the background reflectance.

Figures 3A and 3B demonstrate a similar conclusion with an individual Pseudopleuronectes.

The same conclusion can be demonstrated with a second procedure: by measuring reflectance directly from the fish. A Gossen Lunasix light meter was mounted over the holding chamber. Three additional P. americanus individually were placed in a 35 cm dish and placed over three different Coloraid papers whose reflectance varied in the measured proportions 1:8:250. The area between the three caudal dark spots was measured. Each fish displayed a change in skin reflectance that paralleled the reflectance change in the bottom.

		I	II	III	Fish No.
Back-ground	250	6	7	14	
	8	3	3	2	
Reflectance	1	1	1	1	

Table 1: Each value indicates the increase in reflectance compared to the least value.

Thus, the qualitative conclusions drawn from photographs are equivalent to those drawn directly from skin measurements.

Figure 2A: Adaptation of Paralichthys lethostigma to uniform backgrounds of different reflectances.

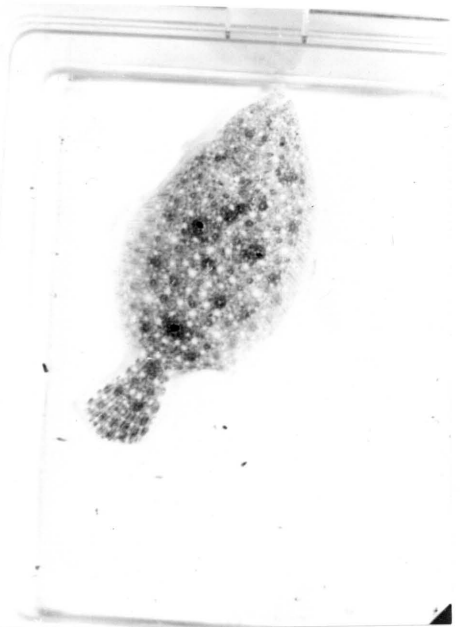
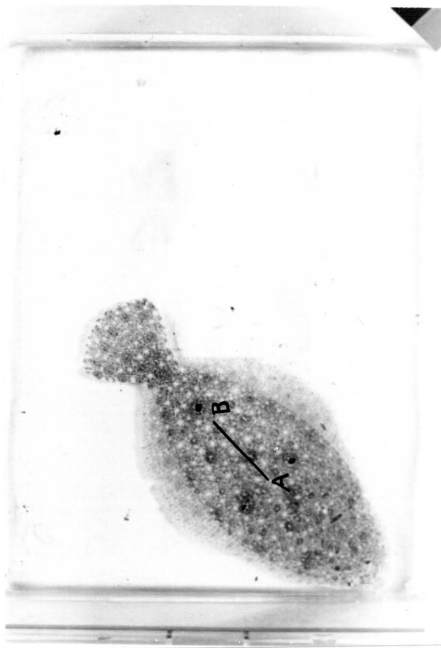
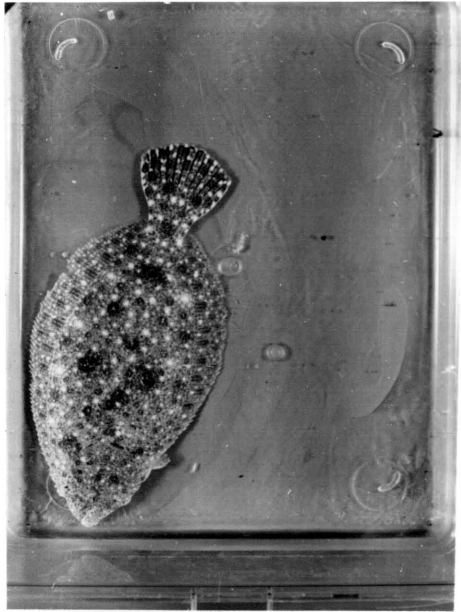


Figure 2B: Reflectance measures of Figure 2A

Graphs measure the reflectance of the path between points A and B (as indicated in the upper left, Figure 2A) of the four adaptations in Figure 2A. Ordinate represents the variations in reflectance measured between points A and B. No reflectance is indicated by the uppermost straight line; increasing reflectance is indicated in the downward direction.

Area under each curve was measured to determine the average reflectance for each curve. Average reflectance is indicated by the black bar on the righthand side of the reflectance curves. Background reflectance is indicated at the far right. (Reflectance from the white background exceeded the scale.)

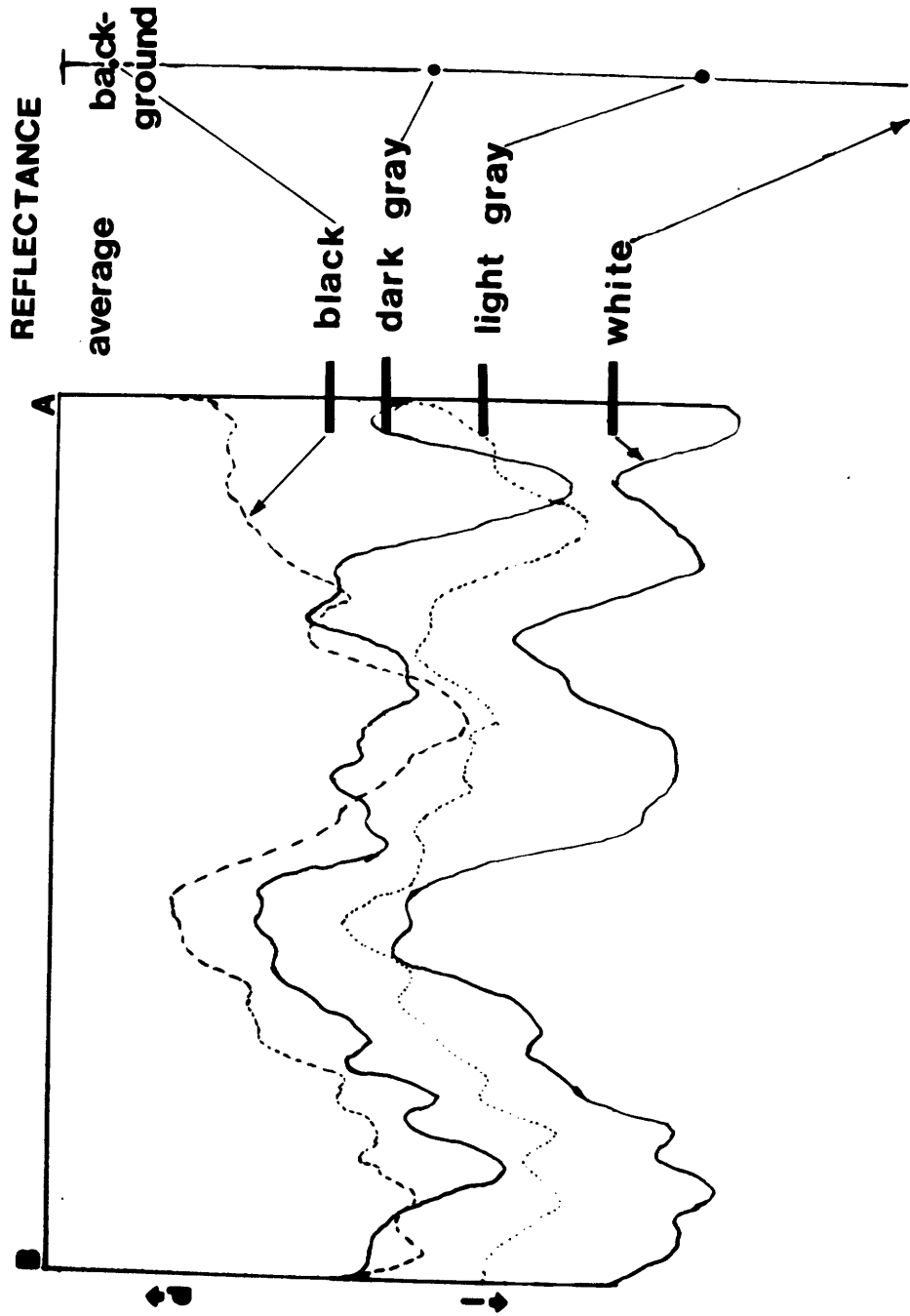


Figure 3A: Adaptation of Pseudopleuronectes americanus to uniform backgrounds of different reflectances.

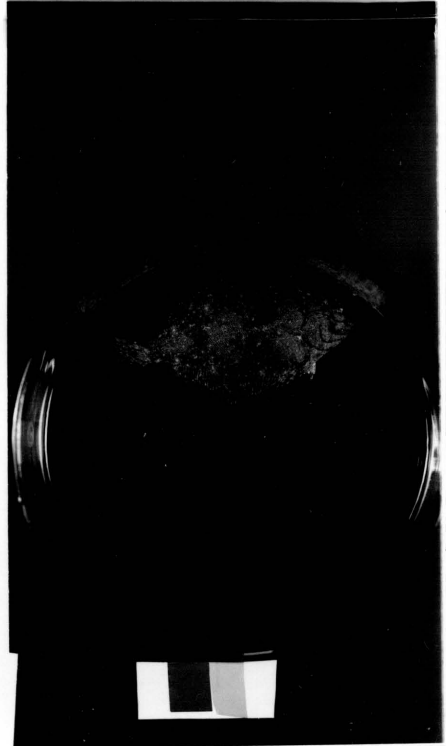
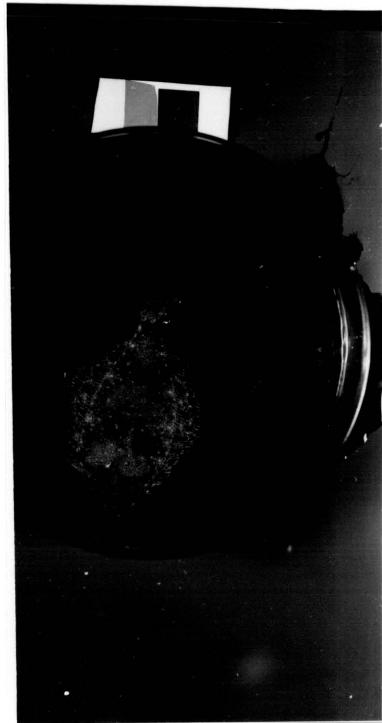
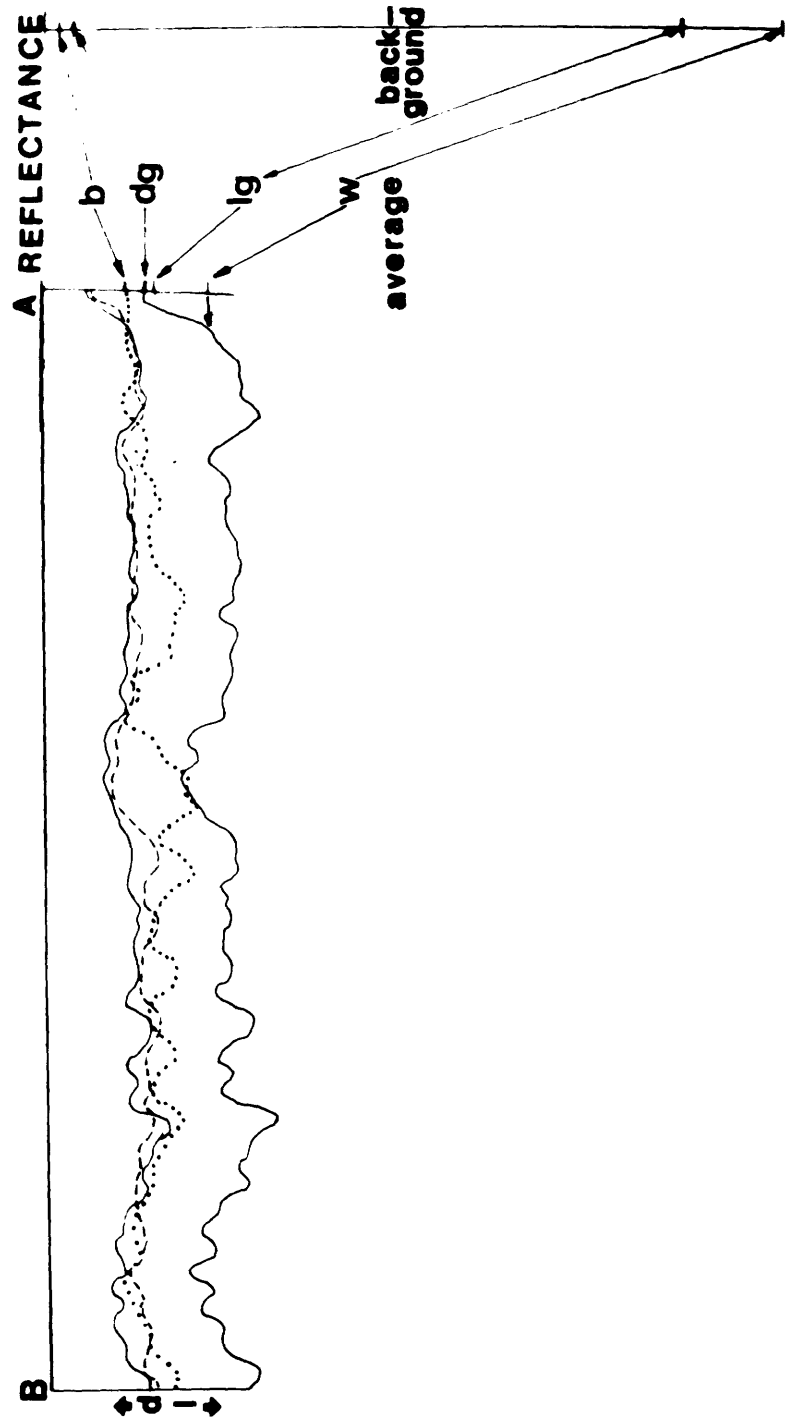


Figure 3B: Reflectance measures of Figure 3A

Graphs measure reflectance of the path between points A and B (as indicated in the upper right, Figure 3A) of the 4 adaptations in Figure 3A. For additional details, see legend for Figure 2B.

Abbreviations:	Symbol:
w, white background.....	lowest solid line
lg, light gray background...	dotted line
dg, dark gray background...	dashed line
b, black background.....	upper solid line



The next figure (4) shows that the response due to a patterned background is different in character from the response due to a uniformly light background. The same fish was adapted to each background for about 30 minutes before the fish was photographed. Reflectance curves were measured between points A and B of the figures. They show that the variation from maximum to minimum reflectance is greater in the patterned response than in the response to the uniform background. The difference between the minimum and maximum reflectance of the patterned response is equivalent to a 0.8 N. D. filter; that of the uniform background, about a 0.2 filter. If the two curves are compared at point C, the background reflectance has been lowered less than a 0.1 N. D. filter. Thus the change in the extent of variations between the two responses cannot be accounted solely by a change in the background reflectance.

Figure 5 illustrates the results of adapting the same fish to two patterns, one whose proportion of white is three times that of black, and the other, with reversed proportions. As can be seen (Figs. 5A and 5B), average reflectance of the fish adapted to the lighter pattern is more than that of the darker pattern. Figure 5C shows the reflectance graphs of paths A-B indicated on figs. 5A and 5B. Two points may be noted. First, the average reflectance of fig. 5A is less than that of 5B. Secondly, the contrast between the maximum and minimum reflectance of fig. 5A is less than that of fig. 5B. Since the minimum reflectances are nearly equal, a change in contrast between the spots and body reflectance may be achieved by only increasing body reflectance.

These figures immediately demonstrate that the fish varies two features of its appearance: body reflectance and spot contrast. In Part Three, I

Figure 4: Response of Paralichthys lethostigma to a patterned and unpatterned background

Above: White and patterned backgrounds and responses

Below: Graphs measure reflectance of the path between points A and B (as indicated in upper left figure) of the 2 adaptations.

The scales are calibrated in terms of the neutral density filter values interposed between the illuminator and the photograph. One should note that the excursion from lightest to darkest reflectance in the patterned response exceeds that in the uniform response by almost 4.5 times.

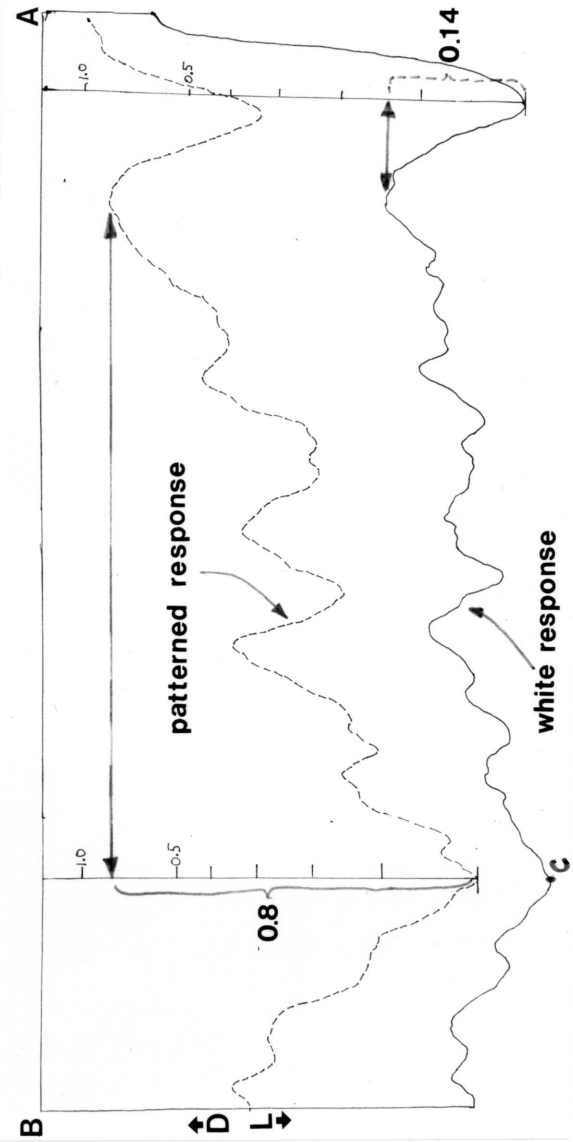
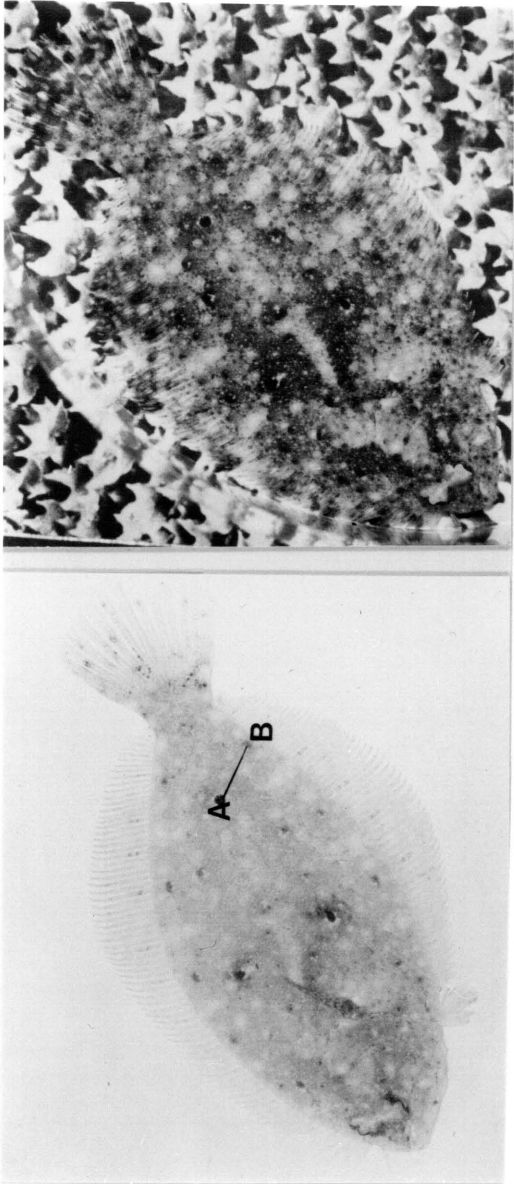


Figure 5: Adaptation of an individual P. lethostigma to backgrounds whose proportion of white to black are reversed.

A, Above: Black:White::3:1

B, Below: White:Black::3:1

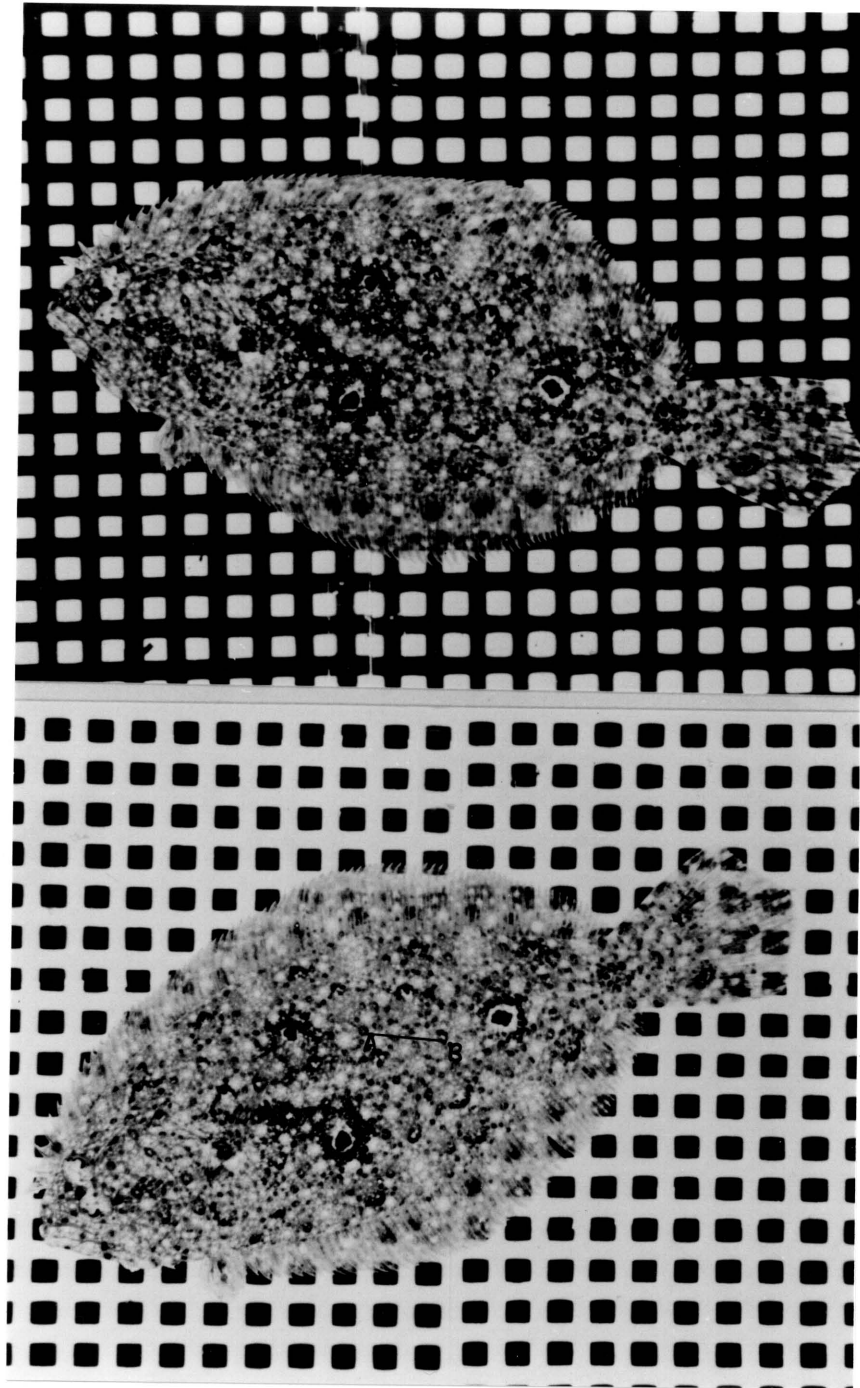
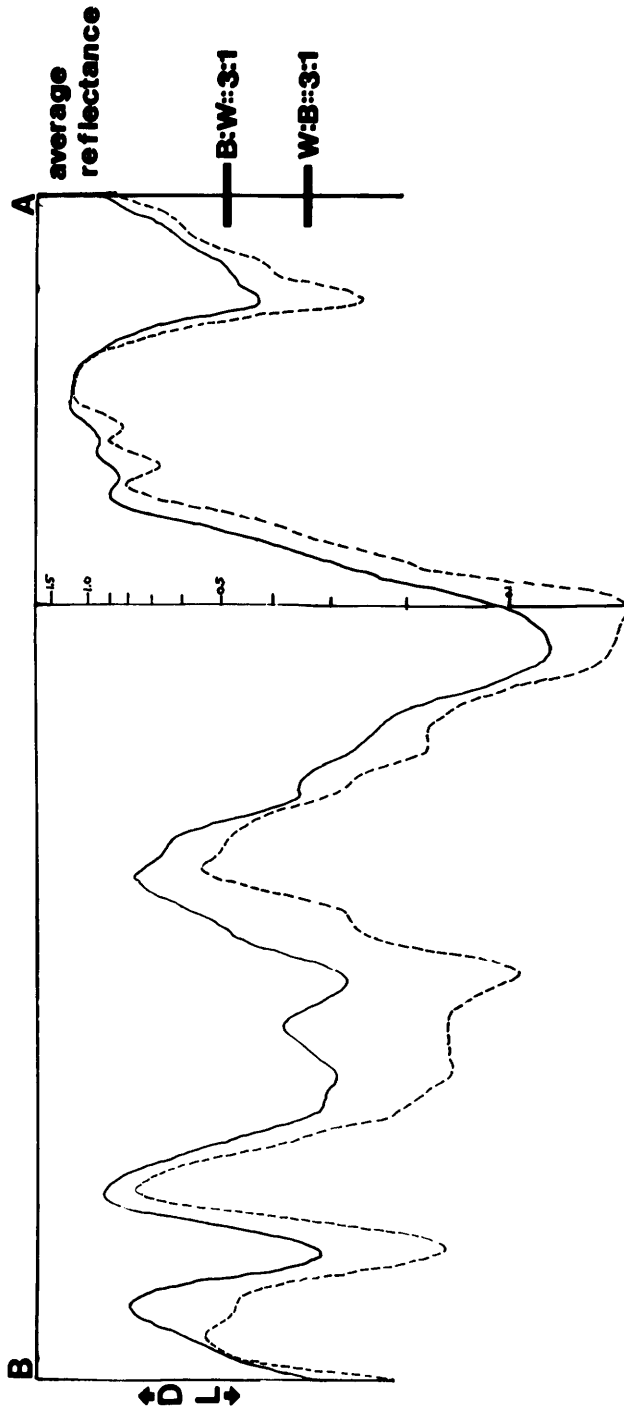


Figure 5C: Reflectance measures of Figures 5A and 5B

Graphs measure reflectance of the path between points A and B (as indicated in Figure 5B) of the 2 adaptations in Figure 5B. For additional details, see legend for Figure 2B.

