EVIDENCE PROVING THE MELANOPHORE TO BE A DISGUISED TYPE OF SMOOTH MUSCLE CELL

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TWO FIGURES

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PRELIMINARY

The melanophores of the lower vertebrates, fish, amphibians and reptiles, are described by most authors as modified connective tissue cells (Fuchs '14). The evidence for such a conception rests chiefly upon a morphological basis. Leydig ('89, '92), showed that no sharp morphological distinction exists between unpigmented, connective tissue cells, inactive, pigmented connective tissue cells and chromatophores (melanophores) containing actively migrating pigment granules.

During the past four years I have been carrying on a series of experiments on the direct physiological responses of the melanophores in fish, chiefly in *Fundulus*. These experiments

have presented an opportunity for occasional morphological and embryological observations also. In attempting to categorize any type of cell it is necessary to consider not only its morphology, but its development and physiology as well. An examination of many data of previous investigators, supplemented by my own observations has led me to the conclusion that the melanophores of lower vertebrates do not belong in the category of connective tissue, but are to be considered functionally modified smooth muscle cells. The object of this paper is to summarize the evidence for my position.

MORPHOLOGICAL EVIDENCE

For our present discussion the following occurrence of involuntary or smooth muscle in vertebrates is of importance, viz., the digestive tract, the vascular system, the eye and the integument. The units of which smooth muscle tissue is composed consist of elongated or spindle-shaped cells which may be branched or forked, containing a single or several oval or elongate nuclei. In certain cases (sphincter pupillae) the cytoplasm is filled with a dense mass of melanin pigment granules. We may therefore say with Franz ('06), (p. 452): "Es ist daher wohl eine zulässige Ausdrucksweise vom Sphincter als von einer Anhäufung spindleförmiger Pigmentzellen zu sprechen."

In the lower vertebrates the melanophores occur chiefly in the derma, more rarely in the epidermis (Pouchet '76, in Rhombus; Müller '60, Eberth '67, Pouchet loc. cit., Biedermann '92 and many others in the frog; Schmidt '12, in *Phelsuma*), on the mesenteries and peritoneum and associated with the blood-vessels. Morphologically we find an entire series extending from relatively simple cells with two or three processes to the familiar, extensively branched forms found in the derma. Zimmerman ('93), for example, noted elongated melanophores between the fin rays in several adult teleosts and I have observed similar simple cells in the tail region of adult *Fundulus* and other teleost species. Franz ('08) has recorded the same condition in the edges of the fins in the larva of *Pleuronectes platessa*. On the

surface of the iris in the eel Steinach ('92) has observed a transition from the stellate melanophores to the spindle shaped cells of the sphincter pupillae. He says (p. 515): "Die verästigten sternförmige Pigmentzellen . . . gehen dicht am Pupillar- rand in einen Ring von besonders dunkeln Pigmentzellen über deren plumpe Körper sich mehr der spindelform nähern."

We may conclude then, that, as regards external and nuclear form and a melanin pigment content, no sharp morphological distinction can be made between melanophores and the smooth muscle cells of the sphincter pupillae.

EMBRYOLOGICAL EVIDENCE

Smooth muscle may be formed either from the mesoderm (mesenchyme), the commonest source, or from the ectoderm, (Nussbaum, M. '01).

Investigations upon the development of the dermal melanophores show a satisfactory agreement. With a single exception¹ they have been found to originate from mesodermal cells (Wenckebach '86, Bolk '10: Ehrmann '96, Eycleshymer '06, Weidenreich '12: Kerbert '77, Todaro '78, Zenneck '94). The chief controversial point in the case of the epidermal melanophores has been as to whether these cells develop from the epidermis in situ, or whether, as unpigmented mesenchyme cells, they wander into the region of the epidermis and become pigmented secondarily. In the first case their origin would be ectodermal and in the second, mesodermal. My own observations are limited to several species of teleosts. In the case of *Fundulus*, epidermal melanophores do not occur, but the origin of the dermal melanophores from wandering mesenchyme cells has been clearly demonstrated by Stockard ('15). Dr. Stockard has very kindly shown me a number of his own preparations and unpublished drawings of the developing melanophores in *Fundulus* embryos. His studies, made in connection with the developing circulatory system, have shown that in this species the yolk melanophores first appear as wandering, actively amoeboid, unpigmented mesen-

¹ Borcea, '09.

chyme cells. From the region of Kupffer's vesicle there develops a fan-shaped area of migrating cells which rapidly spread out on the surface of the yolk. These cells eventually give rise to connective tissue, the yolk melanophores, the endothelial cells and possibly also to the smooth muscle cells in the walls of the larger blood vessels. For some time after the pigment has begun to develop within the wandering cells that are destined to become melanophores, they continue to show active amoeboid movements. In a transparent pelagic egg like that of the scup (*Stenotomus crysops*), these wandering cells at the posterior end of the embryo may be observed with startling clearness.