Scale: Change in brightest reflectance by interposing a neutral density filter between the illuminator and the photograph. Values of scale in terms of the neutral density filter values.



will show that body reflectance corresponds to the distribution of dermal melanophores and spot positions correspond to the distributions of epidermal melanophores.

Figure 6 is a reproduction of Sumner's original checkerboard experiment. Figure 7 is a reproduction of the results of Mast's experimental duplication. I photographed the journal figures on Kodachrome film (asa 25). Those images were projected upon Kodak Continuous Tone Copy Film (no. 4125) to make a continuous tone black and white negative. To my eye, these figures resemble the journal figures well.

Naively, from examination of these two figures, the fish appeared to respond to the different backgrounds as if they partitioned their skin to match spatial frequencies. I attempted several reproductions of this experiment. Figure 8 is characteristic. (It may be directly compared with figure 7 since both fish belong to the same genus.) The fish responded on the largest check as if the contrast were diminished, a result seen often on large partitioned fields. When the background is masked (fig. 8B), the other three adaptations appear similar. Close examination shows a) the background of fig. 8B-2 is lighter than that of figs. 8B-1 and 8B-3; b) the contrast of fig. 8B-1 with respect to the small white spots is visibly greater than in 8B-2 or 8B-3; and c) both the background reflectances and contrast relationships of fig. 8B-3 are diminished compared to the other two figures. The changed variables, however, are no different from the ones illustrated in figures 2-5.

What then accounts for the degree of difference and impressions between my figure 8 and figures 6 and 7? Let me emphasize that the difference is one of degree, not quality.

Two separate problems need to be distinguished at this juncture. They

Figure 6: Above: Reproduced illustration of Sumner's checkerboard experiment(1911).

Figure 7: Below: Reproduced illustration of Mast's checkerboard experiment(1916).

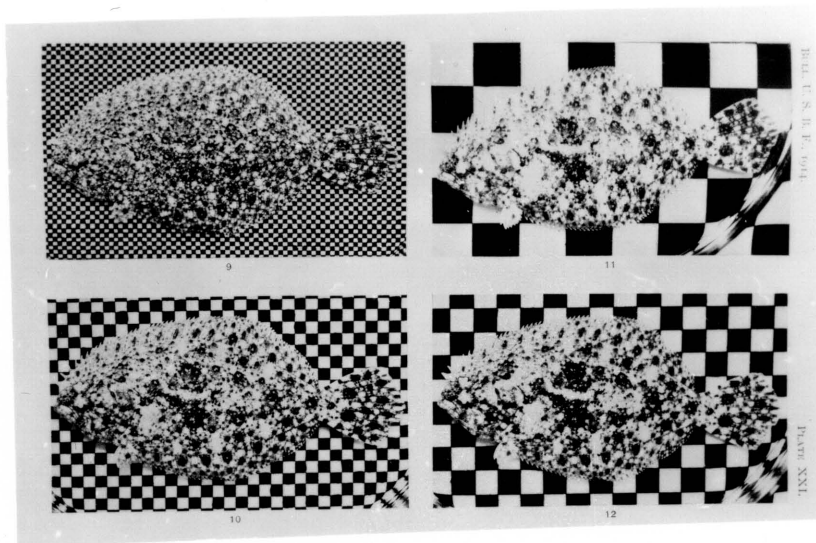
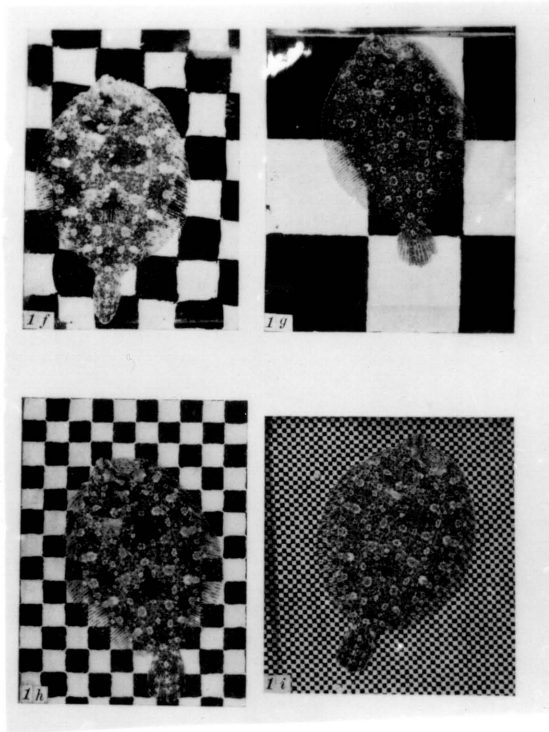
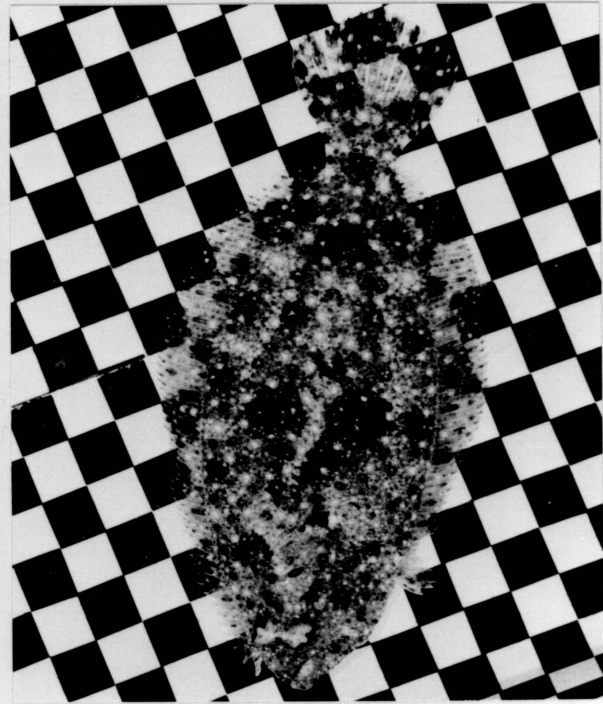
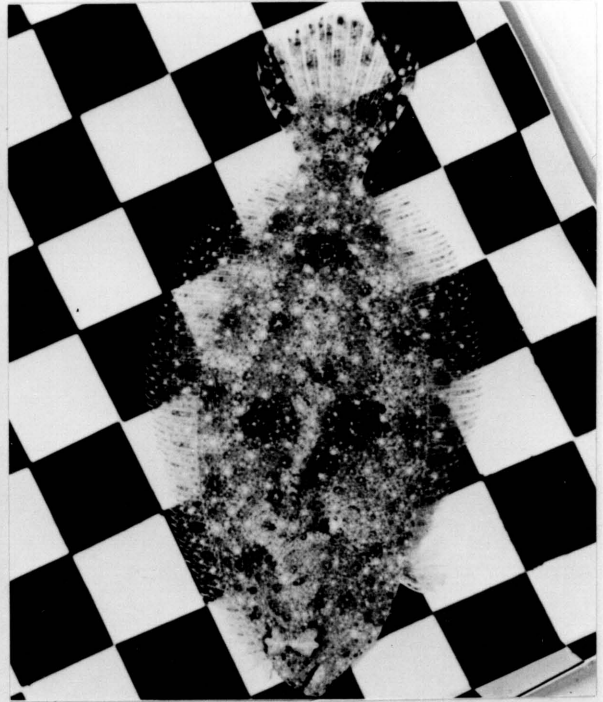
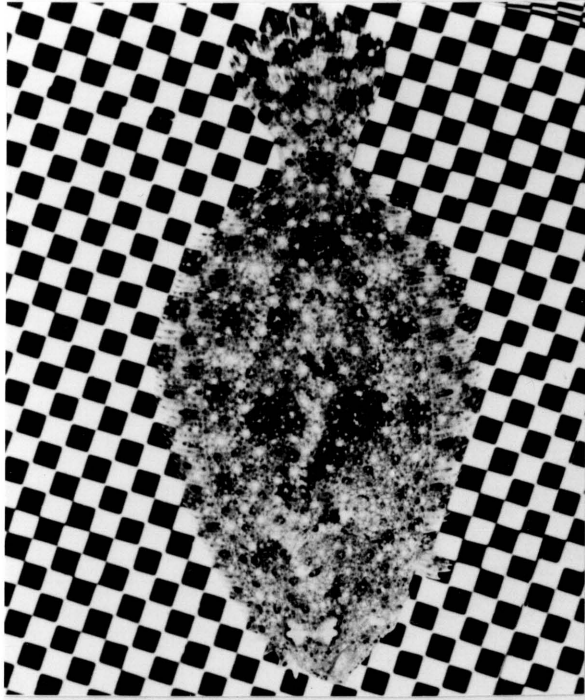
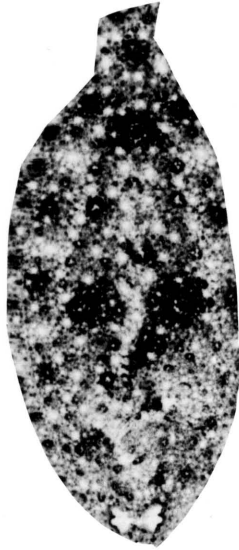


Figure 8A: Response of Paralichthys lethostigma to checkerboard textures of different spatial frequencies.

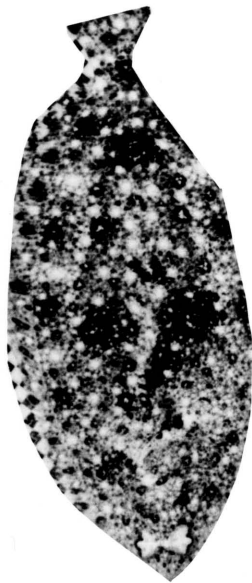
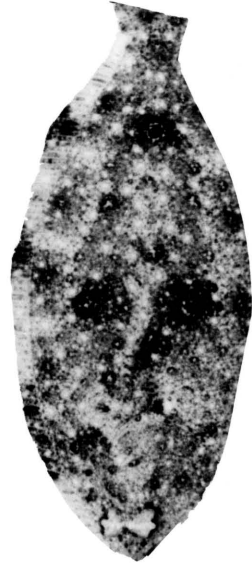
Figure 8B: Figure 8A with the background eliminated. See page 44 for discussion.



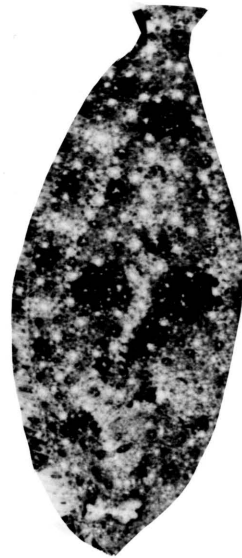
2



4



1



3

are 1) the production of the image, and 2) the production of the impressions that led to the image.

Consider the first. The two variables I identified in figures 2 through 5 and 8 appear exaggerated in figures 6 and 7. This effect can be accounted for by examining photographic procedures during development. (See Neblette, chaps. 20 and 22 for additional details). The range of object luminances recorded on an emulsion is restricted by the properties of the emulsion and its backing. The recording properties of each emulsion are defined by curves relating density of silver to log exposure, the H log E curve. This curve is an ogive, consisting of 4 regions, a foot region, a region of constant gradient, a shoulder region and a region of reversal. The first three are commonly considered.

Luminance values are proportionally recorded only in the linear or constant gradient region. Outside this region distortion of luminance values occurs. These distortions always lead to higher image contrast at both ends of the dynamic range.

The maximum range of proportional luminance values represented photographically occurs when the average luminance value is represented midway on the linear portion of the H log E curve. Additional distortion will occur--and the maximum proportional representation will shrink--if the average luminance is placed elsewhere than on the linear portion of the H log E curve.

Thus Sumner and Mast's end products, figures 6 and 7, could have been engineered to match their written impressions without actually representing accurately the fish's tonal variations. Careful examination of figure 7 suggests that photographic distortions did occur.

Mast's images contain little gray. In addition, no detail such as edges

of scales in the white areas can be seen. These defects are characteristic of underexposed and/or underdeveloped images. Mast's figure cannot be an accurate representation of his experiment.

Both figures show evidence of uneven lighting. The anterior edge of the upper left image in figure 6 and the dorsal edge of all four images in figure 7 are lighter than the ventral edge.

Thirdly, the changes in Sumner's figure, contrast between body reflectance and the spots of the right hand pair, and body reflectance in the left hand pair, are no different from the changes I saw in figures 2 through 5. Our interpretations, though, widely vary. What would have led Sumner to think the fish texture matched its surround rather than changed (non-randomly) in response to the surround? Let me defer a discussion of this problem to Part Five.

Behaviorally, flatfish appear to exercise physiological control over two parameters of their appearance: the general skin reflectance and the contrast relationships of a set of spots and the skin reflectance. The next section identifies these changes as being related to changes in the adaptive state of the dermal and epidermal melanophores respectively. The variations in contrast at other than the morphologically fixed spots are related to reflectance changes inherent in variations of the melanophore-iridophore complex located in different parts of the skin.

PART THREE: SKIN MORPHOLOGY WITH SPECIAL EMPHASIS ON
MELANOPHORE RELATIONSHIPS

MORPHOLOGY: The skin of Pseudopleuronectes americanus is like other teleost skins, a bilayered structure in which the chromatophores, the functional elements leading to changes in appearance, are embedded. The skin in cross section (Fig. 9) consists of two principle layers overlying the scales, the epidermis and the dermis separated by a basal lamina. Epidermis forms a sheet overlying every scale. Scales are embedded in dermal pockets. The suprascalar dermal layer over one scale is connected with both suprascalar and subscalar dermal layers associated with adjacent scales. Pigment cells relating to neuronal adaptive changes lie in the suprascalar dermis. Other melanin-containing pigment cells reside in the epidermis and the subscalar dermis.

The epidermis is stratified into three subdivisions: a superficial layer, an intermediate layer and a basal layer (Brown and Wellings, 1970; Roberts et al., 1971). The superficial layer consists of a single layer of squamous and mucal cells. Several layers of squamous cells form the intermediate zone while a single row of ovoid cells sits adjacent to a basal lamina. The cells interdigitate with each other through extensive cellular infoldings and connect via numerous desmosomes. As a cell ages, it migrates through the intermediate zone and the degree of interdigitation decreases. The exposed surface of the superficial squamous cells is covered with microridges (Whitcar, 1970; Hawkes, 1974a) that perhaps anchor mucus onto the skin. The exact number of cell layers depends upon the area and species of fish sampled (Van Oosten, 1957; Brown and Wellings, 1970). Epidermal melanophores are found between cells of the intermediate zone.

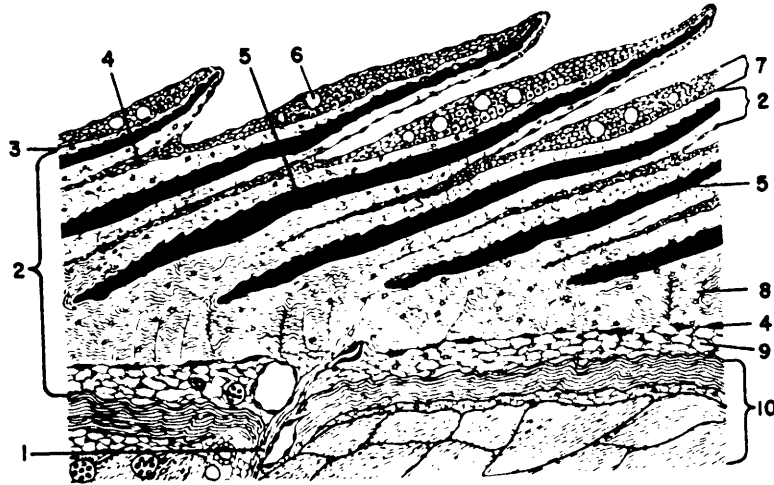


Figure 9: Schematic cross section of fish skin (after Van Oosten, 1957).

1. Septum
2. Dermis
3. Basal Membrane
4. Pigment cells
5. Scales
6. Mucus cells
7. Epidermis
8. Dense collagenous tissue
9. Subcutis
10. Muscle

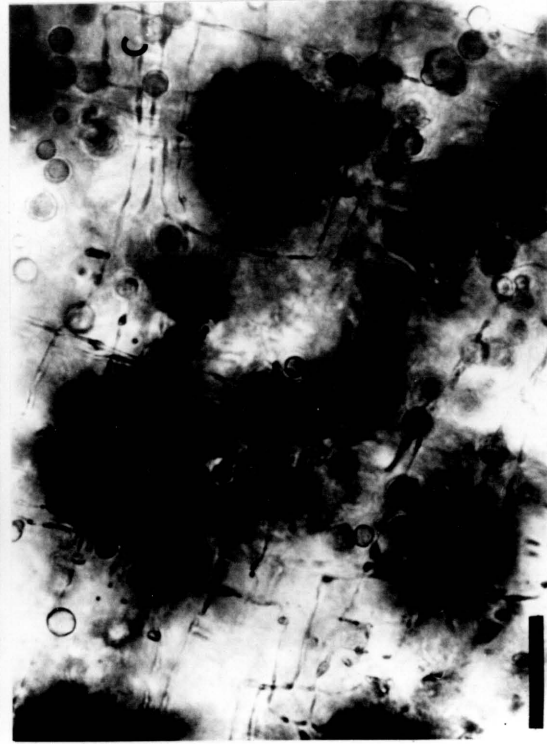
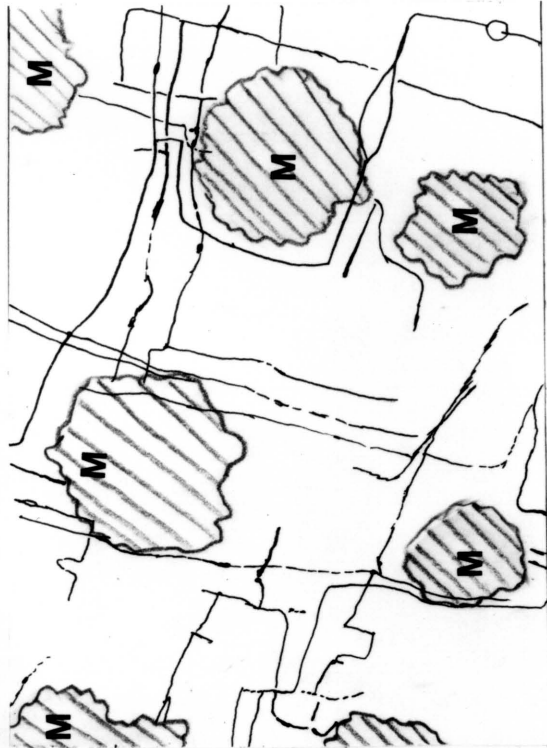
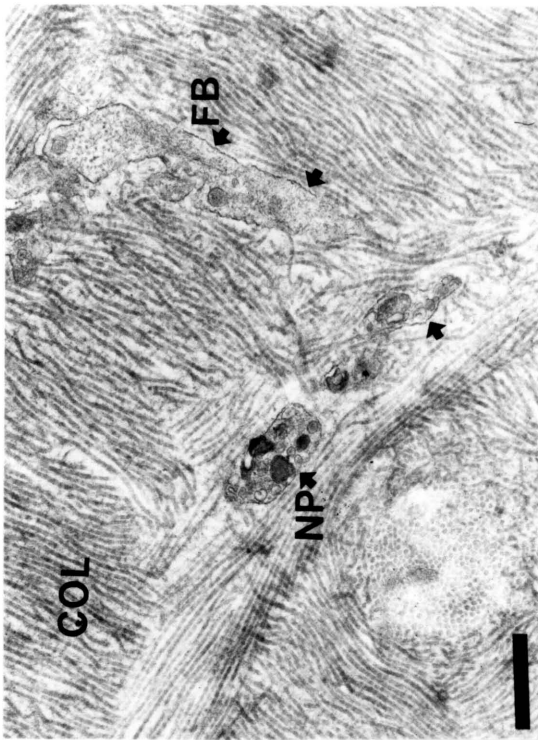
Separating the epidermis from the uppermost limit of the dermis is an electron translucent band, the basal lamina (Fawcett, 1966; Fujii, 1968). Below it lies the dermis, stratified into two subdivisions: the stratum spongiosum (collagen and suprascalar dermis) and the stratum compactum (the subscalar dermis). The collagen layer, distalmost in the dermis, is divided into a number of lamellae, with adjacent layers perpendicularly oriented (Fujii, 1968; Brown and Wellings, 1970; Hawkes, 1974a; Fig. 10a). Within each lamina, the collagen fibrils are oriented in parallel. Nerve fibers descending into the cell-containing dermis and ascending into the epidermis, and fibroblasts, can be found lying between adjacent lamellae and not within a single layer (Brown and Wellings, 1970; Fig. 10b). The fine structural basis of the teleost sub-epidermal nerve plexus (Fig. 10c-d) is possibly derived from the geometric organization within the collagen layer (as suggested by Whitear, 1952).