It is impossible to draw any sharp morphological or embryological distinction between certain pigmented connective tissue cells and chromatophores (Leydig loc. cit.) just as there are morphological transitions between connective tissue and smooth muscle cells (Flemming '97). We can say, then, that typical connective tissue cells, melanophores and smooth muscle cells are all known to develop from mesenchyme and furthermore, that smooth muscle cells (sphincter pupillae) and possibly also melanophores (Borcea loc. cit.) may develop from the ectoderm. The embryological evidence is thus at least not negative.²

PHYSIOLOGICAL EVIDENCE

By far the most striking evidence of the parallelism between smooth muscle and melanophores is furnished by physiological experiments. Franz ('06) first recorded the striking similarity between the physiological responses of the sphincter pupillae in *Acanthias* and the dermal chromatophores of the frog. He noted 1) that both respond to light by contraction, 2) that indifferent gases like N and H elicit no contractions of the chromatophores and that these gases prevent the contraction of the sphincter pupillae upon illumination; 3) that after death, the pigment cells and sphincter are both contracted. He then says (p. 453): "Wir können also in mancher Hinsicht Analogien zwischen den

² It is certainly not without interest in this connection that the chromatophores of cephalopods have been found to develop from single smooth muscle cells by a complicated metamorphosis (Chun '02).

Sphincterfasern und mesodermalen Chromatophoren aufstellen;” and again: “Denn es scheint in mehr als einer Beziehung, dass die Chromatophoren nichts anderes als verkappte Muskelzellen sind.”

Many of the older investigations upon the responses of the melanophores to various stimuli were carried out upon living animals. Here the situation was exceedingly complex since so many physiological factors had to be controlled. In a previous paper ('13 b) I have called attention to this objection and have described a method whereby the melanophores of teleosts, especially *Fundulus heteroclitus*, may be removed from the fish and stimulated in a variety of ways without the slightest mechanical injury in manipulation. The technique is exceedingly simple, consisting of the careful removal of the scales with their superficial sheets of dermal melanophores. These scales are readily transferred from one solution to another and, by selecting adjacent scales from the same fish, we obtain very satisfactory physiological units whereby it is possible to test the effects of a series of solutions—the degree of expansion or contraction of the pigment serving as an indicator of relative stimulation. In preparations of this sort in which the circulation and nervous control have obviously been eliminated, I have found that chemical stimuli such as 0.1 N KCl, heat (30° C.), ultra-violet light and induction currents all bring about a more or less rapid contraction of the pigment granules. Furthermore, in indifferent media like Ringer's solution or olive oil, the melanophores remain expanded for long periods. It therefore seems justifiable to consider the contracted phase that of stimulation, since the melanophores respond by contracting to the above familiar series of physiological stimuli. Throughout the following discussion I shall consider the contracted phase of the melanophores as corresponding, physiologically, to the contraction in smooth muscle. The objections to the application of the term 'contraction' in the case of the melanophores, I shall take up at the close of this section.

I. Innervation

The activity of vertebrate smooth muscle is normally controlled through fibers of the sympathetic nervous system. Voluntary motor connections do not ordinarily occur.

The innervation of the melanophores has been satisfactorily demonstrated both histologically (Ballowitz '93) and physiologically (Pouchet '72 and '76, Von Frisch '11, Spaeth loc. cit.) in several species of teleosts. v. Frisch has recently corroborated and amplified the original observations of Pouchet, who first claimed the innervation of the melanophores to be sympathetic. I have repeated the striking experiments of v. Frisch upon *Phoxinus* with *Fundulus*. In this experiment one of the two branches of the sympathetic system is severed immediately behind the body cavity in the region of the haemal arch. Fish so treated lose their power of color adaptation posterior to the point of incision on the operated side, but continue to show normal motor responses. Reciprocally, severing of the spinal cord eliminates motor responses but, provided the operation has been carefully performed, the sympathetic adaptations to different colored bottoms remains normal.

In *Fundulus* I have also found at the base of the medulla a 'contraction center' corresponding with that found by Lode ('90) in the trout and by v. Frisch ('11) in *Phoxinus*. A lightening of the entire body of the fish follows the electrical stimulation of this center.

No satisfactory histological demonstrations of the nerve endings in the melanophores of amphibians and reptiles have been recorded. Bimmerman ('78), Biedermann, loc. cit. and more recently Hooker ('12) have however, all demonstrated, physiologically, the sympathetic innervation in the melanophores of the frog and Carlton ('04) has made similar observations in *Anolis*. There is thus a very satisfactory unanimity of opinion on this question.

II. *Effect of Light*

Through the investigations of Arnold ('41), Brown-Sequard ('47), Steinach ('92), Magnus ('99), and Franz (loc. cit.) we know that the excised iris of certain elasmobranchs (*Acanthias*), teleosts (*Anguilla*) and amphibians (*Rana*) responds to illumination by a contraction of the sphincter pupillae.

Hertel ('07), first succeeded in demonstrating a direct response to stimulation by light in the chromatophores of the cephalopods *Sepiola*, *Octopus* and especially *Loligo*. In this case the reactions of the chromatophores are due to contractions and relaxations of the radially arranged smooth muscles. The active phase is here the expanded one, elicited by the contracting radial muscles i.e., it is the reciprocal of the condition in the melanophores of lower vertebrates. Hertel found that ultra-violet light of 280 $\mu\mu$ produced an almost instantaneous local expansion of all chromatophores. Blue rays of 440 $\mu\mu$ and yellow rays of 558 $\mu\mu$ of equal intensity also gave a distinct, but somewhat slower expansion.

I have shown (loc. cit.) that the expanded melanophores of *Fundulus* respond to ultra-violet light (280 $\mu\mu$) by a rapid and reversible contraction. I was unable however, in a series of trials with different regions and intensities of the visible spectrum to obtain contractions of *Fundulus* melanophores. Following practically the same technique as in my experiments with *Fundulus*, Laurens ('15) has recently reported contractions by ultra-violet light in expanded melanophores in pieces of the skin of *Amblystoma* larvae. Hertel (loc. cit.) had previously found that the melanophores of *Triton* larvae responded to ultra-violet, blue and yellow rays by contracting. He used the same wave lengths and intensities as in his experiments with cephalopods (vide supra). In this case, as in so many of the older experiments, the presence of a complete nerve mechanism and blood supply presents the possibility of secondary complications. In the case of *Fundulus* and *Amblystoma*, however, I believe the results to be free from this objection.

A direct response to light has not thus far been demonstrated in the melanophores of reptiles, though as Fuchs (loc. cit.) has emphasized, it will in all probability be found to exist.

We may now summarize the foregoing observations as follows; 1) in certain species of fish and amphibians the sphincter pupillae contracts in response to direct stimulation by light; 2) in certain other species of fish and amphibians the melanophores also respond to direct stimulation by light by contracting.

III. Effect of Electrical Stimulation

Induction currents of sufficient intensity and duration produce contractions in smooth muscle. Such contractions may be seen in strips of frog's stomach prepared according to Meigs ('12), or in preparations of the digestive tube of several species of teleosts.³ Beer ('94, '98) observed that the sphincter pupillae of the eel and the frog (*Rana*) contracted when stimulated electrically. The radial muscles of the chromatophores of cephalopods also respond to electrical stimulation by contracting (Brücke '52, Keller '73, Fredericq '78, Klemensiewicz '78, Pouchet '76, Krukenberg '80, Phisalix '92, Steinach '01, Hofman '07, '10, and Fuchs '10). Klemensiewicz (loc. cit.) records similar contractions in isolated pieces of the skin of *Loligo*.

Lode (loc. cit.) first showed that in excised pieces of the skin of the trout the melanophores contracted upon being stimulated by an induction current. In my own experiments with *Fundulus* the melanophores invariably contracted, reversibly, when proper strength and duration of the current and salt concentration of the mounting medium were selected.

Winkler ('10) found that the melanophores of *Rana esculenta* and *Hyla arborea* contracted when directly stimulated by an induction current. Laurens (loc. cit.) has recently verified this observation in large larvae of *Amblystoma opacum*. He finds also that: "When various portions of the body are cut out and directly stimulated either with the central nervous system in-

³ Unpublished observations made upon the stomach muscle of *Stenotomus*, *Tautoga*, *Centropistes* and *Fundulus*.

tact or destroyed, a slight contraction of the melanophores is usually induced" (p. 610).