The basal edge of the collagen layer abuts the dermal chromatophore region. Each chromatophore type is uniquely positioned with respect to the others (Cunningham and MacMunn, 1893; Kuntz, 1917; Hawkes, 1974b). Globular iridophores are found in association with dermal melanophores. The melanophore soma sits beneath the iridophore, but its processes ascend to overlap the associated iridophores. The light reflecting and scattering properties of iridophores are blocked when melanosomes fill the melanophore processes and are exposed when the processes are emptied. The appearance of the fish then is not only related to the pigment granule position within the adapting cells, but also to the extent of iridophore exposure.

Adjacent to iridophores, depending upon skin position, lie the bright

Figure 10: Collagen layer and the sub-epidermal nerve plexus

- A: Upper left: Cross section of the collagen(COL) lamellae located between the epidermis (EP) and the dermis (MS, melanosome of a dermal melanophore; BL, basal laminar). scale: 1 micron
- B: Upper right: Electron micrograph of a nerve process(NP) and a fibroblast process (FB) interdigitating between collagen laminae(COL). scale: 0.55 micron
- C: Lower left: ZrO_2 preparation illustrating the sub-epidermal nerve plexus. scale: 30 microns
- D: Lower right: Tracing from previous figure showing the geometric regularity of the sub-epidermal nerve plexus (M, melanophore located deeper within the dermal layer).



colored cells. These cells act as color filters. A fish's chromatic appearance depends upon the adaptive state of the two types of cells with migratory granules, and their interactions with the stable iridophores. The concept of the Dermal Chromatophore Unit (Bagnara, Taylor and Hadley, 1968; Taylor and Bagnara, 1972) applies to fish (Hawkes, 1974b) as well as to amphibians.

Below the dermal chromatophores lies the scale and below that, the stratum compactum. Within the stratum compactum lies a melanophore-like cell whose structure and geometry resemble an epidermal melanophore, not a suprascalar dermal melanophore. Epidermal melanophores have been considered to be degenerative suprascalar dermal melanophores (Kuntz, 1917). It is unlikely that epidermal melanophores are degenerative for several reasons: 1) Dermal melanophores are found everywhere in the skin—epidermals are not; 2) Their appearances differ; 3) Epidermal melanophores display repetitive adaptations characteristically like a viable cell; and 4) Since the epidermal melanophore resembles the subscalar melanophore, it is more likely that the epidermals are derivative of the subscalar cells.

Basal to the stratum compactum is a layer of connective tissue holding the dermis to the underlying muscle. In some areas a superficial blood vessel with its iridocyte covering may add to the reflectivity of a region when a fish exhibits a pale adaptation. These structures are particularly prominent below the white marginal spots.

The distribution of chromatophores and associations between them is not uniform within the skin. This observation plays an important role in the adaptive changes of flatfish, although in fish with stable patterns (for example Coral Reef fishes of the families Chaetodontidae and

Pomacanthinae), this non-uniformity has been mentioned (Norman, 1975, p. 213). Sumner explicitly rejected this argument in explaining the changes of flatfish appearance.

ANALYSIS OF THE MELANOPHORE-IRIDOPHORE DISTRIBUTIONS:

Consider the distribution of epidermal melanophores of P. americanus. Five skin areas were examined for density of epidermal melanophores. The areas as defined by Mast (1916) are (Fig. 11) 1) black central spots; 2) dark annulus around the black central spots; 3) white marginal spots; 4) black marginal spots; and 5) an interregion between regions 1) and 4) not including regions considered in category 2). Burton (1975) divided the skin into only two discrete regions thereby obscuring the relationships described in figures 12-15 and tables 2-4.

Suprascalar pieces of skin from each region of five specimens were excised, placed in flounder saline (Cobb, Fox and Santer, 1973) containing 10^{-5} M L-epinephrine (Sigma) for 2 - 5 minutes, and fixed in 2% gluteraldehyde in saline, placed on a standard 25 x 75 mm. glass slide and immediately coverslipped. Clear nailpolish applied to the margins of the coverslip permanently sealed the specimen to the slide. Epidermal melanophores within an area 0.11 mm^2 above a scale were counted to within 0.5 of a melanophore when a cell was partially included within the circumscribed area.

The epidermal melanophore density among the different regions varied in a predictable fashion (fig. 12). The absolute number of cells did not vary with respect to any found parameter such as scale size, fish size, etc. (see Table 2). When the numbers from each region of a single fish were normalized by dividing by the number from area 1, a consistent

Figure 11: Skin markings of Pseudopleuronectes americanus

The numbers correspond to the areas analyzed for chromatophore densities in Figures 12-15.

1. Black central spot
2. Surround of black central spot
3. White marginal spot
4. Black marginal spot
5. Interspot or background region

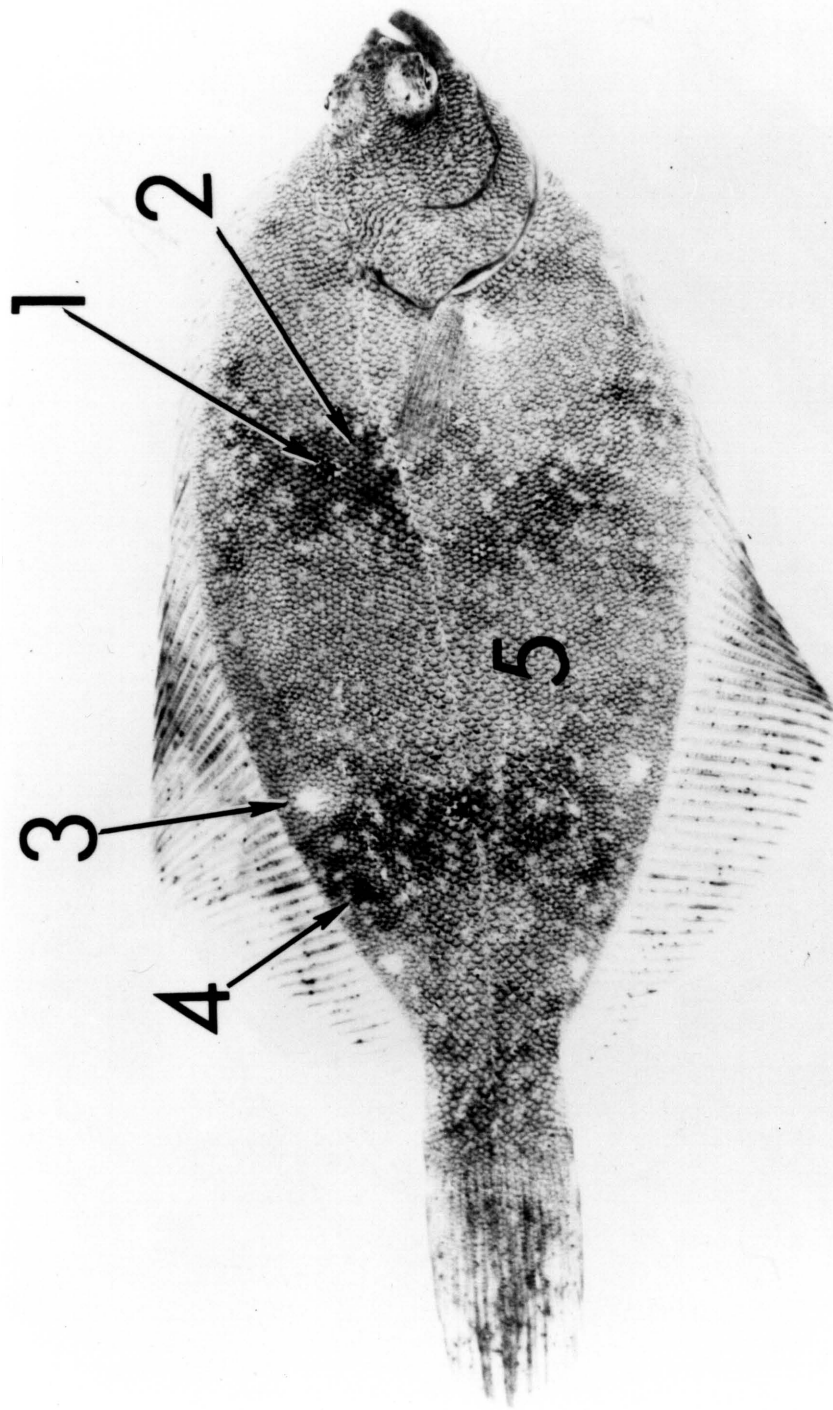


Figure 12: Comparison of the epidermal melanophore densities in the areas indicated in figure 11 for five separate fish. Densities are displayed proportional to that of area 1.

RELATIVE EPIDERMAL MELANOPHORE DENSITY

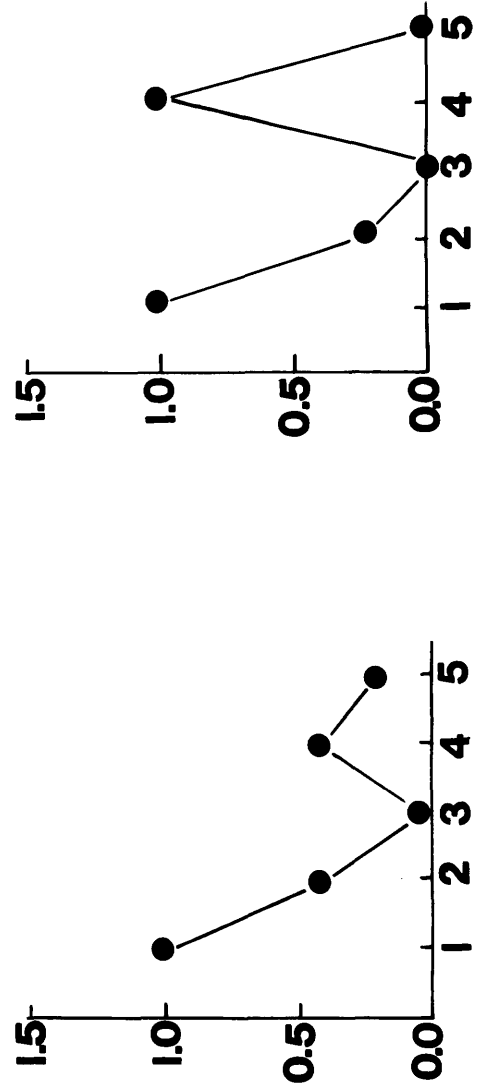
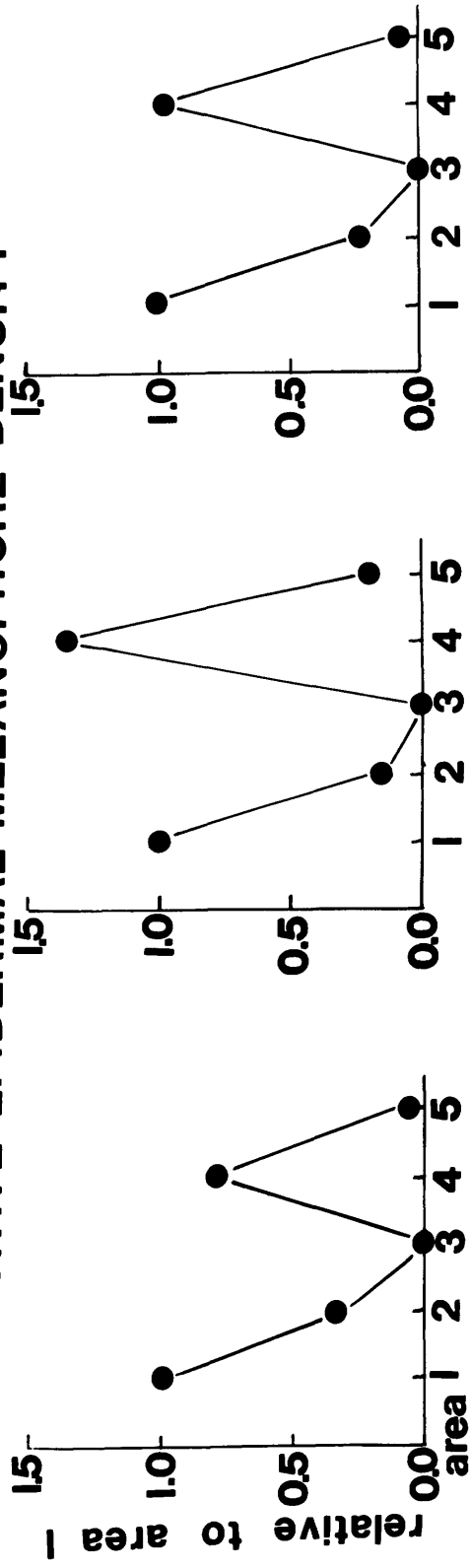


Table 2: Epidermal Melanophore Density (per 0.01mm²)

Fish No. (standard length)	Region(see Figure 11)				
	1	2	3	4	5
1 (14.6 cm.)	1.64	0.55	0.00	1.28	0.09
2 (19.1 cm.)	0.91	0.14	0.00	1.23	0.18
3 (21.6 cm.)	1.64	0.36	0.00	0.96	0.14
4 (15.2 cm.)	1.70	0.72	0.09	0.79	0.43
5 (12.1 cm.)	1.50	0.35	0.00	1.50	0.00

Table 3: Dermal Melanophore Density (per 0.01mm²)
(same specimens as in Table 2)

1	6.74	5.22	5.66	5.76	6.09
2	7.50	6.41	6.38	7.39	7.29
3	9.57	7.18	6.09	6.42	6.08
4	10.00	6.74	7.28	8.33	8.41
5	10.13	6.06	7.39	8.63	8.34

Table 4: Iridophore/Dermal Melanophore Density (per 0.01mm²)

1	$\frac{10.13}{6.30}$	$\frac{18.60}{5.17}$	$\frac{31.41}{6.61}$	$\frac{9.36}{5.77}$	$\frac{21.58}{8.93}$
2	$\frac{5.67}{5.84}$	$\frac{17.77}{5.68}$	$\frac{29.35}{6.51}$	$\frac{4.96}{5.89}$	$\frac{25.01}{6.10}$
3	$\frac{14.05}{6.51}$	$\frac{23.77}{5.58}$	$\frac{35.22}{6.82}$	$\frac{16.33}{8.99}$	$\frac{24.39}{6.41}$
4	$\frac{13.95}{7.54}$	$\frac{20.01}{6.20}$	$\frac{27.78}{5.08}$	$\frac{14.47}{5.68}$	$\frac{25.83}{7.75}$

relationship appeared. This relationship was constant among the different individuals examined. Epidermal melanophore density in area 2 was always less than area 1. Area 3 contained none except in one fish. Area 4 resembled area 1.

These observations require comment. Such counts obscure the particular positions the epidermal melanophores may take with respect to the underlying scales. In area 5, the epidermal melanophores were predominantly found overlying the scale margins almost as if they emerged from the margin. In both areas 1 and 4, the skin overlying a scale was filled with epidermal melanophores. If the sample from area 3 was one scale away from the center of the spot, a few epidermal melanophores might be found. The relationship presented in figure 12 appears rigid.

The distribution of dermal suprascalar melanophores was counted in the identical preparations. All melanophores, fractions included, were counted within an area 0.023mm^2 . Two areas per scale were counted. Fig. 13 and Table 3 represent the results. Again, no relationship between the absolute number of these cells and some physical parameter belonging to the fish was found. However, when the densities in each area were normalized by dividing by the density of area 5 (the background), a relationship was found. The density of dermal suprascalar melanophores in winter flounder is uniform (20%). The deviations were found in area 1. There, the density tended to be somewhat higher.

An iridophore count was made in material separately prepared. Suprascalar skin pieces were excised from the same five regions from four fish, but immersed in 3% EDTA and 0.44% NaHCO_3 (Stone and Chavin, 1974) to separate dermis from epidermis. After 5-10 minutes, the epidermis was removeable. Tissue was then placed in 0.2M KCl to aggregate the melan-

Figure 13: Comparison of the dermal melanophore densities in the areas indicated in figure 11. Same preparation as figure 12. Densities are displayed proportional to that of area 5.

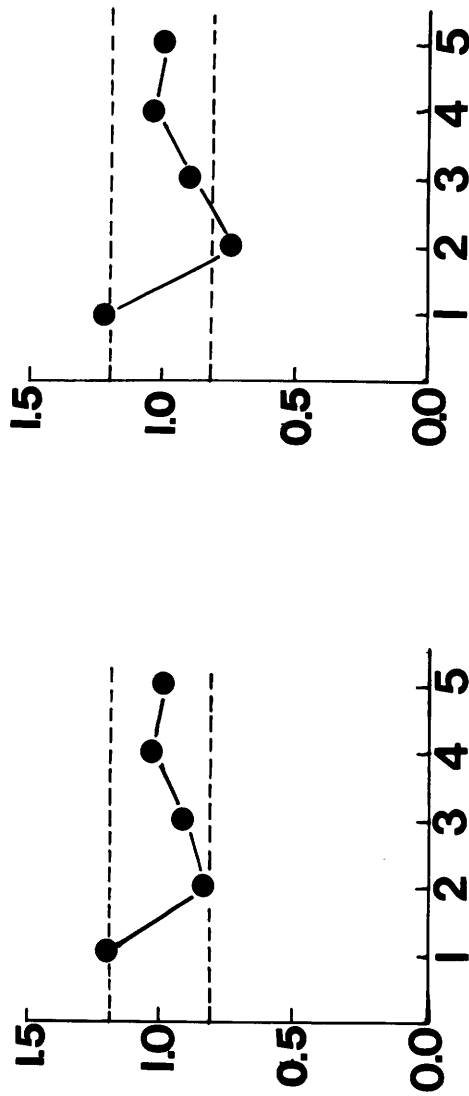
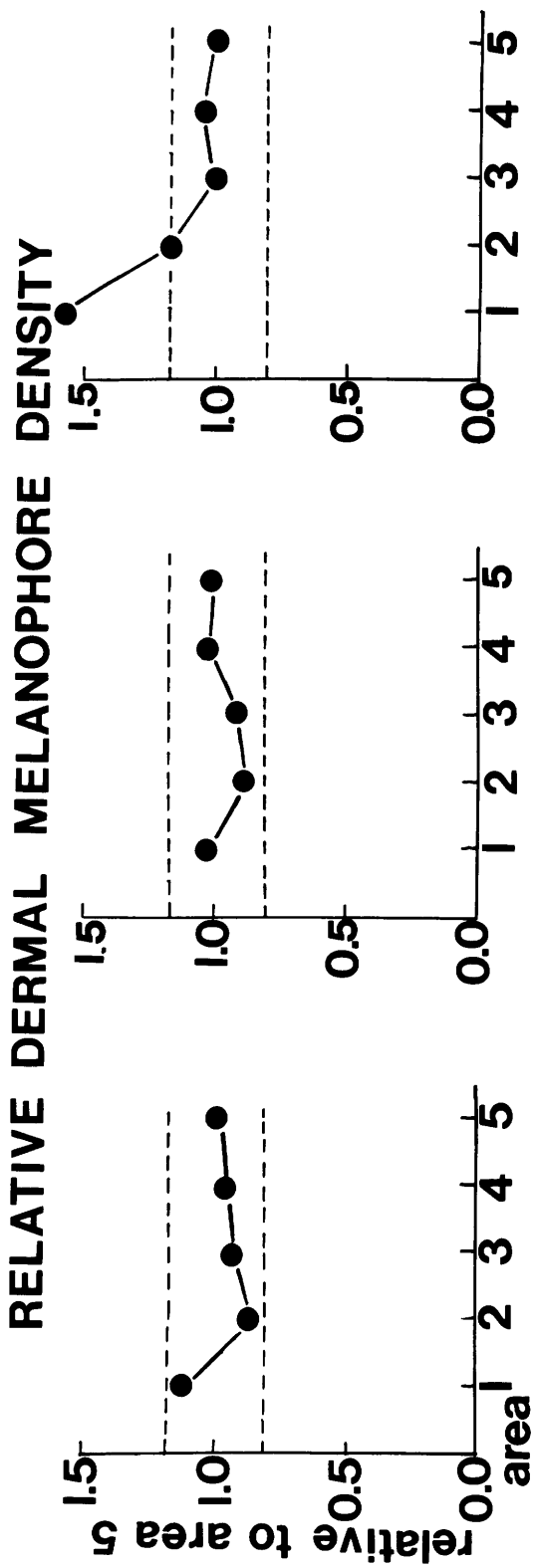
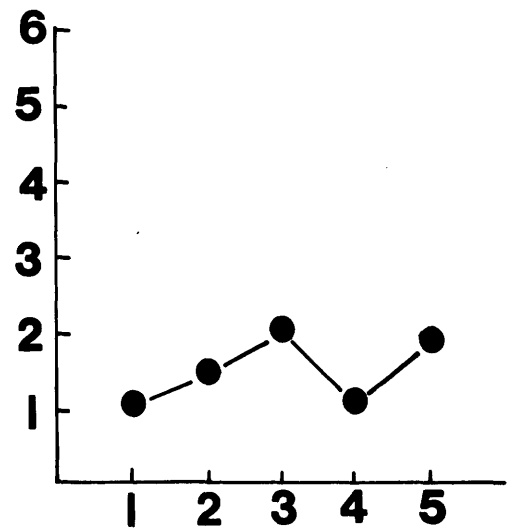
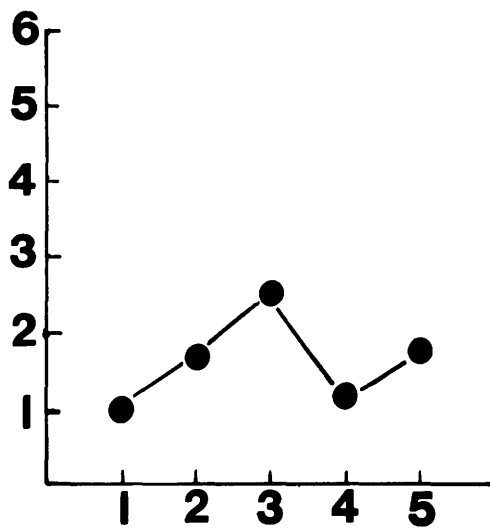
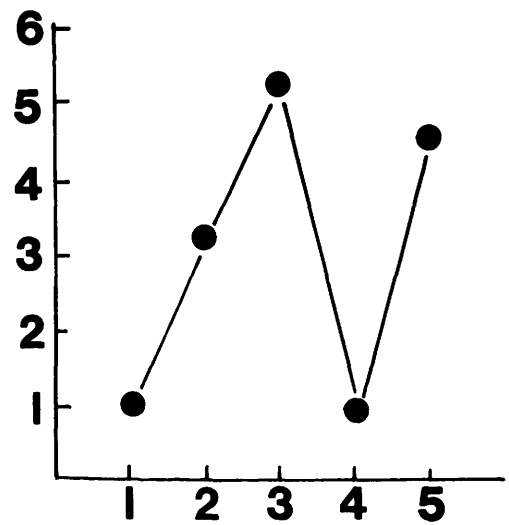
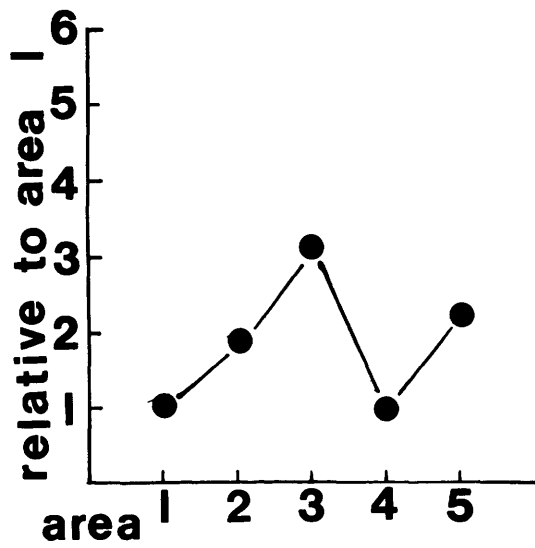


Figure 14: Comparison of the iridophore densities in the areas indicated in figure 11 for 4 specimens. Densities are displayed relative to that of area 1.