In the case of the reptilian melanophores Bert ('75) and Kruk-enberg ('80) were able to corroborate the original observation of Brücke ('52) who found that direct faradic stimulation of excised bits of dark skin in the chameleon produced a lightening, i.e., a contraction of the melanophores.

Thus the responses of several types of smooth muscle, as well as of the melanophores, in representatives of all three groups of lower vertebrates show a satisfactory agreement in their contraction to faradic stimulation.

IV. Effect of Mechanical Stimulation

By gently pinching or stretching excised pieces of frog or fish³ stomach and oesophagus, powerful contractions may be induced, which are reversible provided the stimulus has not been too violent. Precisely the same reaction follows a similar treatment of portions of the skin of *Loligo*; the chromatophores expand widely. I have verified this observation by Klemensiewicz loc. cit. and all of the more recent investigations upon cephalopod chromatophores record a similar phenomenon in *Loligo* and other species.

Fuchs (loc. cit) has called attention to the objection against the expansion observed by many of the older investigators after the surfaces of various teleosts had been more or less violently 'stroked' with a needle. He says (p. 1432): "Aus allen Beobachtungen geht unstreitig hervor, dass einwandfreie Beobachtungen über die direkte mechanische Reizbarkeit der Fischchromatophoren nicht vorliegen." I have shown (loc. cit.) that by selecting scales from the lateral portion of *Fundulus* where the melanophores are relatively far apart, it is possible to stimulate a single melanophore repeatedly by exerting a gentle pressure with a fine, fire-polished glass needle. Great care must be exercised not to rupture the delicate cells, for by so doing, the melanin granules are scattered and produce the effect of an 'expansion' recorded by the older observers. In this case it is certain that

mechanical stimulation (gentle pressure) produces a reversible contraction of the melanophores.

As a result of local pressure or pinching with forceps, and along the margins of an incision, the melanophores in the frog contract (v. Wittich '54, '71, Hering l. c., Lister '58 a, '58 b, Fuchs '06, Étérnod and Robert '08). The darkening of the skin in *Polypodates Reinwardtii*, "nach leichtem Kratzen mit einer Nadel," observed by Siedlecki ('09), is doubtless another case of ruptured melanophores.⁴

The above observations warrant the conclusion that in the radial smooth muscles of the chromatophores of cephalopods, in the smooth muscle of the digestive tract in certain teleosts and amphibia, as well as in the melanophores of these two vertebrate groups, mechanical stimulation (gentle pressure) is followed by a reversible contraction.

V. Effects of Chemical Stimulation

A great many experiments have been carried out upon the effects of various widely differing chemical stimuli upon the melanophores of vertebrates. The method of procedure has been, in most cases, as follows; 1) the substances were brought directly upon the skin of the normal or operated (pithed, etc.) animal, the color-change being considered the criterion of the action of the chemicals; 2) the substances were injected into the circulation, the body cavity, or subcutaneously, the color-change again serving as an indicator of chemical stimulation; 3) the substances were added to the water of the environment, in aquatic forms. In relatively few instances have excised pieces of skin been immersed in the fluid to be tested. As I have repeatedly emphasized, this is the only satisfactory method of determining the direct effect of any stimulus upon the melanophores, for it is only in this way that the circulation and all central nervous

⁴ Observations upon the response to pressure in reptilian melanophores are contradictory and unsatisfactory. Most of the experiments have been made with living animals and the few trials with excised bits of skin were all carried out without regard for the possibility of a darkening resulting from the destruction of the melanophores (Milne-Edwards '34 a, '34 b, Brücke '52, Carlton '04).

control may be simultaneously and effectively eliminated. The objection may be raised that even in excised pieces of the animal it is impossible to destroy the ultimate nerve terminations, hence we are unable to state with certainty that the effect is a direct one and not transmitted through the cut stumps of the sympathetic nerves which remain in such a preparation. I have elsewhere (loc. cit.) adduced evidence to show that this objection is certainly invalid in the case of the isolated melanophores of the scales of *Fundulus*. I shall limit the following discussion of the reactions of melanophores to chemical stimuli to cases which are as nearly as possible comparable to the chemical stimulation of isolated smooth muscle.

A. *Inorganic Substances*. Schultz ('97) observed that distilled water acted as a weak contracting stimulus in opened ring preparations of the stomach of the frog. Meigs ('10) has shown that distilled water gives a typical curve of contraction in preparations of longitudinal strips of the frog's stomach.

I have found (loc. cit.) that the melanophores of *Fundulus* slowly contract when brought from the living dark fish or from 0.1 N NaCl to distilled water (fig. 1).

Schultz (l. c.) noted a relaxation and swelling of the smooth muscle of the frog's stomach in 10 per cent NaCl solution. Meigs (loc. cit.) has shown that in solutions of KCl, the stomach muscle of the frog (*R. pipiens*) slowly contracts and loses weight while in NaCl it elongates and absorbs water.⁵ I have observed that the chromatophores in pieces of the mantle of *Loligo* expand immediately upon being immersed in 0.1 N KCl solution. This is obviously due to a contraction of the radial smooth muscles. Furthermore, provided the exposure to KCl has not been too long, when such chromatophores are returned to 0.1 N NaCl or better, Ringer solution, they contract again, the radial muscles are relaxed.

The melanophores of all the species of teleosts with which I have experimented, showed a contraction in 0.1 N KCl solution and an expansion or relaxation in 0.1 N NaCl, dilute sea-water,

⁵ Zoethout ('02) found that KCl produced a contraction in the gastrocnemius of the frog and NaCl a relaxation.

or Ringer's solution.⁶ The effects of other neutral salts of the alkalies were also studied in considerable detail but, since, so far as I am aware, no parallel experiments have been performed upon smooth muscle, a discussion of these results would be irrelevant. It is, however, of interest that both cations and anions of the neutral salts show the same order of physiological effect, the so-called 'lyotropic order,' as in experiments upon striated muscle (Overton '04 and Sewartz '07) and certain colloids (Hofmeister '91, Pauli '99, Höber '14, Chapter 7).

Fig. 1 The slow contraction of a melanophore in distilled water. The upper line represents the movements of the terminal pigment granules in one process of the cell. The lower line indicates time in minutes. At D. W. the cell was immersed in distilled water (from 0.1 N NaCl). The melanophore was completely contracted in twenty minutes. In this case the migration of the pigment granules was followed by means of an ocular micrometer carrying a moveable scale. An empirically selected line of the micrometer scale was kept tangent to the terminal pigment granules by turning the adjusting screw of the ocular. The motion of the screw was transmitted to a set of pulleys and a heart lever and the curve was recorded on a kymograph in the usual way. The actual path of the pigment migration was 0.104 mm. which makes the magnification of the reproduced figure approximately $\times 534$. A detailed description of this apparatus will appear shortly in the American Journal of Physiology.

Recently⁶ I have found that the alkaline earths produce a contraction of the melanophores in *Fundulus*. The time for this contraction in isotonic solutions of the neutral chlorides varies in the order—

In strontium, calcium and magnesium chloride, the contractions are all reversible, the time required for complete recovery varying in the reciprocal order—

The behavior after treatment with 0.1 N BaCl_2 for ten minutes is peculiar in that, frequently, upon being returned to 0.1 N NaCl , no sign of recovery appears for as long as thirty minutes. After such a longer or shorter period of quiescence, the peripheral melanophores suddenly expand slightly and contract again almost immediately. After a brief interval of inactivity, a second expansion wave appears at the periphery, but this time the melanophores lying nearer the centre of the scale, which had previously remained contracted, show a slight expansion and those at the periphery expand further than the first time. In this way there is set up a rhythmic expansion and contraction wave which, creeping towards the centre of the scale, gradually comes to include all the melanophores. In the course of an hour after being returned to sodium chloride, all the melanophores of a scale are slowly pulsating. This pulsation may continue at ordinary temperatures for as long as four or five hours, the expansion gradually becoming less complete and frequent and eventually ceasing, the melanophores remaining contracted. In cases where the immersion in BaCl_2 has lasted only about five minutes, the pulsations appear upon being returned to NaCl , but

⁶ In scale preparations of *Fundulus*, the small dermal blood vessels always contain a few corpuscles. When a scale is immersed in a KCl solution these corpuscles begin to move within the vessels and this movement continued approximately as long as the melanophores are contracting. On being returned to NaCl , the melanophores expand and the corpuscles return to their original position or pass beyond it. This movement of the corpuscles may be due to a contraction and relaxation of the walls of the larger blood-vessels.