RELATIVE IRIDOPHORE DENSITY

ophores so as to expose the iridophores. Tissue was fixed and mounted as before. This preparation allowed for easy iridophore visualization by Nomarski interference microscopy.

Iridophores and dermal melanophores were counted in an area 0.0081mm^2 , four counts per scale. Counts were normalized to 0.01mm^2 (Table 4) so as to be directly comparable to tables 2 and 3. Figure 14 reveals that iridophore distribution is the inverse of the epidermal melanophore distribution. This figure represents iridophore counts in the five regions normalized to area 1. Areas where many epidermal melanophores exist (1 and 4) are areas of fewest iridophores. The area of fewest epidermal melanophores (3) is the area containing most iridophores.

When the association between the dermal melanophores and iridophores is examined, it is apparent (fig. 15) that the relationship iridophore no. per dermal melanophore (I/D.M.) varies with position. On the basis of figs. 13 and 14, I would have predicted this joint relationship. Fig. 15, however, was derived from the same material. Not only do the number of iridophores in association with individual melanophores vary with the position in the skin, but as figure 16 shows, their position with respect to the melanophores varies. In area 1, iridophores occupy positions between melanophores (fig. 16, A), while in area 3, a melanophore is surrounded by iridophores (fig. 16, B). In area 5 the relationship is intermediate between these two (fig. 15, C).

The close melanophore-iridophore association provides a chromatic structure whose function cannot be explained by the functioning of one cell alone. These associations have been termed chromatosomes (Parker, 1948). Ballowitz (1914) considered the melaniridosome the most common type although every other chromatophore combination has been described at one time or another.

Figure 15: Comparison of the ratio of iridophores per dermal melanophore in the areas indicated in figure 11. Same preparation as figure 14. Densities are displayed proportional to that of area 1.

RELATIVE I/D.M. DENSITY

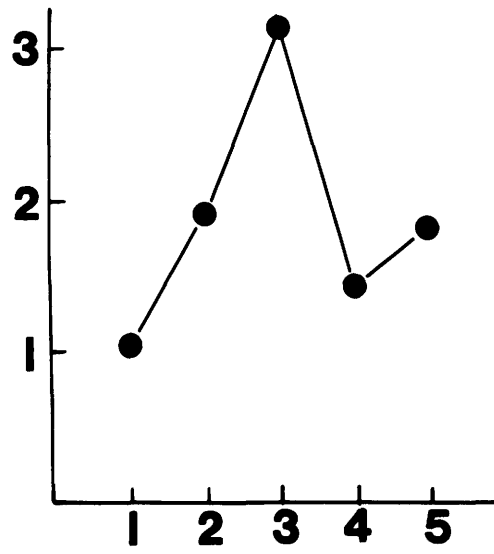
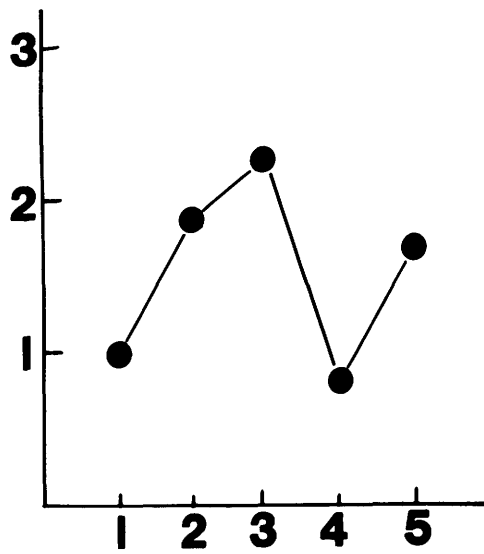
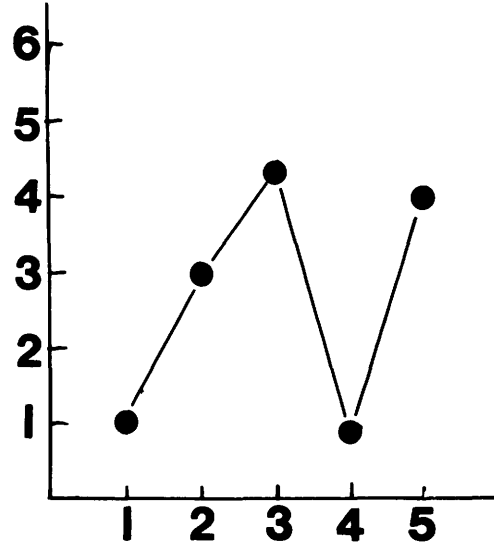
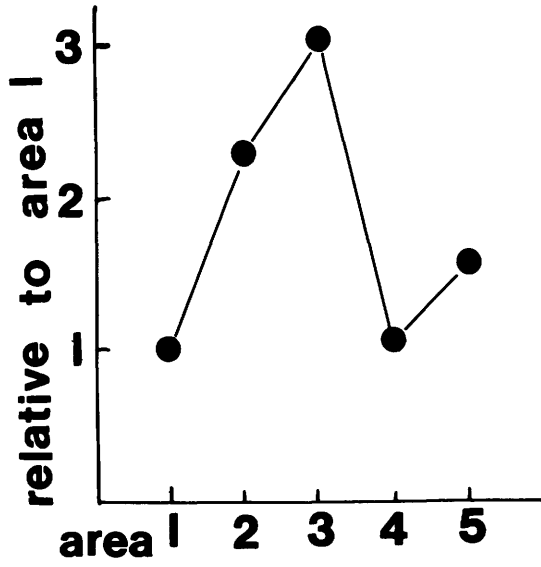


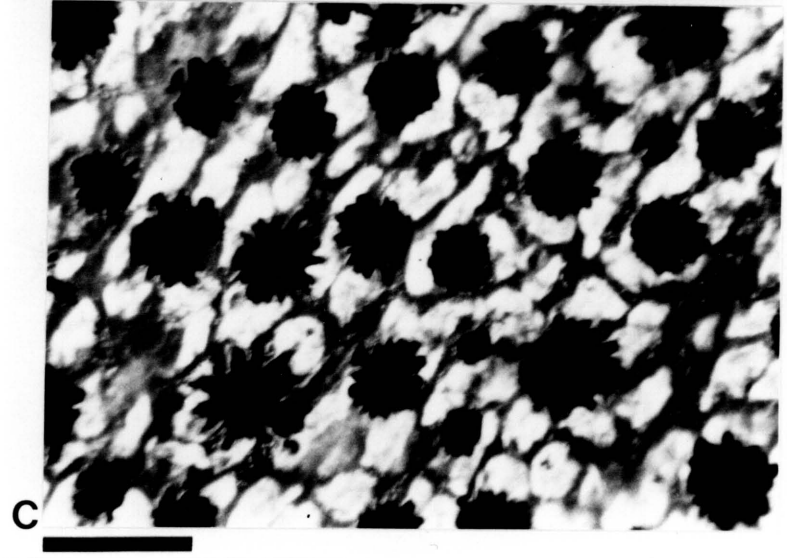
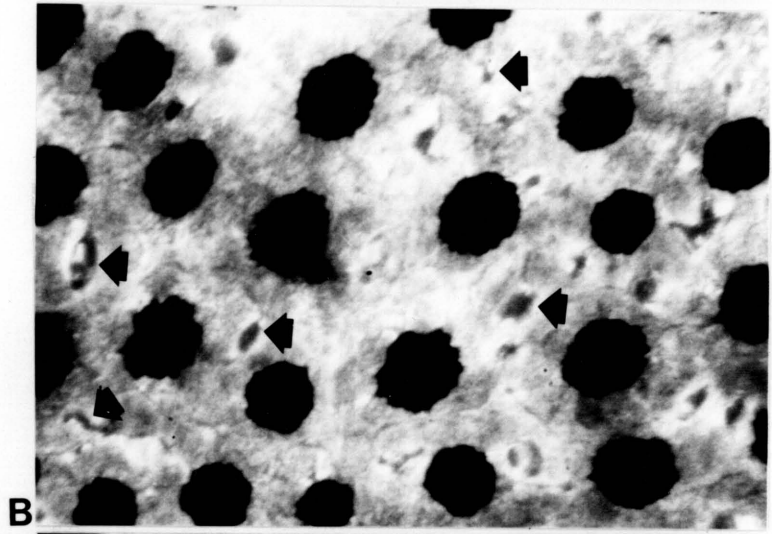
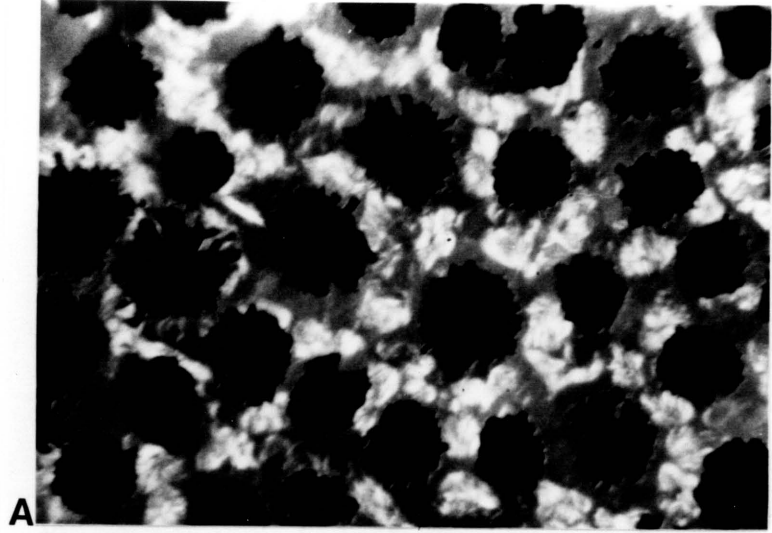
Figure 16: Dermal melanophore-iridophore complexes from areas
of the skin (as indicated in figure 11).

A, Above: Area 1 melaniridosomes.

B, Middle: Area 3 melaniridosomes. Arrows point to spaces
between iridophore sheet.

C, Lower: Area 5 melaniridosomes.

Scale: 100 microns



It is apparent that iridophore reflectance will be determined by the degree of pigment aggregation or dispersion in both melanophores. The two types of melanophores, however, perform different functions. Epidermal melanophores act to shield the dermal melanophore-iridophore complex. When dispersed, epidermal melanophores block both incident and reflected light regardless of the reflectance value from the melanophore-iridophore complex. Dermal melanophores modulate the reflectance from the surrounding iridophores as a function of melanosome positions. When melanosomes are dispersed into the processes, reflectance by the surrounding iridophores is low. When the granules are aggregated, reflectance is high. For the same degree of melanophore adaptation, reflectance varies with the density of iridophores. Small changes in local skin contrast depend only upon reflectance differences due to different iridophore densities at different states of melanophore adaptations.

A final experiment was performed to demonstrate the validity of this analysis. As before, suprascalar skin was removed from a fish from areas 1, 3, and 5. The epidermis was removed and the dermis was floated in a drop of 10^{-7} M, 10^{-6} M or 10^{-5} M norepinephrine dissolved in flounder saline. After 2-5 minutes, the specimen was fixed in 4% gluteraldehyde in saline, immediately dehydrated and coverslipped. Reflectance was measured in the reflecting densitometer. The % reflectance decrease was determined by the neutral density filter necessary to convert the 10^{-5} M reflectance value from area 3 to that of the others. The results of this experiment are represented in figure 17. The following points should be emphasized: 1) reflectances of the three different regions measured at 10^{-5} M norepinephrine are not equal; 2) reflectances of the three

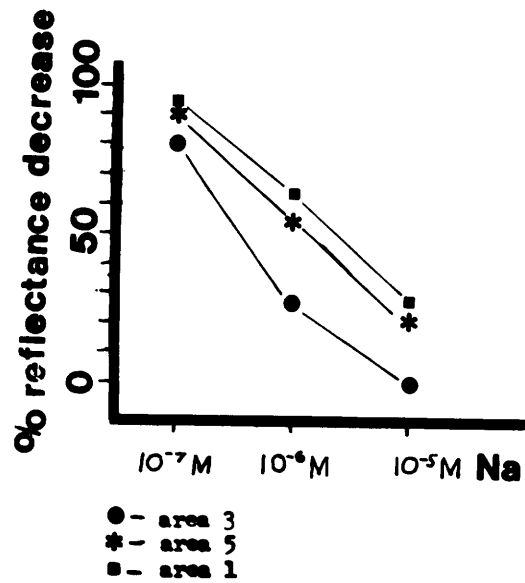


Figure 17: Change in dermal skin reflectances at three concentrations of norepinephrine in saline.

Absissa: Concentration of Norepinephrine (Na)

Ordinate: % reflectance decrease calculated by comparing the neutral density filter value necessary to convert area 3 reflectance value at $10^{-5} M$ to the reflectance values of the other points.

different regions measured at 10^{-7} M are not equal either; 3) the differences in reflectance change disproportionately when the cells are partially aggregated; and 4) the relative reflectances of the three areas are maintained regardless of the degree of melanophore adaptation. Melanophores in 10^{-5} M norepinephrine are maximally aggregated; in 10^{-7} M, they are nearly maximally dispersed. At no time does a fish skin reflect light equally from all its parts. Whether the differences are noticed, however, depends on factors unrelated to the skin.

SUMMARY: This section has shown that the variations in flatfish appearance strictly follow from the morphological constraints inherent in the skin. Dense distributions of epidermal melanophores coincide with the observed positions of 'spots'. A uniform distribution of dermal melanophores modulates reflectance due to the distribution of iridophores; and local variations in iridophore distribution account for changes in apparent patchiness of the skin.

PART FOUR: ANATOMY AND PHYSIOLOGY OF THE NERVE-MELANOPHORE RELATIONSHIP

The functional relationship between the activity of the melanophore and its innervation is the subject of this section.

HISTORICAL SUMMARY: Two different models of the neural system modulating melanophore adaptations have evolved from experiments conducted during the last 100 years. The first may be called the 'tonic aggregating influence'; the second may be described as 'competing influences.' Brucke (1852) claimed that in chameleons, when chromatic nerves ceased to fire, melanophores dispersed. This notion was revived by Gray (1956) while examining denervated melanophores in Phoxinus. Pouchet (1876) demonstrated on flatfishes of the family Turbot that severing the autonomic chain led to a darkening of the skin distal to the point of section. This cut eliminates nerve impulses to chromatophore innervating cells in the chain distal to the cut (Iwata and Fukada, 1973). Von Frisch (1911), by progressive transections and stimulations of the medulla of Phoxinus located the pre-ganglionic cells that project to the autonomic chain in the anterior medulla. Gentle (1972) using a degeneration technique claimed to have located the medullary paling cells of Phoxinus somewhere in the rostral half of the medulla.

If this pre-ganglionic chromatic nucleus is eventually located in the brainstem, it will be the only sympathetic pre-ganglionic group within the brain stem. Pre-ganglionic sympathetic nuclei have always been located in the intermediolateral region of the spinal cord (Ruch et al., 1965, p. 228 ff.).

Fibers from the supposed medullary group descend into the spinal

cord (Burton, 1964, 1969), exit via a species specific vertebra,³ and pass rostrally and caudally along the autonomic chain, innervating cells in the different ganglia (Nicol, 1952). The synapse between the pre- and post-ganglionic cells has been identified as cholinergic (Scott, 1965; Wilhelm, 1969).

A dark fish is produced when the spinal cord is sectioned anterior to the outflow vertebrae. Section of the spinal cord posterior to the outflow vertebrae does not hinder a chromatic response. Section of a peripheral root distal to the entry of the gray ramus produces a dark, wedge-shaped area, corresponding to the dermatome innervated by that root (Van Herk, 1929).

Electrical stimulation of the medulla, the spinal cord rostral to the outflow vertebrae and the autonomic chain or gray ramus induces aggregation in a region corresponding to that darkened by nerve section. Thus nervous activity in the pigmentomotor nerves corresponds to the lightening of the fish (melanophore aggregation) while elimination of activity by nerve section corresponds to darkening of the fish (melanophore dispersion).

Bert (1875) proposed that antagonistic nerve fibers controlled the aggregation and dispersion of melanophores. Parker (1948) later championed that notion. This notion has been a constant and unproven proposition in the subsequent two decades of melanophore research.

Parker found that denervated skin regions lightened over several weeks. He observed that when the fish was placed in a light tank, a subsequent cut distal to the first and within its limits redarkened the denervated region. He postulated that the second cut reactivated the dispersing fibers. Both Mills (1932) and Abramovitz (1935) noticed at the edges of denervated or regenerating zones that melanophores may

function in any of four manners; dispersed cells that would aggregate; unresponsive dispersed cells; aggregated cells that would disperse; and unresponsive aggregated cells. They both interpreted this result to mean that dispersing fibers regenerated independently of the aggregating fibers. Healey (1967) used the same argument to support the recovery of chromatic behavior in spinalized Phoxinus phoxinus.

Reports of electrical stimulation of dispersing fibers are scarce. Parker and Rosenblueth (1941) claimed to have selectively stimulated Ameiurus dispersing fibers only after fifteen minutes of continuous stimulation. Kinoshita and Ueda (1970) reported selective stimulation of dispersing fibers in Oryzias latipes immediately prior to aggregation. Von Gelei (1942) using the same transection technique as von Frisch traced what he claimed was a pathway of dispersing pigmentomotor fibers that closely followed the aggregating fiber route. Fujii and Novales (1972) suggested that the lack of success in preferentially stimulating dispersing fibers by most researchers was due to the simultaneous stimulation of the two sets of parallel nerves innervating the melanophores. The action of the aggregating transmitter released during stimulation overwhelmed the dispersing transmitter. Fujii also presented synaptic profiles in the electron microscope that he characterized as perhaps cholinergic because they contained no small dense core vesicles (Fujii, 1966a; Fujii and Taguchi, 1970).

Thus, two possibilities concerning the functioning of the nerve-melanophore physiology have been proposed to explain teleost melanophore adaptations: (1) activity in the fibers acts to aggregate melanosomes which disperse when the nerves are inactive or (2) adaptive changes in pigment distributions within the melanophore are due to the

opposite actions of two sets of nerve fibers.

Experiments were developed to resolve the two possibilities. In Section A, the electron microscopic description of the nerve-melanophore junction was examined for normal synapses and vesicular contents of the preterminal enlargements. Material was also examined with the Champy-Maillet stain for light microscopic analysis. Both techniques were then applied to material treated with 5- and 6-hydroxydopamine (-OHDA). 6-OHDA is a potent chemical sympathectomy agent (Tranzer and Thoenen, 1968). Any fibers remaining following application of 6-OHDA should not be catecholamine-containing.

In Section B, I examine the normal physiology of melanophores. Inhibitors of oxidative phosphorylation were found both to aggregate melanosomes and to prevent their redispersion.

SECTION A: ANATOMY

Ballowitz (1893a, 1893b) and Eberth and Bunge (1895) demonstrated anatomically that fish melanophores were nervously innervated. Each melanophore was contacted by more than one fiber; each fiber made multiple contacts with each melanophore; and each fiber contacted more than one melanophore. Jacobowitz and Laities (1968) and Falk et al. (1969) using the histochemical fluorescence technique for catecholamines showed the pattern of catecholamine-containing fibers innervating melanophores resembled those described by the techniques of Ballowitz and Eberth and Bunge. These light microscopic demonstrations of melanophore innervation did not exclude the presence of a non-catecholamine containing nerve innervation.

Vesicle characterization has been used to identify catecholamine and

non-catecholamine nerve fibers in the electron microscope. Nerve profiles with a vesicular content consisting of small agranular vesicles ($\leq 500\text{\AA}$) and occasional large ($\approx 1000\text{\AA}$) vesicles each with a density in its core have been identified as cholinergic nerves; nerve profiles with a vesicular content consisting of either large dense core vesicles and small dense core vesicles or large and both small agranular and dense-core vesicles have been identified as catecholamine containing nerve fibers (de Robertis and Pellegrino de Iraldi, 1961; Wolfe, 1962; Richardson, 1966). A number of fish melanophores have been examined with the electron microscope. Nerve profiles and close appositions have been identified in association with melanophores from the following fish: Fundulus heteroclitus (Bikle, Tilney and Porter, 1966; Junqueira and Porter, 1974), Lebistes reticulatus (Fujii, 1966b; Fujii and Taguchi, 1970), Chasmichthys gulosus (Fujii, 1966a), Myxine glutinosa (Holmberg, 1968), Pleuronectes platessa (Roberts et al., 1971) and Tilapia melano-pleura (Castrucci, 1975a). Nerve profiles were not seen around melanophores of Pterophyllum scalare (Schliwa and Bereiter-Hahn, 1973). Characteristics such as close nerve appositions ($\leq 200\text{\AA}$), en passant appositions, varicosities separated by thin, unmyelinated nerve segments, nerve profiles nested within grooves or depressions in the target organs, little or no post-synaptic specialization, and small dense core vesicles and mitochondria within a nerve process have all been identified in a variety of autonomic target organs.⁴ Nerve processes adjacent to fish melanophores exhibit all these features. (This provides an interesting addendum to the early contention by Spaeth (1916) that melanophores were 'disguised' smooth muscle fibers.)

Fujii (1966a) and Fujii and Taguchi (1970) noted a number of profiles

containing only agranular vesicles. They related these structures to Parker's postulated cholinergic dispersing mechanism (1948). Their conclusion can be challenged on methodological grounds (see discussion).

METHODS: Dermal skin from regions above scales of Pseudopleuronectes americanus was examined specifically for nerve-melanophore relationships in the electron microscope. Normal tissue pieces were immersed in 4% paraformaldehyde-2% glutaraldehyde in 0.1M Sorenson's phosphate buffer or 0.1M sodium cacodylate buffer with 0.1M sucrose for 0.5 to 2 hours, washed in buffer overnight, post-fixed in 2% osmium tetroxide in buffer for 0.5 to 1 hour, rinsed in buffer, dehydrated in a graded series of ethanols, and embedded in an Epon-Araldite mixture. Some fish were injected with 5-hydroxydopamine one to two days prior to fixation. This procedure enhances the visualization of the small dense core vesicle core (Tranzer and Thoenen, 1967 ; Tranzer, 1969). Some fish were injected more than once with 6-OHDA 3-8 days prior to fixation in an attempt to eliminate the adrenergic nerve component (Tranzer and Thoenen, 1968). Ultrathin sections (gold and silver) were cut both parallel and perpendicular to the skin surface on an LKD Ultramicrotome. Sections were collected on bare 300 mesh copper grids, stained with lead citrate (Reynolds, 1963) and uranyl acetate (Watson, 1958), and examined in a JEOL-100B electron microscope operated at 60 or 80 kv.