Fig. 2 A typical case of rhythmic pulsation in the melanophore after treating with BaCl_2 . The curve represents changes in the diameter of a single melanophore in the course of an hour. The maximum diameter of this cell was measured by means of an ocular micrometer at various times. These diameters are plotted against the time; 33 divisions of the micrometer represents complete contraction and 130, extreme expansion. The treatment of this cell was as follows: Experiment S, 15, 20. June 21, 1915.

7.43 a.m. Scales from an 8.5 cm. female *F. heteroclitus* were immersed in 0.1 N NaCl .

8.14 a.m. 3 scales to 0.1 N BaCl_2 .

8.27 a.m. One scale returned to 0.1 N NaCl ; temperature 19.4°C .

9.14, 15. First observation of rhythmic contractions.

the contractions between expansions are incomplete, the reduction being in this case in the contractions. The disappearance of the pulsations leaves the melanophores expanded instead of contracted. We may therefore say that the melanophores may recover after having been contracted in BaCl_2 , provided the time of immersion in the BaCl_2 has not been more than about five minutes (fig. 2).

Automatic pulsations or rhythmic contractions occur not uncommonly in several types of smooth muscle. Among the lower vertebrates, for example, Schultz (l. c.) has described this phenomenon in ring preparations of the stomach of the frog and Stiles ('01) has studied it in similar preparations of the frog's oesophagus. An examination of their figures shows that the rapidity and frequency of the contractions is not unlike those occurring in the melanophore after having been treated with BaCl_2 and returned to NaCl .

B. *Organic Substances.* Krukenberg ('80) carried out a series of experiments upon the reactions of the chromatophores of *Eledona*, a cephalopod, to organic substances. He found that 5 per cent ethyl alcohol, ether and chloroform (strength not stated in either case) produced a contraction of the radial smooth muscles i.e. an expansion of the chromatophores. When pieces of the skin were washed or returned to sea-water, the chromatophores contracted and regained their irritability. Klemensiewicz ('78) observed a lightening of the skin in *Loligo* and *Eledona* when subjected to vapors of amyl nitrite. The chromatophores in isolated pieces of skin showed the same reaction. Amyl nitrite is a familiar vaso-dilator. The relaxation of the radial muscles in the chromatophores of cephalopods therefore suggests that this may be a typical response for physiologically widely differing varieties of smooth muscle.

I have exposed the melanophores of *Fundulus* to alcohol, ether and chloroform vapors. The scales bearing these melanophores were placed upon glass slides and just covered with Ringer's solution. The slides were then placed over Syracuse glasses containing the organic fluid to be tested and both were covered with small, ground-edged, bell jars and sealed with vaseline.

Under these circumstances the melanophores always contracted.⁷ Control cells under identical sealed jars, remained expanded.

I have also exposed contracted melanophores of *Fundulus* in a contracting mixture of 4 parts of 0.1 N NaCl + 1 part 0.1 N KCl to the vapors of amyl nitrite, using the same technique as in the trials with ether and alcohol. All these melanophores expanded promptly whereas control cells in the same contracting mixture, under a second sealed bell-jar, showed no change.

Weak solutions of atropine or atropine sulphate (0.00025 M in 0.1 N NaCl) produce a prompt expansion of the melanophores in *Fundulus*. The dilating action of this alkaloid upon the pupil is a familiar physiological phenomenon.

Lieben ('06) found that in *R. temporaria*, subcutaneous injections of adrenalin produced a complete contraction of the melanophores in 15 to 20 minutes. The local application of adrenalin upon the swimming web also produced a contraction. These contractions Lieben believed to be independent of the constricting action of the adrenalin upon the blood vessels. When the melanophores of *Fundulus* are immersed in solutions of adrenalin hydrochloride of different concentrations⁸ they invariably contract. Even at a dilution of one part to one million (in 0.1 N NaCl) a contraction appears, though not always in all the melanophores of a single scale.

From the foregoing observations it appears that certain organic substances which typically produce contractions in smooth muscle, cause a contraction in the melanophores of *Fundulus*. Reciprocally, certain other organic substances which produce an expansion or relaxation in isolated smooth muscle, produce an expansion in the melanophores of *Fundulus*.

⁷ The contraction depends, however, upon the amount of organic fluid in the Syracuse dish. Under the conditions of these experiments, 3 to 5 drops produced a contraction. Larger amounts inhibited the contraction and produced a narcosis. This became evident when the cells were removed from the bell jars, for a transient contraction appeared, the effect of the dilute stimulus.

⁸ A Parke-Davis preparation of adrenalin hydrochloride 1 : 1000 in physiological salt solution was used as the starting point in these experiments. This was diluted with 0.1 N NaCl to the required concentration.

AN ANALYSIS OF THE CONTRACTION IN THE MELANOPHORE

A structural and functional parallelism between smooth muscle and melanophores can scarcely be considered complete without a comparison of the contractions in the two cases. At first sight they have little in common. Indeed the exact mechanical nature of the contraction in the melanophores is still a disputed question. Certain facts are established beyond any reasonable doubt viz: 1) the proximal and distal pigment migrations invariably occur along fixed paths (Kahn u. Lieben '07, Spaeth '13 b); 2) in cases where living cells have been observed under high magnifications it has appeared that the pigment granules are not merely carried along passively by a streaming or flowing of an extremely liquid sort of protoplasm, but that they exhibit active, independent movements within the cell processes (Ballowitz '13 a and b, Degner '12). Exactly what occurs after the pigment granules have reached the centre of the cell, has not been determined as yet with certainty. Either 1) the cell processes remain in situ or 2) the more fluid protoplasm follows the migrating pigment granules and eventually the entire cell becomes rounded up as in a contracted amoeba. In the latter event it is difficult to imagine how the protoplasmic processes find their way back again to the original contour of the expanded cell. Hooker ('12 and '14) has explained away this difficulty by assuming, from histological preparations, the existence of lymph spaces into which the pseudopodia of the amoeboid melanophore creep or flow. Hooker believes these lymph spaces to be fixed and the melanophore to be a typical amoeboid cell. There are serious objections to this view. 1) Hooker's evidence is adduced from fixed preparations and he has been unable to corroborate his observations upon living adult cells; 2) in the many hundreds of living melanophores that I have observed under high magnifications and the most favorable optical conditions (reflected and transmitted light, dark-field illumination, etc.) I have never been able to detect the secondary migration of the fluid protoplasm, though I have repeatedly watched the same cell in a series of expansions and contractions with this as the sole object; 3) the his-

tological preparations of Ballowitz ('93) show nerve terminations on the processes of the melanophores which could not have any physiological significance if the entire cell were withdrawn from these processes; 4) finally I have repeatedly corroborated in *Fundulus*, the observation of Ballowitz ('13) concerning the position of the nucleus following a proximal migration of the pigment granules. Not infrequently in an expanded melanophore, the nucleus may be seen to lie far out from the centre in one of the processes. If such a cell be contracted, all the pigment granules accumulate at the centre, but the nucleus remains in its original position in the process. Under favorable conditions it may actually be seen in the contracted cell, lying in the process, with a considerable area of pigment-free cytoplasm between it and the central mass of melanin granules. In any case, upon reexpansion, the nucleus is found practically to have retained its original position. It is difficult to correlate these facts with Hooker's conception.

At present it is, then, impossible to say that the melanophores are certainly not amoeboid cells in the mature condition, though the weight of the evidence appears to be against such a view.

The question now arises as to what a proximal and distal migration of pigment granules within a stellate cell can have in common with a 'contraction' in smooth muscle. A physical-chemical analysis of the melanophore may serve to clarify the comparison.

Considered as a physical-chemical system the melanophore consists essentially of a colloidal suspension of melanin granules (the disperse phase) in a dispersion medium which is itself an exceedingly fluid sort of protoplasm i.e., an emulsoid sol. 'Contraction' of the melanophore consists of an aggregation of the disperse phase of melanin granules and in 'expansion' there is an increased dispersion of the melanin granules. In other words, when the melanophores are stimulated to contract, we observe the first step in a reversible aggregation or coagulation process, i.e., the aggregation of the melanin granules. Is it possible to consider this phenomenon a reversible coagulation such as occurs commonly in emulsoids? The size of the melanin granules is relatively so large (ca. 0.0004 mm.) that we should rather expect