RESULTS: Dermal melanophores of Pseudopleuronectes americanus are densely surrounded by nerve profiles. Nerves are found near the tips of melanophore processes (Figs. 18, 20A, 27, 29, 30D, 31A) and as noted by Fujii (1966b), at the basal portion of the process (Figs. 19A-B, 22A-B, 24, 26A-B, 29). They were found along the middle portion

of a process as well (Figs. 18, 21, 22C-F, 25, 29, 30A-B, 31B, 33). In sections cut parallel to the skin surface, nerve profiles were found in longitudinal and transverse aspects as well (Figs. 26A, 29). Occasional fibers were found beneath the deepest portion of the cell (Fig. 34). The electron microscopic evidence of the nerve-melanophore junction supports Ballowitz's original observation that nerves tended to surround the melanophore. A nerve junction could be found associated with any portion of the melanophore.

Close appositions were found between nerve profiles and the melanophore membrane (Figs. 19A-26B, 29, 30A-D, 31A, 33). Appositions closer than 100\AA (Fig. 22) have been seen. En passant fibers were found adjacent to melanophore membranes. Fig. 25 illustrates a fiber apposed to the melanophore membrane for 2.5 microns; NP₃ of Fig. 29 paralleled a process for longer than 3 microns. Figs. 31A and B illustrate multiple contacts by single fibers onto two different melanophore processes. Multiple varicosities in single fibers were encountered (Figs. 30B-D, 32, 33). Vesicle-filled varicosities were found connected by thin nerve segments. Mitochondria were often encountered within the varicosities (Figs. 19A, 22F, 26A-B, 27, 29, 30B, 30D, 34A-B).

Vesicular distribution within a closely apposed nerve profile varied. Some transverse profiles closely apposed to melanophore membranes were densely filled with vesicles (Figs. 22B-F), but Fig. 22A shows a distribution in which the vesicles were found preferentially near the post-synaptic membrane. Longitudinal fibers in which no enlargements were evident were densely filled with vesicles (Figs. 18, 30A). Vesicle content of the intersegmental regions between two varicosities was low or non-

existent while the varicosities contained vesicles. Vesicles appeared to accumulate within the enlargement, but Fig. 30C shows vesicles preferentially adjacent to the pre-synaptic membrane.

The melanophore post-synaptic region showed the same lack of specialization characteristic of other autonomic post-synaptic membranes (Burnstock, 1970, p. 43). Slight post-synaptic membrane thickening was apparent in a few contacts (Figs. 23, 26B), but the post-synaptic membrane apposed to the majority of nerve profiles showed no such increase. An occasional sub-membranous sac of endoplasmic reticulum has been described in vas deferens material (Merrillees et al. 1963; Lane and Rhodin, 1964). Unidentified structures, possibly endoplasmic reticulum, were found in a few post-synaptic melanophore cytoplasm (Figs. 22A, 22C). The rarity of these specializations equalled that of other peripheral autonomic synapses.

Pinocytotic vesicles, with a diameter about 800 \AA were found embedded in the melanophore cytoplasmic membrane. They were encountered in melanophore membranes apposed to nerve profiles (Figs. 22B, 22D-E, 24) as well as non-apposed membranes (Fig. 23). Dense matter was occasionally encountered within them (Figs. 22B, 26A). Melanophore membranes apposed to a majority of nerve profiles contained no pinocytotic structures (Figs. 19A, 20A-B, 22A, 22F, 23, 25, 30A-D). The significance of these pinocytotic vesicles in relation to nerve function is unclear.

An examination of vesicular content in more than one hundred nerve profiles (139) shows a scant 3% without any small dense core vesicles. All four nerve profiles without small dense core vesicles lay further than 600 \AA from the nearest melanophore membrane.

Figure 18: Electron micrograph of nerve fibers interposed between melanophore processes. Note the equal distribution of vesicles in these uniform diameter nerve fibers.

scale: 0.5 microns

Small arrow associated with each nerve profile indicates a small dense core vesicle in this and the other electron micrographs.

Abbreviations common to the electron micrographs:

melanophore process: MP

nerve fibers: n, n1, n2

nerve profiles: np, np1, np2

mitochondria: mt

nucleus: nuc

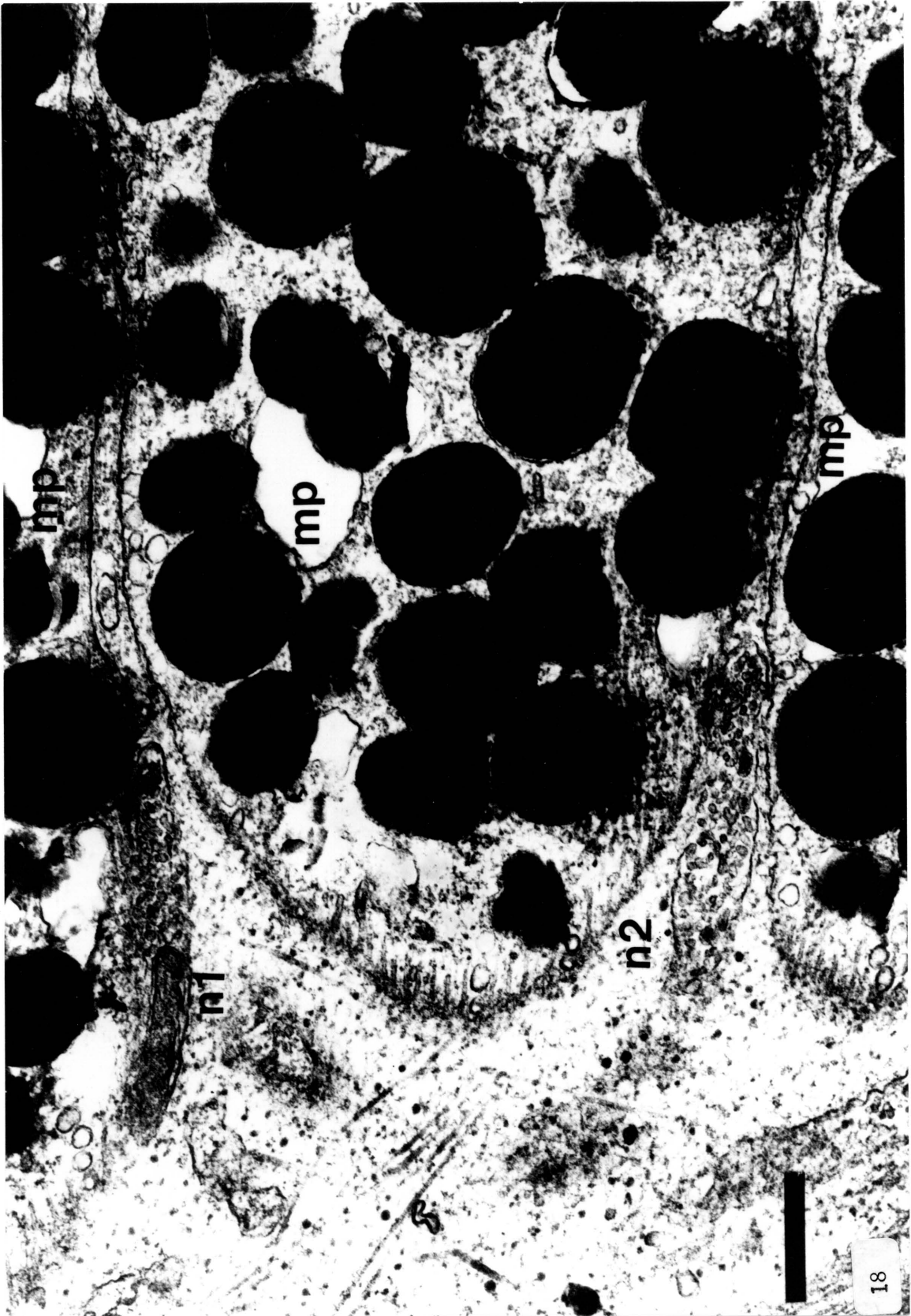


Figure 19A, B: Nerve profiles in melanophore membrane pocket

scale: 0.5 microns

Figure 20A, B: Nerve profiles adjacent to distal end of melanophore
processes

scale: 0.5 microns

Figure 21: Nerve process bending to approach melanophore

This figure was obtained from an animal injected

with 5-OHDA

scale: 0.5 microns

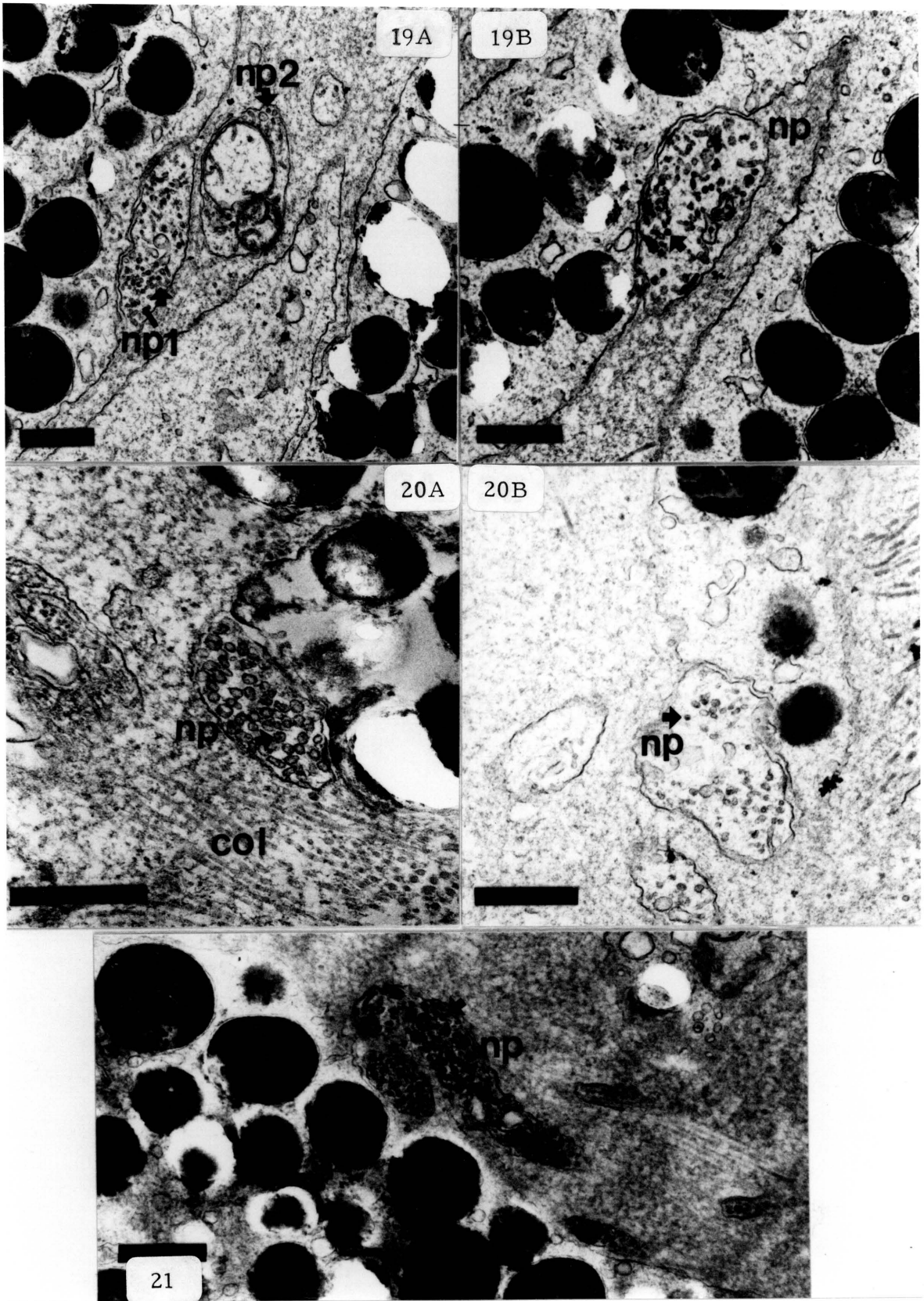
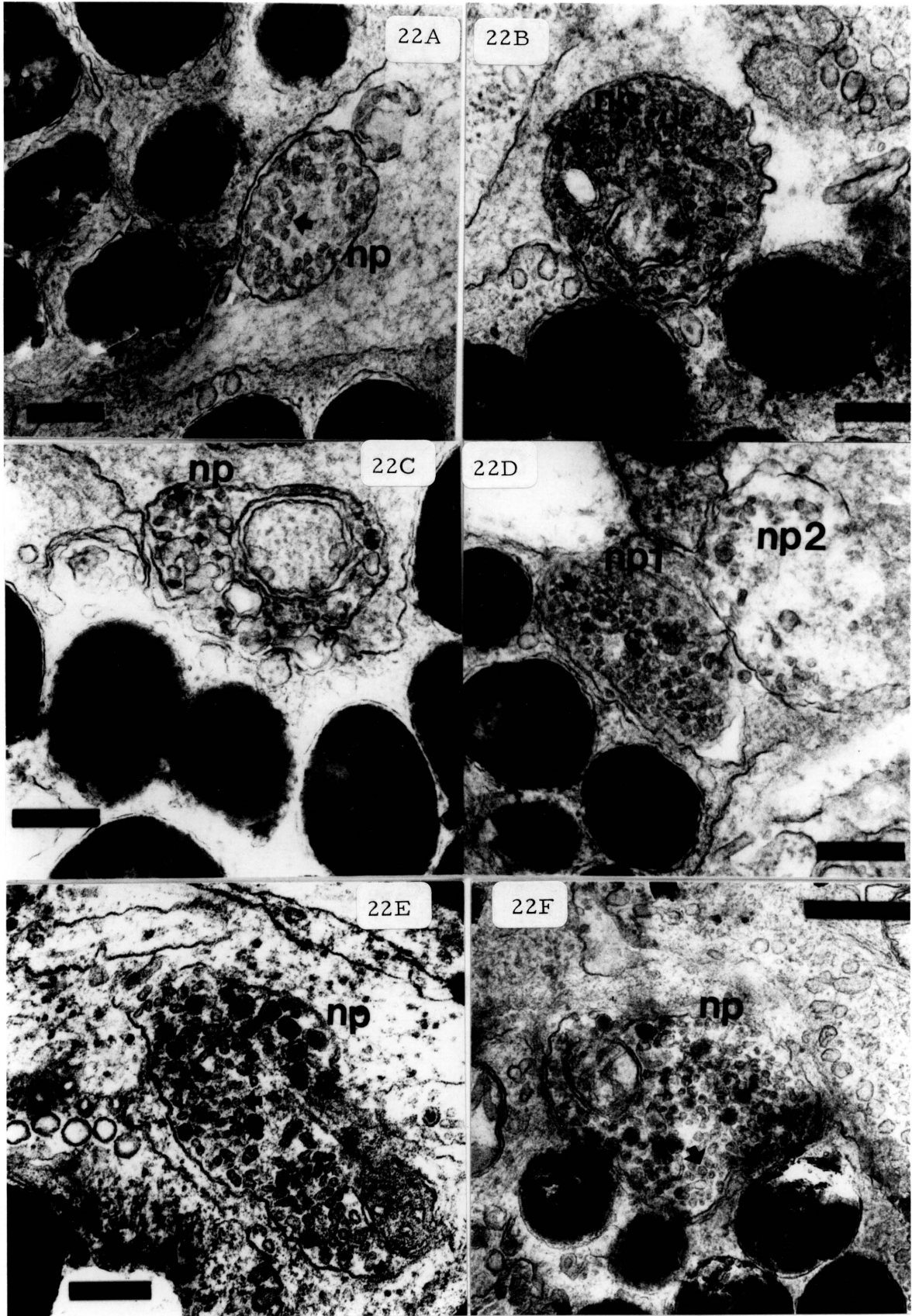


Figure 22A-F: Close appositions between nerve profile and melanophore membranes

Figure 22E was obtained from an animal injected with 5-OHDA.

scale: 0.25 microns, A-E

scale: 0.5 microns, F



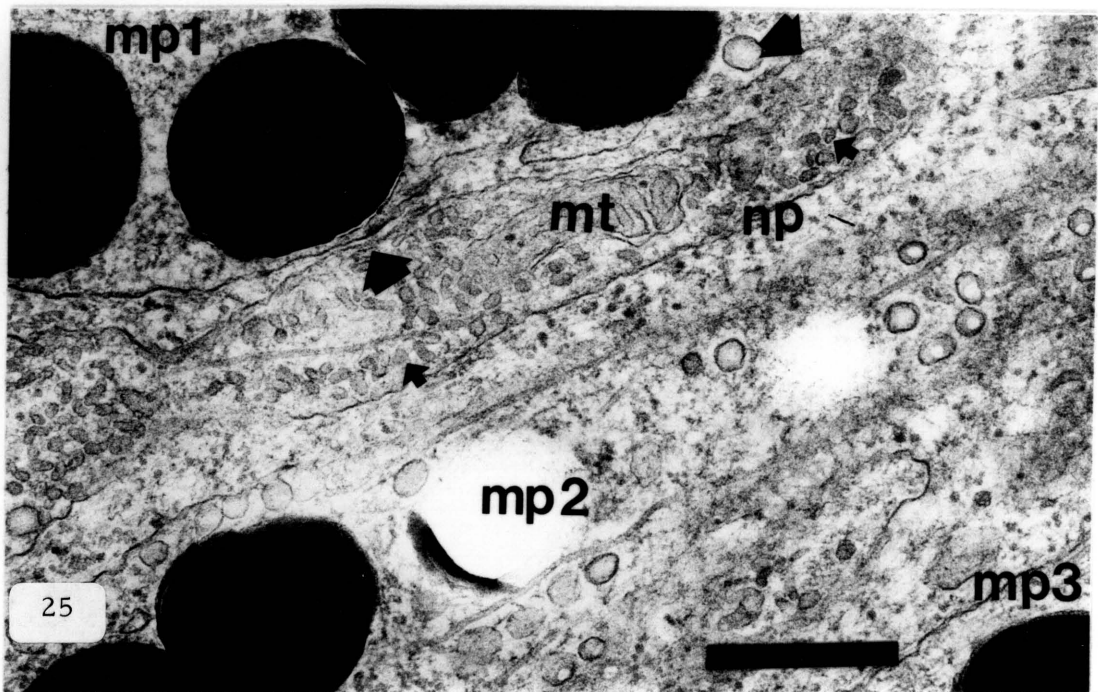
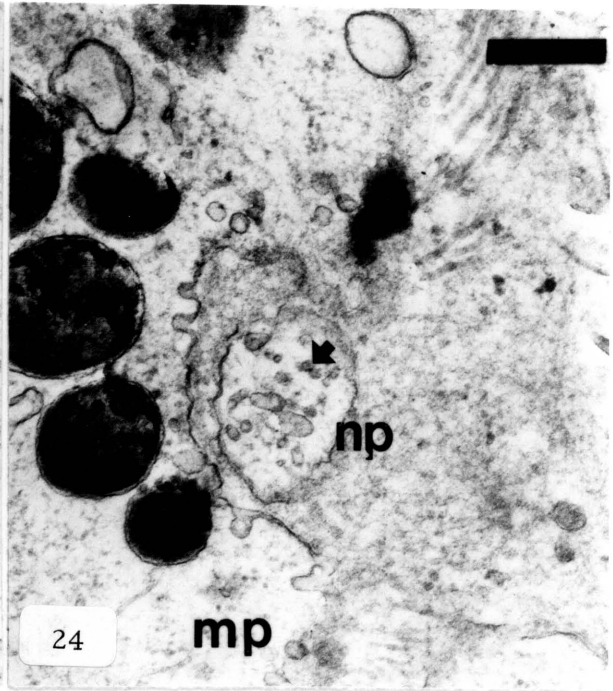
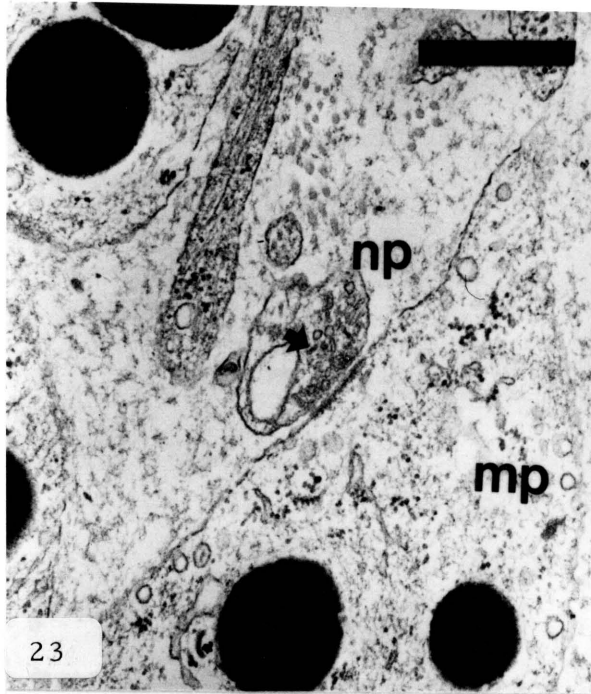


Figure 26A, B: Nerve profiles associated with the centrosphere
at the level of the nucleus scale: 1 micron