an irreversible coagulation as we find among suspensions or suspension colloids. The presence of the fluid protoplasm in which the granules are suspended must here be taken into consideration. It is a well known fact (Bechhold '04) that the disperse phase of a suspension colloid or suspension, adsorbs the particles of the disperse phase of an emulsoid when the two are in 'solution' together. This phenomenon has been termed a 'protection' of the coarser phase; the adsorbed emulsoid is the protecting colloid ('Schutzkolloid' of Bechhold). Under such circumstances, the suspension colloid assumes the character of a true emulsoid, i.e., it is protected against the irreversible coagulative effects of electrolytes, heat, etc. Suspension colloids or suspensions, in the presence of emulsoids, thus become far more stable and plastic systems (Neisser und Friedemann '04). The existence of just such a physical-chemical system within the melanophore is a perfectly demonstrable fact. Its reversibility is equally obvious. Thus far, then, we have but analyzed the physical-chemical conditions within the cell based upon the observed facts.

In the case of an increased dispersion in a two-phase system, there is an increased intimacy of relation between disperse phase and solvent; the system approaches a true solution and we speak of an increased solubility of the disperse phase. Reciprocally, in an aggregation or coagulation process, we have a separation of disperse phase and solvent. There is an accumulation of evidence at hand showing "that during the contraction of smooth muscle there is an exchange of fluid between the cells of the tissue and their surroundings" (Meigs '12, p. 543). It is difficult to imagine how this setting free of fluid can occur in a colloidal system such as a smooth muscle cell, except in connection with a reversible coagulation or aggregation process. In other words, evidence for an exchange of fluid during contraction in both striated and smooth muscle must, at the same time, be considered evidence for a reversible aggregation or coagulation process in the cell colloids. The displacement of fluid is as necessary an accompaniment of the act of contraction as is the coalescence of colloidal particles.⁹

⁹ A discussion of the relative merits of the 'swelling' and 'surface-tension' hypotheses of muscle contraction would be irrelevant (Lillie '12).

In the case of the melanophores there is no direct evidence at hand for a loss or exchange of fluid during contraction. There is, however, a visible and reversible colloidal aggregation of melanin granules following a variety of physiological stimuli, all of which elicit contractions in smooth muscle. Similarly, a number of physiological stimuli that produce a relaxation in smooth muscle bring about a dispersion of the pigment granules in the melanophore. Now, from the evidence cited above, we are forced to assume that, during the contraction of smooth muscle there is an invisible aggregation or heaping up of colloidal particles which becomes reversed in relaxation, i.e., when the muscle relaxes, there is an increased dispersion of the invisible colloidal particles. *The aggregation of melanin granules within the melanophore must therefore be considered a visible expression of the colloidal phenomenon that occurs, upon stimulation, in the micro-homogeneous colloidal content of a smooth muscle cell.*

GENERAL APPLICATION OF THE CONCLUSION

Although much of the evidence in the preceding sections has been obtained from experiments with a single species of teleost, *Fundulus*, there is no valid reason for doubting that the mechanism of the responses in the chromatophores of crustaceans and the melanophores of fish, amphibia and reptiles, is identical.¹⁰ In all these cases the color-changes are brought about by an aggregation and dispersion of pigment granules. Chun (l. c.) has shown that in cephalopods the chromatophores develop from a single smooth muscle cell. We may therefore say that it seems highly probable that color changes in crustaceans and cephalopods, as well as in the three groups of lower vertebrates, are brought about by the physiological responses of specialized smooth muscle cells.

The physiological modification in the case of the melanophore may be compared with that of the electric organ in fish where a striated muscle cell becomes so far modified that the normal mo-

¹⁰ The term 'chromatophores' can not be applied to the case of the lower vertebrates until more is known of the physiology of the xanthophores.

tor function disappears completely and the action current, which is normally of relative insignificance, becomes a physiological end in itself and a formidable means of protection. Similarly in the melanophore, the motor function of the smooth muscle cell is lost and there is developed a modified motility, a migration of pigment granules, which may again serve as a means of protection.

SUMMARY

1) Morphological, embryological and physiological evidence is advanced to prove that the melanophores of fish, amphibia and reptiles are not connective tissue cells as has been tacitly assumed heretofore, but functionally modified smooth muscle cells.

2) In the contraction of the melanophore there is an aggregation of melanin granules which is to be considered the visible counterpart of an aggregation of colloidal particles that occurs during the contraction in smooth, and possibly striated muscle.

3) It seems highly probable that color-changes in crustaceans and cephalopods, as well as in the three groups of lower vertebrates, are brought about by the physiological responses of specialized smooth muscle cells.

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