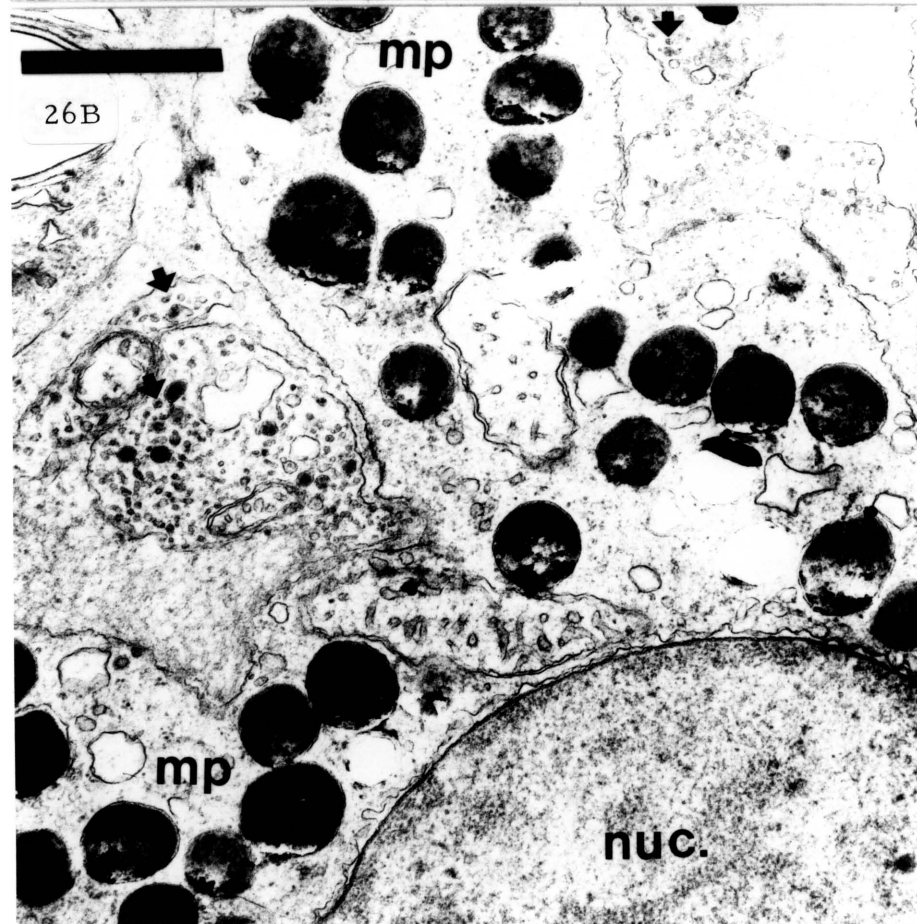
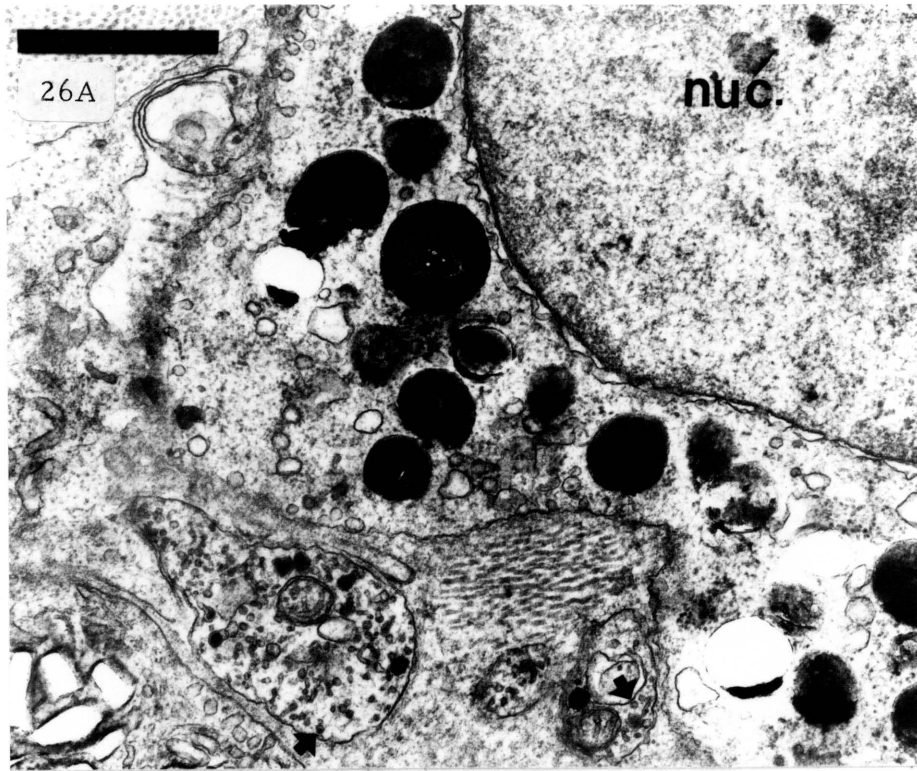


Figure 27: Cluster of nerve processes associated with a
melanophore

Obtained from a 5-OHDA injected fish. scale: 1 micron

Figure 28: Cluster of nerve processes associated with a
melanophore

Obtained from a 5-OHDA injected fish. scale: 0.5 microns

Figure 29: Three nerve profiles associated with a single
melanophore

scale: 1 micron

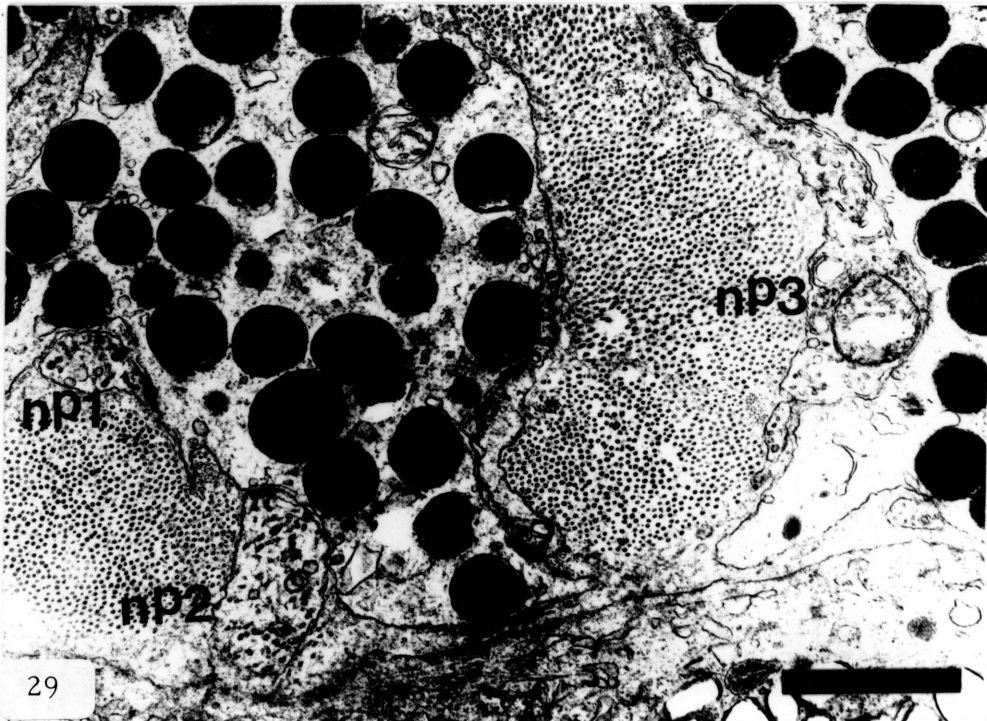
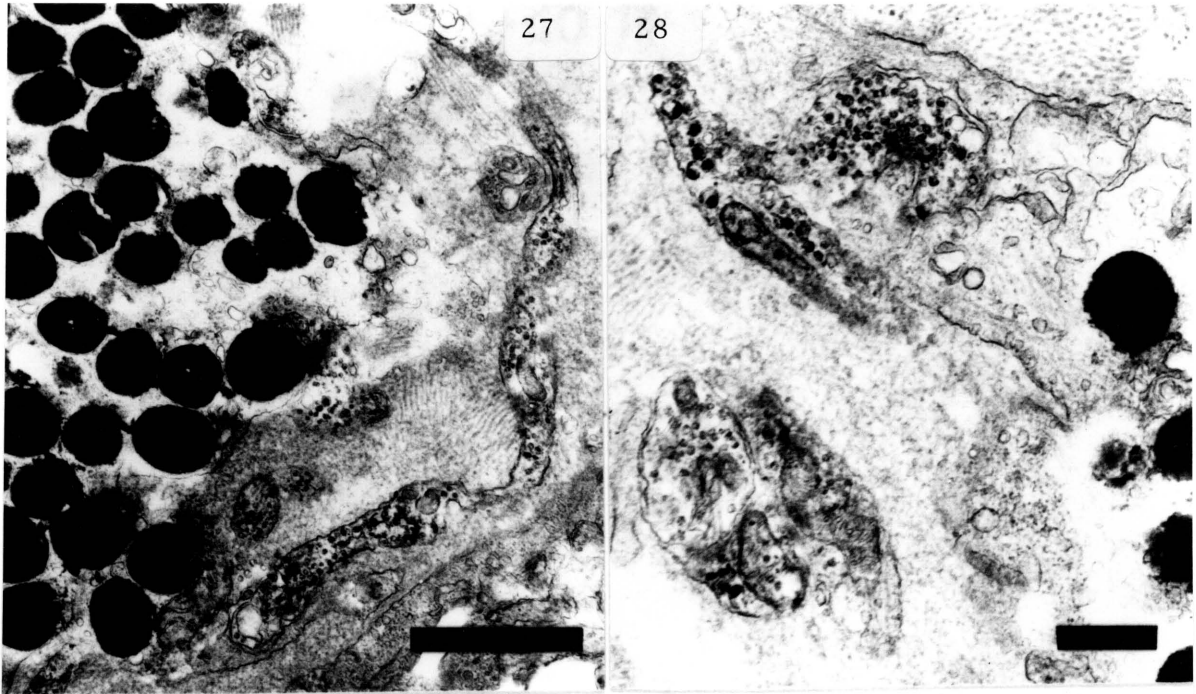


Figure 30 A-D: Examples of single fibers making multiple melanophore appositions

A: Uniform diameter fiber densely filled with vesicles

B: Two varicosities joined by ultrathin segment

C: Fiber showing segmented distribution of vesicles

D: Single fiber making contact with two processes

Scale: 0.5 microns

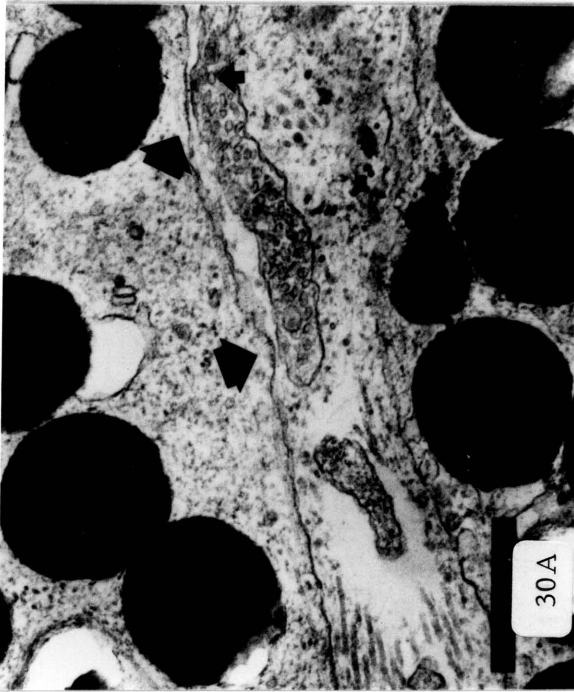
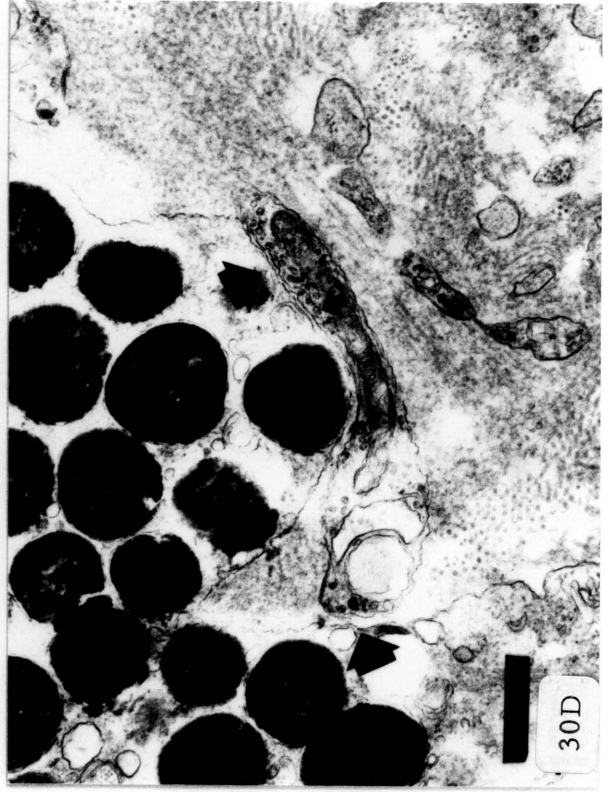


Figure 31A, B: Single fiber making close contacts with two
melanophore processes

Large arrows: points of close contact

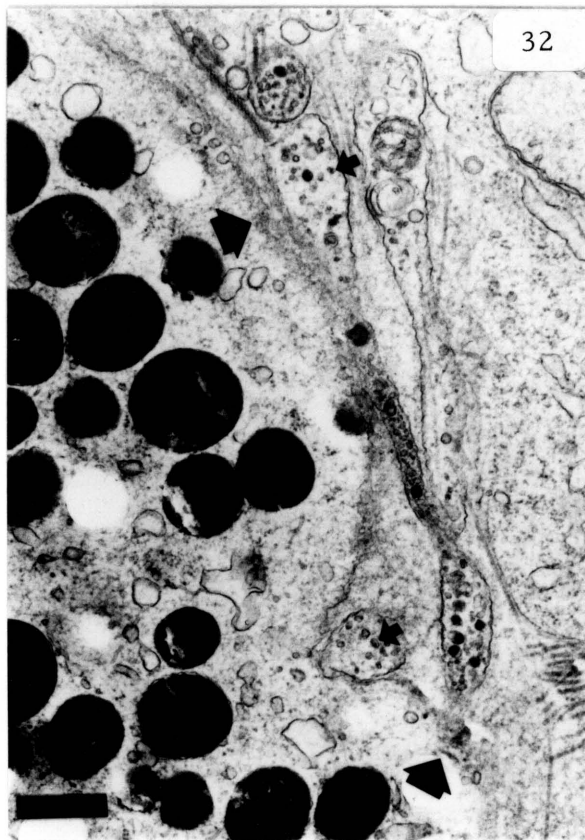
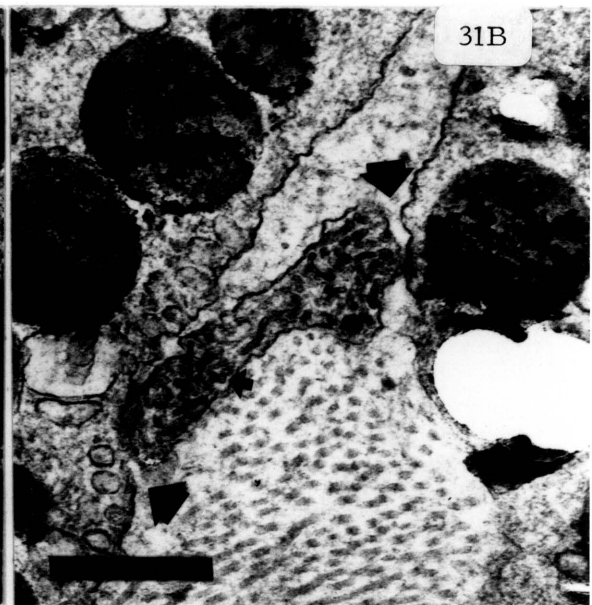
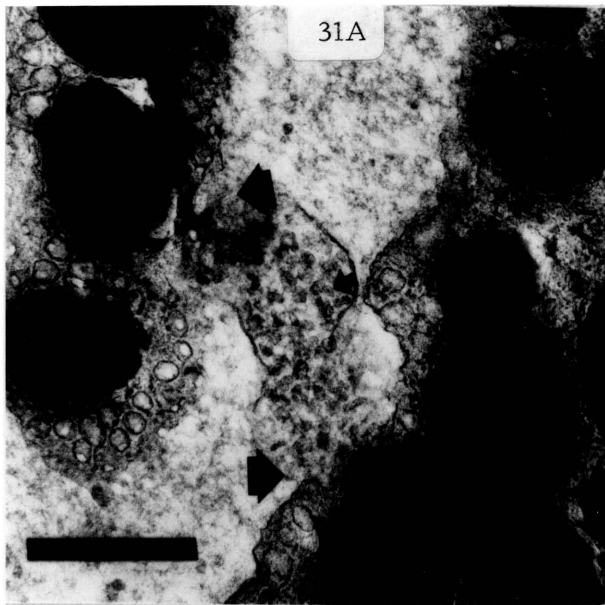
scale: 0.5 microns

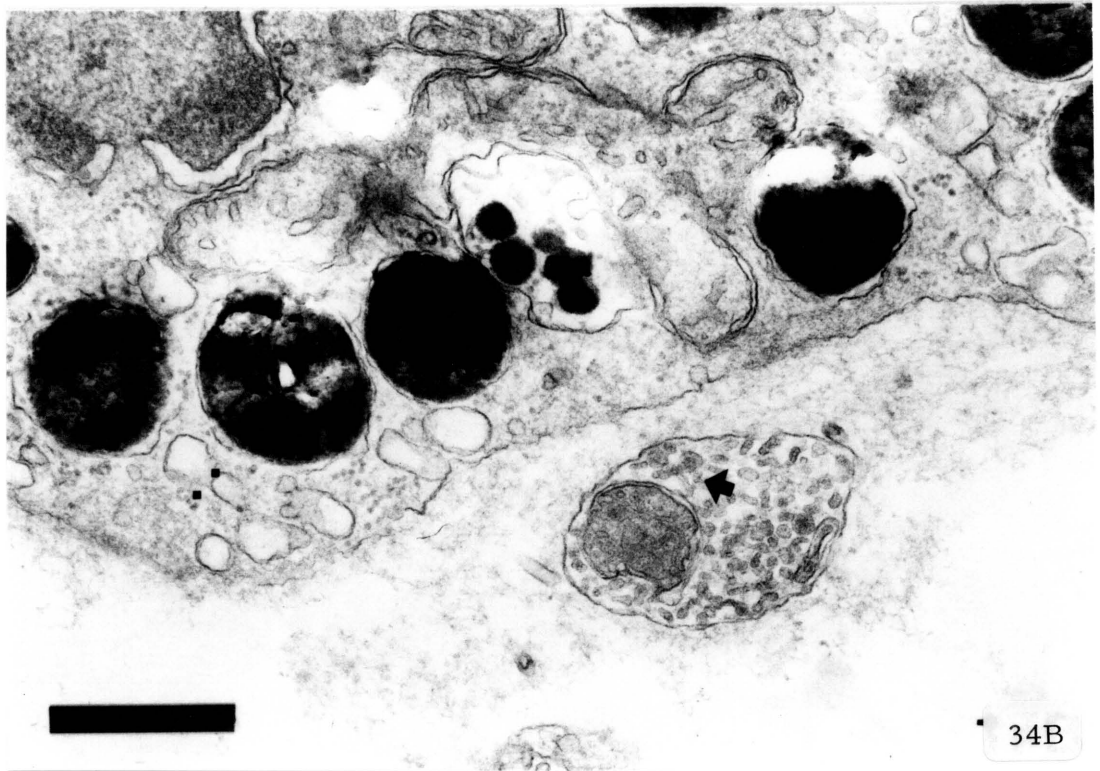
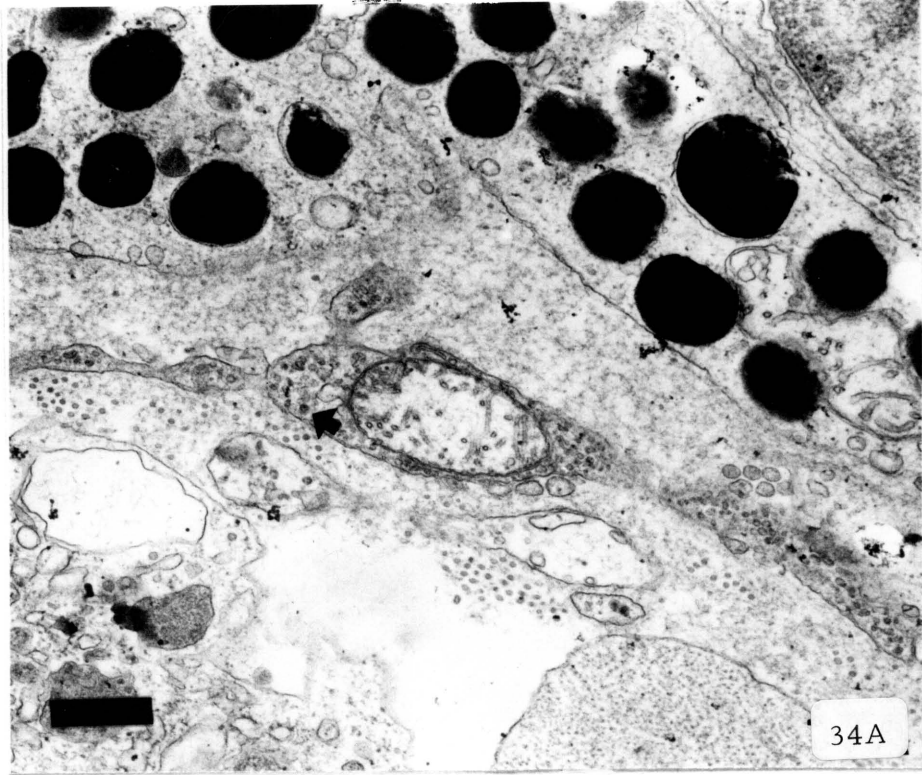
Figure 32: 6 micron fiber containing two varicosities and
intersegment Large arrows: points of close contact

scale: 0.5 microns

Figure 33: Fiber containing two varicosities that curls around
melanophore membrane

scale: 0.5 microns





Since preference was made during selection of material for close appositions, the data sample should be skewed towards closer approaches. This procedure should have enhanced the finding of close non-catecholamine fibers.

The line on Fig. 30C slices a nerve identifiable as containing small dense core vesicles at point A where no dense core vesicles were present. In inopportune section, this fiber would appear as a close agranular vesicle-containing nerve profile. The visualization of agranular small vesicles alone cannot then be considered (as Fujii did) as a demonstration of non-adrenergic nerve fibers in this material.

Follenius (1971) first showed that 6-OHDA affected the melanophore system of the stickleback Gasterosteus. Following an initial paling response, a fish darkened for two weeks after which it slowly began to recover chromatic adaptability. This recovery time was similar to that reported for the regeneration of chromatic fibers following nerve section (Parker and Porter, 1933; Abramowitz, 1935).

Flounders injected with 6-OHDA responded in a similar fashion. An initial paling lasting for less than four hours was followed by a long term dark phase. After three weeks, if the fish survived (5 out of 8), it began to recover its chromatic response. At no time following an injection did the fish exhibit a patterned response characteristic of an adaptive change. Some lightening was apparent when a fish was transferred from a black tank to a white one, but the delay was between one and four hours indicating a non-neural influence.

Experiments with the pharmacological agents 5-OHDA and 6-OHDA did not produce the uniform results suggested by their activity in the central nervous system (see 6-Hydroxydopamine and Catecholamine

Neurons, ed. Malmfors, T. and Thoenen, H., Elsevier, N. Y., 1971).

Material examined in the electron microscope following a sub-cutaneous or i.p. injection of 5-OHDA (80 mg./kg. body weight) 3-4 days prior to fixation showed a denser filling of the small vesicles within the nerves surrounding the dermal melanophores (Figs. 21, 22E, 27, 28). This material does support the prior findings on normal material that all nerves close to melanophores were probably catecholamine-containing.

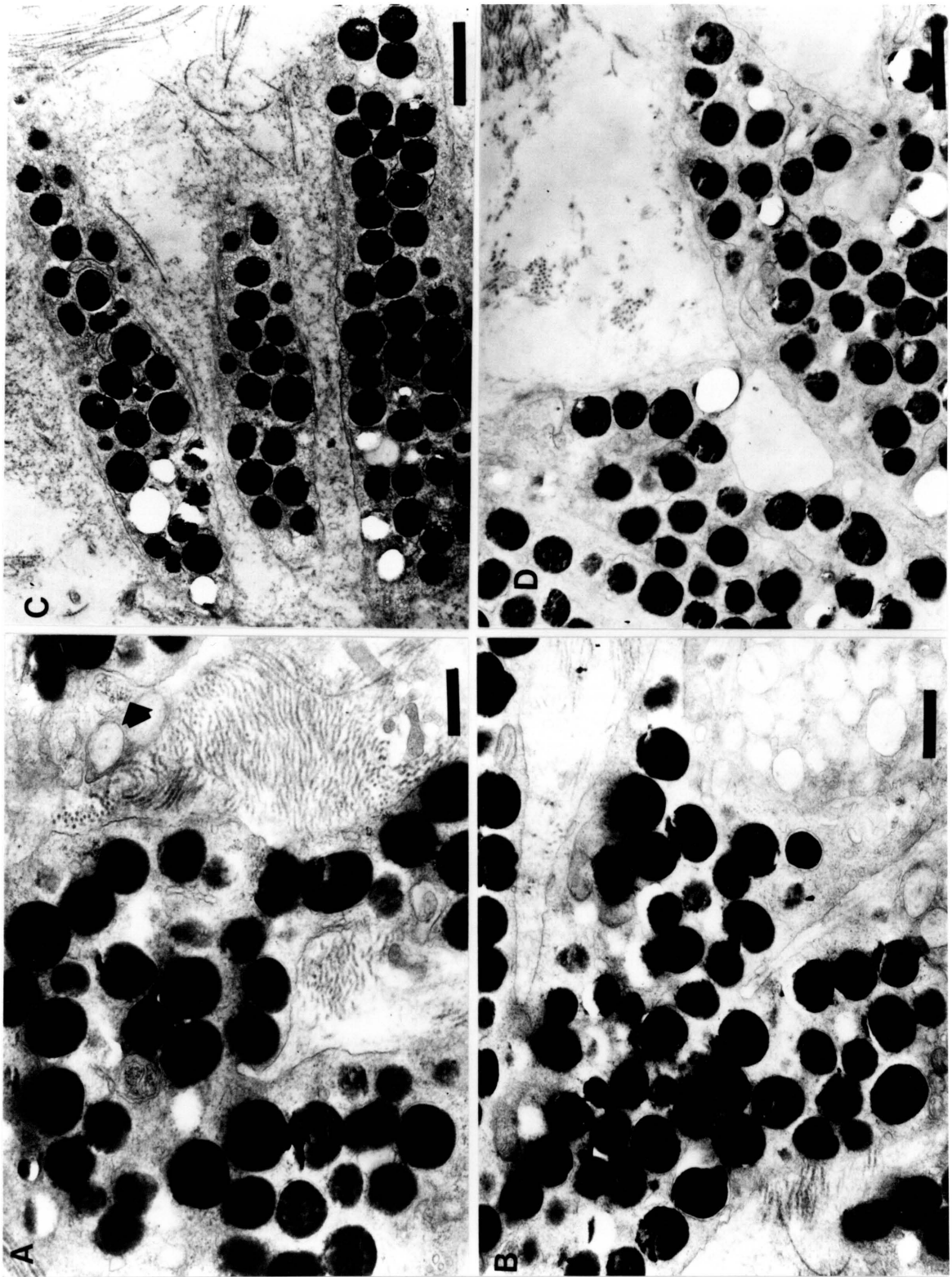
The action of 6-OHDA in large doses (100-130 mg./kg) 5-10 days prior to fixation was not completely conclusive. Not all the nerve terminals containing small dense core vesicles were eliminated following sub-cutaneous or intraperitoneal injections. Figs. 31A, 34B, and 35A (arrows) show remaining nerve terminals. However, Figs. 35B-D show examples of melanophore membranes from three different melanophores with no opposed nerve profiles. Nerve profiles were absent from the areas where they were found in normal material. This result is consistent with a sub-lethal injection of 6-OHDA (Thoenen and Tranzer, 1973). When injected sub-lethally, 6-OHDA acts in a fashion similar to 5-OHDA. The density of the dense core vesicles increases without inducing the degenerative changes. This finding, the simultaneous absence of some nerves and presence of filled dense core vesicles in other nerves, is consistent with a multiple catecholamine nerve innervation to a single melanophore. In addition to the electron microscopic examination, material was examined in the light microscope. Whitear (1952) had previously reported difficulty in staining fish skin nerves, but the Champy-Maillet method for boutons and small fibers worked in this material. The stain according to Pellegrini de Iraldi (1977) resembles a methyl blue preparation.

Figure 35: Effect of 6-OHDA on the location of nerve profiles near melanophores.

A: Arrow (upper right) points to a remaining nerve profile in treated material.

B-D: Absence of nerve profiles in relation to melanophore processes.

scale: 1 micron



Supra-scalar skin separated from the scale was immersed in freshly prepared zinc iodide-osmium tetroxide (ZI-OsO₄) according to Akert and Sandri (1968) for 4 to 7 hours, dehydrated and mounted whole or embedded in Epon-Araldite and sectioned at 1-4 microns.

Normal skin showed the characteristic surrounding nerve plexus (Fig. 36A). Features such as the geometry of approach, the surrounding 'net-like' nerve presence, and multiple nerves per melanophore and multiple melanophores per nerve are distinguishable in this material. The sub-epidermal nerve plexus is densest above each melanophore in thin section (Fig. 37A).

In material from fish injected with 6-OHDA, fibers were not found in the stratum spongiosum adjacent to the dermal melanophores (Fig. 36B), although remaining fibers can be found in the sub-epidermal nerve plexus (Fig. 37B). Some of these fibers (arrows) appear broken as if degenerative remainders were present. Fibers were not found between melanophores and between processes of a single melanophore, and branches from the remaining sub-epidermal nerve plexus were not seen descending into the stratum spongiosum as was the case for normal material. This observation suggests that the sub-epidermal nerve plexus contains nerve types other than autonomic fibers related to melanophore control. Fibers have been found that enter the epidermis from the sub-epidermal nerve plexus to form free nerve endings and innervate skin taste buds (Whitear, 1971).

DISCUSSION: The evidence derived from these two anatomical techniques reinforces the notion that the peripheral nerve innervation of melanophores is of a single character, and that character is catecholaminergic in this fish. The evidence does not support the notion of a dual nerve innervation.

Figure 36: ZrO_2 preparation of melanophore nerve plexus
before and after 6-OHDA treatment

A, Above: Normal whole mount preparation.

B, Below: Whole mount preparation after 6-OHDA treatment.

scale: 30 microns



A



B

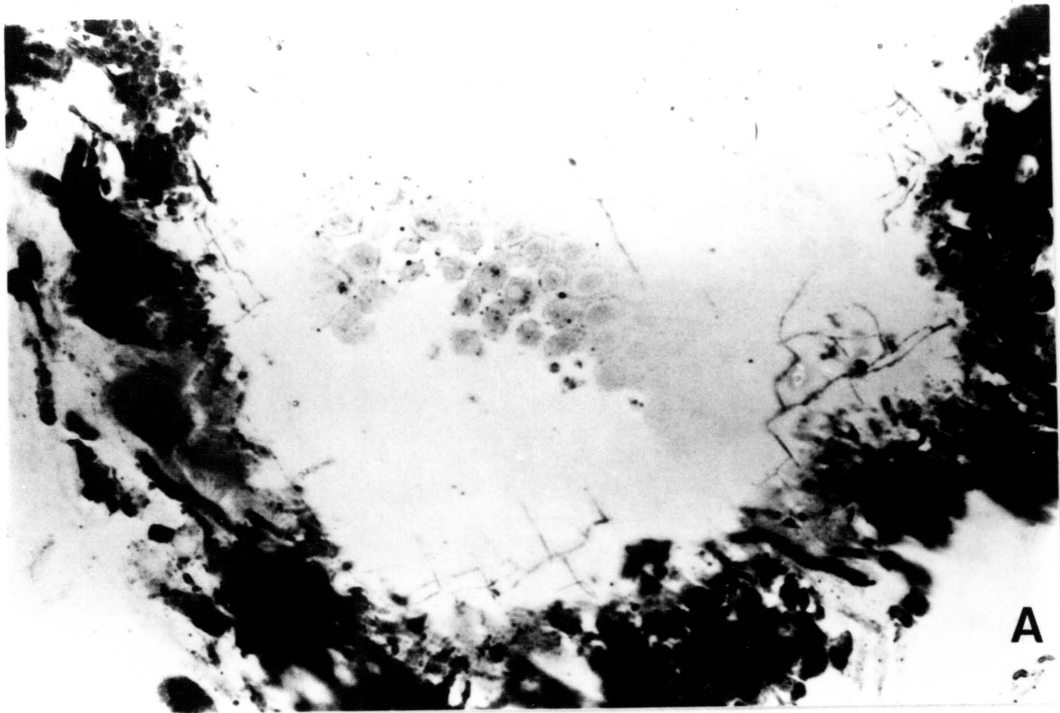
A

Figure 37: ZrO_2 preparation of sub-epidermal nerve plexus before and after 6-OHDA treatment.

A, Above: Normal sub-epidermal nerve plexus; 1 micron section of Epon-Araldite embedded material.

B, Below: Remaining sub-epidermal nerve plexus after 6-OHDA treatment; 1 micron section of Epon-Araldite embedded material. Arrows point to particulated appearing fibers.

scale: 50 microns



Anatomically, differences in states of adaptations at denervated or regenerating chromatic nerves were attributed to a disturbed neural functioning (Mills, 1932; Abramowitz, 1935; Healey, 1967). Melanophores were considered passive responders.

The other anatomical argument has been presented with electron microscopic evidence. Fujii (1966b) and Fujii and Taguchi (1970) identified non-catecholamine fibers by the presence of small agranular vesicles. Their conclusions can be questioned on technical grounds. For consistent fixation of the dense core of small dense core vesicles, the material must be fixed by gluteraldehyde followed by an osmium postfixation (Hokfelt, 1966; Tranzer and Thoenen, 1967; Pellegrino de Iraldi et al., 1971). The material from Fujii's 1966 paper was fixed in osmium alone; Chasmichthys melanophores in the second paper were fixed in gluteraldehyde alone. He could not be guaranteed preservation of the detail in question. Moreover, as Fig. 30C demonstrates, the presence of small agranular vesicles may be due only to the artifact of thin section selection. The presence of small agranular vesicles in a mixed population of vesicles does not exclude identification of that nerve as a catecholamine-containing nerve (Tranzer and Thoenen, 1967.) because small agranular vesicles may be empty of catecholamines rather than full of other substances.

The second type of evidence in favor of dual nerve innervation has been physiological. Following a peripheral nerve section, a dark, denervated band was created in the tail fin of a Fundulus (Parker, 1948). The band faded within a few days after the fish was placed in a light tank. Parker found that a second cut distal to the first within the extent of the original band produced a redarkened denervated band distal to the second cut. He interpreted the result in the following fashion: The first cut

denervated the melanophores in the band. A general hormonal catecholamine action on the melanophores lightened the denervated band after the fish was placed in a light tank. The lightening catecholamine may have been distributed via a vascular system or it may have diffused from the adjacent innervated regions into the denervated band. A second cut 'restimulated' the darkening fibers such that a second dark band was produced. Moreover, the effect of the second cut could be blocked by cold in a fashion analogous to a nerve block induced by cold.

His experiments were repeated often. Some experimenters successfully duplicated this experiment; others did not (see Parker, 1948, p. 126 for a listing). In the most recent attempts, both Scott (1965) and Fujii (1973) reported a failure to duplicate Parker's experiments. Neither could reproduce a dark band following the second cut. These experiments were interpreted with regard to neural functioning. The melanophore was considered a passive cell whose state of pigment granule disposition was determined by the neural (or hormonal) functioning. The cell had no intrinsic activity, so the states of adaptation were under command of a hormonal presence.

As I show in the next section, the teleost melanophore does have an intrinsic tendency to disperse its pigment granules when it is healthy and is isolated from all neural influence. Hence, any interpretation requiring a dispersing neural system is superfluous without direct evidence.

SECTION B: CELLULAR PHYSIOLOGY OF MELANOPHORES

The migration of melanosomes during adaptive changes is a dramatically asymmetric process. Migration from the distal end of a process to the cell center is a smooth and continuous movement. Migra-

tion in the opposite direction is a slower, stepped movement, much like a biased Brownian movement (Green, 1968). The movements appear tracked because melanosomes appeared in the microscope to follow the same intracellular paths in subsequent migrations. Franz (1939) claimed the existence of intracellular channels by which the melanosome path was defined. The channels were defined by a cytoplasmically distributed component because migration of melanosomes occurred in processes isolated from the cell centrosphere (Mathews, 1931; Kamada and Kinoshita, 1944).

Since their visualization in the electron microscope, microtubules were thought to define the intracellular channels (Bikle, Tilney and Porter, 1966; Schliwa and Bereiter-Hahn, 1973). Attachments between pigment granules and the microtubules were thought to provide the motive force, but unlike intracellular transport of mitochondria (Raine, et al., 1971) or intracellular transport of synaptic vesicles (Jarlfors, et al., 1969), no direct attachment between microtubules and melanosomes had been seen in the transmission electron microscope.

The integrity of microtubules has been found to be a necessary requirement for melanosome migrations. Disruption of microtubules by colchicine or vinblastine (Wikswa and Novales, 1969; Junqueira, 1972; Murphy and Tilney, 1974; Murphy, 1975), by cold (Murphy and Tilney, 1974), or by hydrostatic pressure (Marsland, 1944; Murphy and Tilney, 1974) prevents melanosome migration. Very recently Byers and Porter (personal communications) visualized by high voltage electron microscopy of tissue cultured erythrocytes, a series of connections between pigment granules and microtubules via a third organelle. The connections were made by a series of filamentous trabeculae between the

microtubules and melanosomes. These connections were only evident when the melanosomes opposed the microtubules.

Teleost dermal melanophores with only one known exception (see below) aggregate in the presence of catecholamines. In vivo injections of catecholamine or sympathomimetic drugs aggregate fish melanophores (Fujii, 1961; Scheline, 1963; Scott, 1965; Grove, 1969; Reed and Finnin, 1972; Bagnara and Hadley, 1973; Fernando and Grove, 1974a; Castrucci, 1975b). Likewise, melanophores of in vitro skin pieces immersed in saline containing a catecholamine, aggregate (Fernando and Grove, 1974b; Fujii and Miyashita, 1975). Blocking the alpha-adrenergic receptor site on the melanophore membrane prevents catecholamine induced aggregation (Reed and Finnen, 1972; Fujii and Miyashita, 1975). Direct stimulation of the alpha-receptor site by sympathomimetic drugs, isoprenalol for instance, promotes aggregation (Fujii and Miyashita, 1975). Blocking the enzymes that degrade catecholamines potentiates their aggregating actions (Scott, 1965). There is little doubt that pharmacologically melanophores from all fish except one tested so far are catecholamine sensitive. Melanophores respond to the presence of catecholamines by aggregation of melanosomes to the centrosphere. Melanophores of the fish Parasilurus asotus do not show a response to catecholamines, but to acetylcholine (Fujii and Miyashita, 1976). Even in this fish, however, the transmitter substance acts to aggregate the melanophores.

In vivo injections of acetylcholine have been attributed dispersive powers as a result of a direct melanophore response (Parker, 1948; Green, 1968). Scott (1965) noted that pigment-aggregating fibers are excited when acetylcholine is injected into the spinal cord. Grove (1969)

and Wilhelm (1969) pointed out that the pre- to post-ganglionic synapse is cholinergic. Hence, an unambiguous conclusion on the effect of acetylcholine in vivo on melanophore adaptation cannot be drawn.

Junqueira and Porter (1971) adopted a second point of view with regard to melanosome movements. They noted that pigment migration in both directions can continue for hours without an external energy supply. When oxidative phosphorylation was stopped, pigment granules of Holocentrus erythrophores ceased migration under conditions promoting migratory activity. They also noted (1974) when oxidative phosphorylation was inhibited, the extent of melanosome redispersion in Fundulus melanophores was limited.

METHODS: Experiments along these lines were extended. Pieces of skin above a scale were separated from the scale. The epidermis was removed by immersion in 3% EDTA buffered with 0.44% NaHCO₃ (Stone and Chavin, 1974) for 5-10 minutes and reimmersed in Plaiice saline (Cobb, et al., 1973) to maintain melanophore viability. Smaller melanophores would aggregate and subsequently remain aggregated if left in the EDTA solution longer than 15 minutes. Separation of the epidermis from the dermis followed the aggregation of melanophores from one side of the scale to the other.

Isolated dermis was mounted in a perfusion chamber upright on a Leitz Binocular microscope. 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. Voltage between the photocathode and anode varied depending upon the magnification and the number of melano-

phores in the field of view. At 1000 x magnification, 1-3 melanophores were monitored; at 450 x, 5-8 were examined. A diaphragm was mounted at the plane of the real image inside the eyepiece to limit the number of melanophores measured. All records presented correspond to the response of 1-3 melanophores.

The output of the photomultiplier was recorded on an HP 680 chart recorder. A Roscolene number 815 filter was interposed between the light source and the microscope condenser to eliminate light variations due to concurrent erythrophore granule migrations.

RESULTS: Both 2, 4-dinitrophenol (DNP) (Figs. 38B-C) and sodium cyanide (NaCN) induced melanosome aggregation. Melanosome distribution was unaffected when the concentration of either inhibitor of oxidative phosphorylation was below 10^{-4} M. Both DNP (Fig. 38A) and NaCN at concentrations of 10^{-4} M in dispersing saline induced pulsations in the adaptive state of dispersed melanophores. Cells responded by aggregations up to 30% maximum from the dispersed state followed by a slower redispersion. Control material showed no such activity. These pulsations did not appear to correspond with the regular pulsations induced by Ba^{2+} (Spaeth, 1916). Pigment granules would begin to retract smoothly but cease and pulse outward. Not all processes pulsed outward simultaneously as they did when aggregating. Melanosomes did not redisperse in normal saline containing 5×10^{-4} M DNP.

Pigment migration did not cease when the cells were exposed to inhibitors of oxidative phosphorylation. Cessation of migration occurred only after the pigment granules were aggregated. Even then, small movements of individual melanosomes could be seen at the perimeter of the aggregated melanosome mass. However, once aggregated, these

Figure 38: Aggregating properties of 2, 4-dinitrophenol

(Note: Ordinate represents the degree of melanosome aggregation, from 0, i. e., complete dispersion into the melanophore processes, to 100%.)

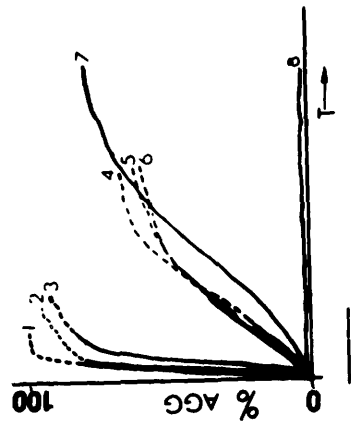
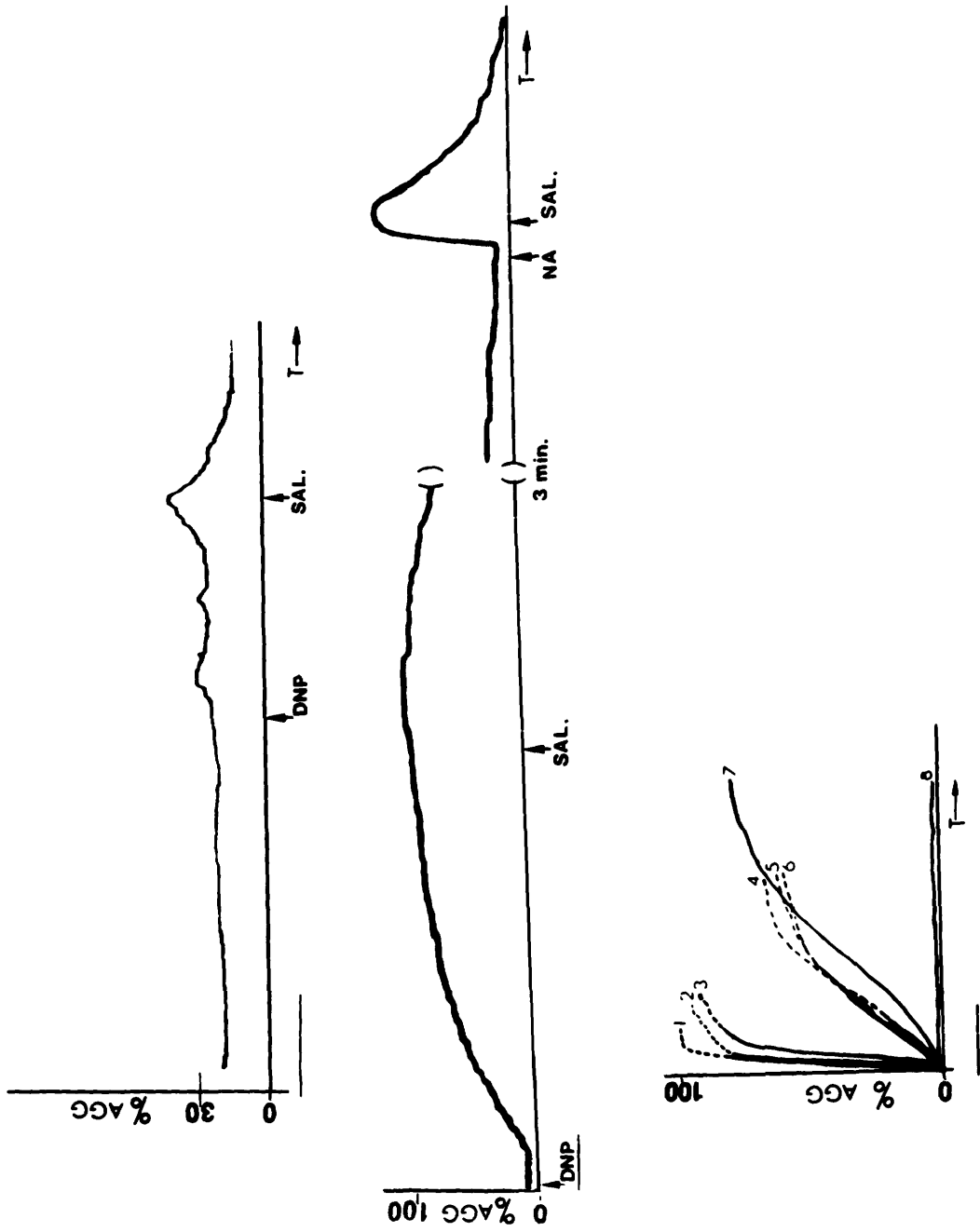
A, Top: Response of partially aggregated melanophores to 10^{-4} M dinitrophenol (DNP) in normal saline. Time scale: 7.5 min.

B, Middle: Comparison between the aggregating effects of DNP (5×10^{-4} M) and noradrenalin (Na) at 10^{-6} M. The onset, rise time and redispersion following saline perfusion are slowed by DNP. Time scale: 30 sec.

C. Bottom: Comparison of the aggregating effects of Na, DNP and tolazoline hydrochloride (TOL). Tracings of records with the initial response placed at t-0.

1. 5×10^{-6} M Na
2. 5×10^{-6} M Na
3. 5×10^{-6} M adrenalin (Ad)
4. 5×10^{-4} M DNP
5. 5×10^{-4} M DNP
6. 5×10^{-4} M DNP
7. 10^{-3} M TOL and 10^{-3} M DNP
8. 10^{-3} M TOL and 10^{-5} M Na

Replacement of experimental perfusate by dispersing saline alone is indicated by the dashed line. Time scale: 30 sec.



melanosomes acted as if they were prevented from entering a process.

The time characteristics of DNP- and NaCN-induced aggregation differed from catecholamine-induced aggregation. Onset time was slower for the oxidative phosphorylation inhibitors than for catecholamines. The rate of the former was approximately 20% that of the latter (see Table 5).

Redispersion following DNP or NaCN treatment was not simply determined by the washout time. The volume of solution contained with the perfusion chamber was approximately 6 ml. Flow rate varied from 8 to 10 ml/min. The rate of redispersion was dependent upon the nature of the prior treatment and the volume of rinse.

Redispersion following DNP-induced aggregation was twice as slow as that for the catecholamine concentration inducing just maximal aggregation. During the course of an experiment the onset of redispersion continually slowed until it exceeded 25 minutes. Experiments were terminated when that time was reached.

To determine if the site of activity of the oxidative phosphorylation inhibitors was the aggregating alpha-receptor membrane site, the action of DNP and NaCN in conjunction with tolazoline hydrochloride (TOL) was tested. TOL is known to block the alpha site on fish melanophores (Fujii and Miyashita, 1975). Consequently it prevents the aggregating effects of noradrenaline and adrenaline. The presence of TOL made little or no difference on the aggregating properties of inhibitors of oxidative phosphorylation. As Fig. 38C shows, TOL blocks the action of noradrenaline (#8) compared with the normal aggregating properties of adrenaline and noradrenaline (#1-3). TOL has no effect on the aggregating properties of DNP (#7) as compared with the aggregation due to DNP in saline

**Table 5: Comparison of Melanophore Aggregation and Dispersion
Response Times following Catecholamine or Oxidative
Phosphorylation Inhibitor Treatment**

	Aggregation	Dispersion
Ratio of Time Intervals Between 20% and 80% Maximal Response: <u>O.P.I.</u> Cat.	$6.1 \leq R_1 \leq 15.5^*$ n=6	$1 \leq R_2 \leq 3.0^*$ n=6
Ratio of Initial Response Delay Intervals: Cat. <u>O.P.I.</u>	0.23 ± 0.08 n=6	0.41 ± 0.2 n=6

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

Cat: Catecholamines

O.P.I.: Oxidative phosphorylation inhibitors

(#4-6).

The membrane alpha-receptor cannot be the site of DNP activity. In addition to blocking the action of catecholamines, TOL has been seen to increase the rate of redispersion (Fig. 39). This result indicates a) catecholamines act at the alpha-receptor site; b) removal of catecholamines does not correspond to eliminating the activity at that site; and c) when the site is blocked, dispersion is accelerated. Since DNP induces aggregation in the presence of TOL, its site of action must lie elsewhere.

I noticed that if the cell was not fully dispersed, low concentrations of epinephrine induced a dispersion immediately prior to its aggregating effects (Fig. 40). This observation is consistent with the action of adrenalin at membranes containing both alpha- and beta- receptor sites.

COMMENT: The results of this section suggest that metabolic energy is required in only one direction, and that is outward. Aggregation appears to be a relaxation process utilizing the potential energy generated during outward migration. That this system is ATP-dependent has been shown directly in an experiment by Miyashita (1975).

She treated melanophore membranes with Triton X-100. Following this treatment, melanosomes were found in an aggregated state. To this preparation she added ATP and Mg^{+2} . ATP alone did not change the adaptive state, but following the addition of both dispersion occurred. Outward migration thus requires energy and the energy is generated via an ATP- Mg^{+2} system.

Perhaps the site of enzymatic activity occurs on the trabeculae visualized by Byers and Porter connecting melanosomes to microtubules.

DISCUSSION: The anatomical and physiological evidence developed in Part Four indicate a system of teleost dermal melanophore control

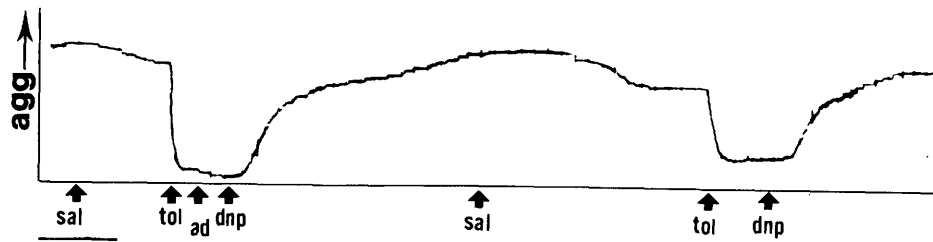
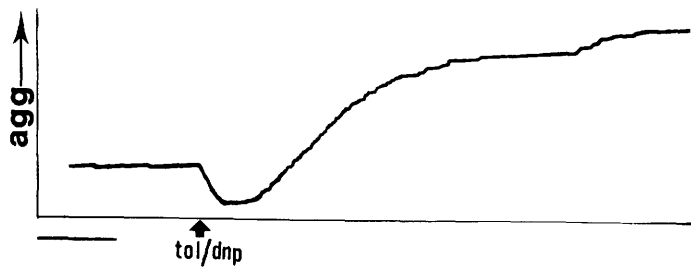
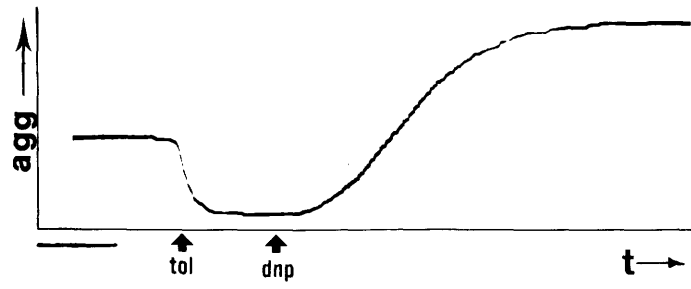
Figure 39: Independent effects of TOL and DNP on melanophore aggregation and dispersion

- A, Top: Addition of 10^{-3} M TOL accelerated redispersion of three melanophores. No hindrance of the aggregating property of DNP was seen when DNP replaced TOL.
- B, Middle: When 10^{-3} M TOL and 10^{-3} M DNP were added simultaneously, the cells responded by first dispersing to the TOL and then aggregating to DNP.
- C, Bottom: After redispersion was initiated by dispersing saline, 10^{-3} M TOL was added. Complete dispersion followed. No aggregation occurred when 5×10^{-6} M adrenalin replaced TOL. Notice the slight additional dispersion following this replacement(see Figure 40). Aggregation followed the replacement of adrena lin by 10^{-3} M DNP.

The second half of this figure repeats the observation illustrated in A,above.

Time scale for Figures 39A, 39B: 1 minute.

Time scale for Figure 39C: 6 minutes.



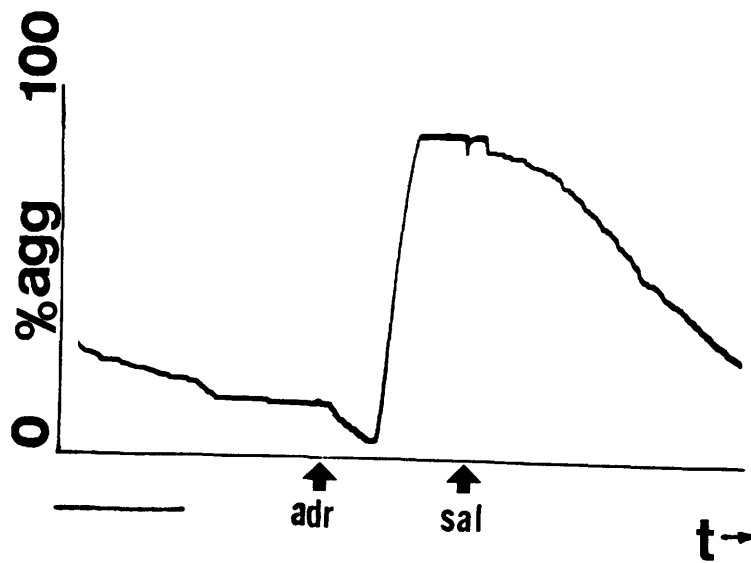


Figure 40: An example of adrenalin reversal at a concentration of 2×10^{-6} M.

This behavior was seen only when the cell was incompletely dispersed.

Scale: 30 seconds

requiring a single catecholaminergic neural innervation for melanosome aggregation. No evidence contradicts this notion. In essence, this theory returns to the original proposal of Brucke with the modification only of the source of the nerve transmitter. He thought the system was part of the skeletal muscle system. Subsequent investigations (Pouchet, 1876; von Frisch, 1911; Parker, 1948; Fujii, 1961; Scott, 1965) have demonstrated that the innervation relating to aggregation was autonomic.

Only one of the evidences in support of dispersing nerve innervation, in my opinion, is a serious challenge to the single nerve hypothesis. That is Kinoshita and Ueda's demonstration of dispersion by nerve stimulation (1970). Parker, Mills and Abramowitz may have seen dispersion following their experimental manipulations, but Junquiera et al (1976) unambiguously has shown that innervation to the fish used by the three (Fundulus heteroclitus) is only adrenergic. Any dispersive effect seen by them could not have been of neural origin.

As has been mentioned, the electron microscope evidence of a non-adrenergic innervation (Fujii, 1966b; Fujii and Taguchi, 1970) cannot be sustained; nor can Parker and Rosenblueth's attempt to stimulate dispersing fibers (1941).

Kinoshita and Ueda's records, however, indicating that they had stimulated what they interpreted were dispersing fibers is not open to methodological problems as were the others. Immediately following onset of nerve stimulation, they showed a degree of dispersion that was followed within seconds by aggregation. They suggested that the dispersing nerves were stimulated first because they have a lower threshold of activity and then the aggregating fibers overwhelmed the effect of the dispersing fibers. The initial dispersion was seen only when stimulating voltage was low.

Above a certain level, it was not seen.

Low concentrations of epinephrine have been shown to act in an antagonistic manner to higher concentrations in smooth muscles of blood vessels (Inness and Nickerson, 1975) and in uterine muscle (Dale, 1906; Hermansen, 1961). Both alpha- and beta-receptor sites are found on these muscles. Epinephrine stimulates both sites, but the threshold for the beta-site is lower than the alpha-site threshold. Beta-receptors will respond to the presence of epinephrine prior to alpha-receptors.

Both alpha- and beta-receptor sites have been reported for fish melanophores (Reed and Finnin, 1972; Fujii and Miyashita, 1975.; Miyashita and Fujii, 1975). As the concentration of epinephrine increases, first the beta-site and then the alpha-site respond. This sequence would be visualized as first a dispersion immediately followed by aggregation.

Melanophores in vitro aggregate in a graded fashion to increasing catecholamine concentrations. Likewise, they aggregate in a graded manner to nerve stimulation as the stimulating frequency increases to 10-20 Htz. (Fujii and Novales, 1969). This parallelism probably represents the accumulation of transmitter locally around a melanophore regardless of the mode of delivery.

Multiple nerve innervation suggests multiple stimulating thresholds. Kinoshita and Ueda's report of dispersing nerves at low threshold is also consistent with a multiple nerve-melanophore innervation, melanophore alpha- and beta-receptor sites, and the known melanophore activity to increasing epinephrine concentrations. Considered in the context of known adrenoreceptor action, their demonstration proves as ambiguous as any other demonstration of dispersing nerve innervation to teleost melanophores. It provides supporting evidence that the nerve innervation is likely based

on the catecholamine epinephrine.

Thus, the overwhelming evidence for a single nerve innervation to dermal melanophores should be sufficient to eliminate the dual nerve hypothesis as a working proposition. As proposed by Bert (1875) and Parker (1948), the dual nerve innervation notion seems incorrect. The demonstration by Fujii and Miyashita (1976) that at least one fish melanophore is mediated not by catecholamines, but by acetylcholine, means that the character of the membrane-site modulating disposition of melanosomes may vary, but the functional relationship between melanophore adaptations and external modulation is universal among teleosts.

AFTERWORD: A NOTE ON THE THEORETICAL BASIS OF
FLATFISH CAMOUFLAGE

An observer views the natural world via the medium of light. He observes an object by the light reflected from that object. For a human eye, the visual world measures about 160° horizontally by about 150° vertically (Le Grand, 1968). Most objects occupy a visual angle less than that, so light from many objects impinges on the retina at any instant of time. However, humans are aware of only one object at a time. That object is usually the foveally-seen one. All other objects become part of a generalized background until each is seen foveally.

If we consider that the intensity of light reflected from an object has its own spatial distribution irrespective of where it lands on the retina, we must conclude that the fovea has an image processing capability somewhat different from the peripheral capabilities. If we call the spatial distribution of light intensities texture, then the fovea abstracts some relationship about objects that the periphery does not. Neurophysiological (Stone and Fukada, 1974) and behavioral evidence (below) suggest that some processing properties of the retina vary with retinal position.

Distinctions between objects depends upon perceived texture differences. In an interesting attempt to discover the parameters of light that induce texture differences, Julesz (1962) and Julesz et al (1973) investigated a statistical distribution of light reflectances. Light and dark areas were classified in terms of a joint probability distribution of orders 1, 2, and 3. Subjects were able to distinguish first and second order differences, but not third order differences. These relationships, however, could not be a complete description of the variables because he illustrated a counter-example (1973, fig. 9b).

The observers' use of foveal vision was implicit in his experiments. The receptive field of the retina between the fovea and perifovea extends to about 10° of the available visual world. The rest of the visual world is seen by peripheral retina. When the same experiments are performed using a peripheral view, the second order variations become increasingly difficult to distinguish. Differences in reflectance, a first order distribution, remain discriminable.

In the periphery, the organization of textures plays a major role in their discrimination. A square and a circle against a uniform background will be seen as different in a peripheral view even when their reflectances are similar. As more squares or circles are placed in the periphery, the surrounded forms become impossible to see. Lettvin (1976) pointed out that as the number of figures increases in the periphery, so does the difficulty in seeing a particular one. If we consider that the visual angle subtended by one object is much less than the subtended angle of the visual world, we can conclude that one object in the periphery is surrounded by many others. In the periphery, an object will not be noticed unless it substantially differs in texture from the others.

Discrimination on the basis of textural differences appears in humans to be an innate retinal function. Von Senden (1960) compiled reports of visual behavior by persons who, for medical reasons, started seeing much later than at birth. After correction of the optical deficiencies, subjects could immediately discriminate texture differences. Hebb (1949) separated the process of figural identification into two steps. The first was separation of a figure from the background (texture differences); the second was identification of the figure (texture characteristics). By deduction, identification of the characteristics of an individual texture,

its contour for example, must be a property of the fovea. Walls (1963) pointed out that fish existing at or near the bottom of the continental shelf, especially the littoral zone, have a fovea or a fovea-like region. This observation suggests a specialized retinal region is needed to discriminate single objects in an isotropically structured visual world.

Finally, one further retinal property must be mentioned. Whereas texture is a visual property relating to a static activity of the entire retina, motion detection is a property relating a change in the activity of one texture 'field' to the activity in the rest of the retina. This property is not retinally segregated, but until foveal attention is paid to the moving object, the object is not directly identified except as something moving. For purpose of identification, saccadic movements may be considered a mechanism for positioning the fovea onto a peripherally signalled movement.

Now let us consider the problem of camouflage as it relates to flatfish. I have shown in this thesis that flatfish modulate two general features of its skin texture: general body reflectance and enhancement or diminution of the contrast between a set of morphologically placed spots and the body reflectance. The features varying in every flatfish examined (Sumner, 1911; Mast, 1916; Hower, 1927, 1931; Osborn, 1939; Chinarina, 1960; and de Groot, 1969) are the same ones. These characteristics belong to Heterosomata regardless of the evolutionary diverse paths the fish may have followed. Norman (1934, pp. 2-4; 35-37) suspected that flatfish may have evolved two or more times, yet the characteristics of these fish are similar. One might suspect that flatfish appearance embodies some general design features that enhance cryptic camouflage.

If one were to measure the distribution of spots in many flatfish

species, one would find a large variation in their descriptions. Consider that variation in relation to the lack of an inherent measure in peripheral retina. All that one could distinguish on the basis of the spot distributions is the texture difference based on its presence or absence. The patterns need not be identical to some surrounding spot pattern because an observer has no capacity to discriminate a measured difference in peripheral vision. A corollary to this conclusion is inescapable: All spot patterns of flatfish in peripheral vision are equivalent when the reflectance of the fish is similar to that of the surround.

More than once, I have been surprised at the density of P. americanus underfoot while SCUBA diving. I had not noticed their presence because I could not see them as long as they were situated within my peripheral vision. Once I became aware of them, I could seek them out, but in doing so, I used my foveal vision.

Peripheral retina can distinguish differences in reflectance. The flatfish, as I have shown, changes its reflectance according to the background. Thus, the two features, skin reflectance and texture contrast, changed by the flatfish are the two features peripheral discrimination operates upon. Based on the lack of perceived differences between a flatfish and its background, it is easy to imagine that a description of the skin might be couched in terms appropriate to the natural surround.

But in foveal vision differences can be discriminated that cannot be in peripheral vision. Consider the structure of a flatfish. The dorsal and anal fins extend the length of the body. These fins in the families Bothidae and Pleuronectidae cease at the caudal peduncle while in the family Psettodidae, the fins merge with the tail fin. Fin skin contains dark and light pigment markings. Between the markings the interray

skin is transparent (Sumner, 1911; personal observation). The underlying substrate can be seen through the skin. Texture of the fins combines both the bottom textures and fish skin textures. The fins act as a texture gradient. The textures of the fish skin and the surround cannot be visually compared because a discrete intersection of the two textures does not occur. Shadows which provide a fine edge demarcation are not formed either. Thus, the structure of the fins adds to two features necessary for peripheral camouflage to aid camouflage in a foveal or near foveal view.

In the case of flatfish, the fins act to limit discrimination between two textures. In the case of the Nandidae, the chromatic details of the fins are used in forcing a specific texture upon the body shape. Simply, those fins that detract from the imagery of a leaf in water are transparent. These fish appear to an observer as a leaf in water. If the anal and second dorsal fins were visible, this appearance would be disrupted. The other unpaired fins are clearly pigmented although the caudal fin of Schomburgh's Leaf Fish (Polycentrus schombungke) and the African Leaf Fish (Polycentropsis abbreviata) of the family Polycentridae are also transparent while that of the South American Leaf Fish (Monocirrhus polycanthus) is not. (See Frank, 1971, figs. 606, 607.)

Flatfish swim in bursts. This motion is especially true of individuals smaller than 14 or 15 cms. Rather than swim, they dart. This behavior hinders tracking because it minimizes moving time and maximizes still time. P. americanus displays an escape behavior based on this activity (p. o.). When chased, an individual swims a distance and then darts to the left or right. The length of the dart is only a fraction of the length of the initial path. After the dart, the fish remains immobile. The length of the dart matters little once the fish is stationary and situated outside the

observer's fovea. In the peripheral retina, its other camouflage properties become operative. (In my own experience, I could only find an individual after it performed this maneuver by stirring up the bottom near where I thought it had settled. If it moved, I would regain view of it.)

Cott (1957) pointed out that birds and lizards have a highly developed still reaction to prevent movement cues while certain fish elaborately camouflaged their movements during predation. He mentioned the drifting of the South American Leaf Fish and the Long-Nosed Armpike.

I may add to the list of camouflaged movements, those of the Southern Flounder as it tracks its prey in the laboratory (Olla et al, 1972; p. o.). Its movements are snakelike. The fins do not rise above the substrate. The body snakes along the bottom and, if not ready to strike, the fish appears to stop if its prey turns in its direction. Successful attacks occur when the prey approaches within some striking distance (as defined by the flatfish) of a motionless flatfish.

It is evident that for camouflage or predation, flatfish exhibit behavioral routines that rely on their physical appearance.

Evolution of appearance is no accident. It is the result of a long process of interactions between the environment of an animal and that animal. This thesis has shown that one visual process, texture perception, must be mappable among the different branches of vertebrates. Some evidence exists that texture perception is also a feature of cephalopod vision (Sutherland, 1963). One must conclude that texture perception is a necessary requirement of biological vision.

FOOTNOTES

1. For example, see comments regarding flatfish in the following books and monographs:

Parker, G. H., Animal Colour Changes and their Neurohumors, 1948, Cambridge University Press, p. 169ff.

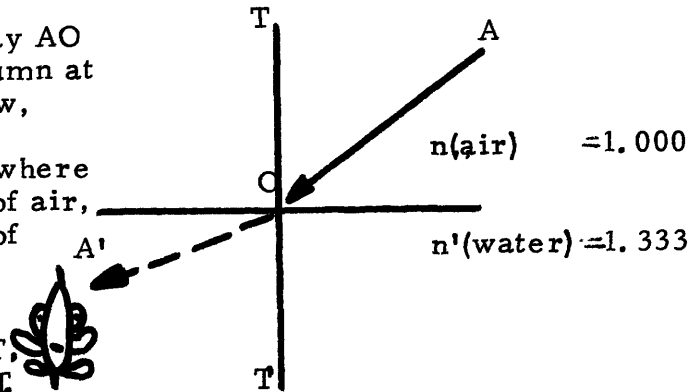
Waring, H., Color Change Mechanisms of Cold-Blooded Vertebrates, 1963, Academic Press, p. 266ff.

Norman, J. R., A History of Fishes, 3rd edition, 1975, Wiley, p. 207ff.

Lagler, K. et al., Ichthyology, 2nd edition, 1977, Wiley, p. 122ff.

2. Consider an incident ray AO entering the water column at point O. By Snell's Law,

$n \sin AOT = n' \sin A'OT'$, where
 n = index of refraction of air,
 n' = index of refraction of water.



$$\sin A'OT' = \frac{n}{n'} \sin AOT, \\ = 0.75 \sin AOT$$

$$\text{But } 0.00 \leq \sin A'OT' \leq 1.00.$$

Case 1: $\sin A'OT' = 0.00$ $\sin AOT = 0.00$, and angle $AOT = 0.00$.

Case 2: $\sin A'OT' = 1.00$. Then $\sin AOT$ is greater than 1.00 which cannot be.

Let $\sin AOT = 1.00$. Then $\sin A'OT' = 0.75$, and angle $A'OT' = 48.6^\circ$.
 Therefore, 0.00 angle $A'OT' = 48.6^\circ$ and the vertical angle of the cone of refracted light is a maximum 97.2° .

3. Fish	Exit Vertebra	Reference
<u>Carassius carassius</u>	V-12	Iwata and Fukada, 1973.
<u>Phoxinus phoxinus</u>	V-13 V-15	Burton, 1964, 1969. Pye, 1964.
<u>Pleuronectes limanda</u>	V-6	Hewer, 1927.
<u>Pleuronectes platessa</u>	V-5-6	Fernando and Grove, 1974a.

4. Autonomic Organ	Animal	Reference
vas deferens	mouse, guinea pig	Merrillees, 1968. Thaemert, 1963.

- dilator muscle rabbit, rat iris
- nictitating membrane cats, rabbits
- urinary bladder and glandular tissues.
- Merillees et al., 1963
Lane and Rhodin, 1964.
- Richardson, 1964
Hokfelt, 1966.
- Evans and Evans, 1964.
- Burnstock, 1970, pp. 52-55.

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BIOGRAPHICAL NOTE

William Saidel was born in Chelsea, Massachusetts in 1947. He attended the Massachusetts Institute of Technology as an undergraduate, receiving a Bachelor of Science degree in 1969. In 1970 he worked in the laboratory of Dr. S. A. Raymond. After working as a photographer, he was employed for a period assisting Fritz Goro, science photographer for Time-Life, Inc. He returned to the study of science under the tutelage of Professor J. Y. Lettvin at M.I.T. in September, 1971. In 1973, he participated in the teaching of a neurophysiology laboratory course. During the winter of 1973-74, he began his study of flatfish camouflage at the aquarium of the National Oceanic and Atmospheric Administration, Wood's Hole, Mass. Following his return to the M.I.T. campus, he participated in the teaching of an I.A.P. course in neuroanatomy(1975) and initiated and taught an undergraduate seminar in animal sensory systems (1976). He was appointed research scientist at the Cohasset Marine Biological Station, June, 1977.

He married Joan Carole Feldman in June, 1974. Their first child, Jeremy Ben Feldman Saidel, was born in August, 1977.

He is an active SCUBA diver and underwater photographer.

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