STUDIES ON PIGMENT MIGRATIONS IN THE MELANOPHORES OF *XENOPUS LAEVIS*[1]

by

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One of the most fascinating activities by which animals can respond to environmental changes is their ability to change the colour of their skin. Among Vertebrates this phenomenon is restricted to numerous poikilotherm animals.

The colour change of the skin is brought about by the activity of specific cells, the chromatophores, which contain large quantities of pigment granules. In view of the colour of this pigment different kinds of chromatophores can be distinguished.

In the present paper we will draw attention to the dermal melanophores of the amphibians, which play the most important part in the colour change of these animals. Melanophores contain blackish brown pigment granules, probably consisting of melanin, which can migrate from the centre towards the periphery of the melanophores ("dispersion") or in the reverse direction from the periphery towards the centre of the cells ("concentration"). As a result of these pigment migrations the animal becomes either dark or pale.

In the first decennia of the 20th century it was generally thought that the colour adaptation of amphibians to their environment was regulated by the eyes and by nervous transmission. Later experiments, however, have shown that also the presence of the pituitary gland is essential for their colour adaptation, for after ablation of the pituitary gland in *Rana*-tadpoles or in adult frogs (ADLER, 1914; ALLEN, 1916; KROGH 1929, p. 150) the experimental animals invariably show a very pronounced irreversible pigment concentration in their melanophores,

[1] This paper gives a survey of the investigations, which have been carried out in our laboratory and chiefly relate to the endocrinological aspects of the physiology of the melanophores.

i.e., they remain pale. It was, therefore, supposed that the pituitary gland is the source of a powerful pigment dispersing substance. That this supposition is correct was proved by the results of experiments in which extracts of frogs' pituitaries were injected or pituitary glands were transplanted into hypophysectomized animals.

Moreover, it was found that the pigment dispersion activity of extracts of intermediate pituitary lobes was much greater than that of extracts of neurohypophyses (GIUSTI and HOUSSAY, 1924; VAN DYKE, 1926) and also that suppression of the development of only the intermediate lobe in *Hyla*-tadpoles resulted in albino-like animals (BURCH, 1938).

These and other experiments showed that the hormone responsible for the melanin dispersion in amphibian melanophores is located in the intermediate lobe of the pituitary gland. After its source ZONDEK and KROHN (1932) named it "intermedine". It is also called "pigment dispersing"- or "melanophore expanding"-hormone. The release of this hormone into the blood is thought to be controlled by the central nervous system.

Before reporting our investigations, we will draw attention to a paper of HOGBEN and MIRVISH (1928) in which these authors described a distinct melanin concentration in the dermal melanophores after electric stimulation of the palate of the Cape Chameleon. This phenomenon was called excitement pallor. Later, similar sudden colour changes caused by excitement-stimuli, e.g. rough handling, the prick of a needle or the injection of a physiological saline solution were named excitement colour reactions (BURGERS, 1956); they can be divided into excitement pallor and excitement darkening reactions.

In *Rana*-species an excitement pallor, obtained by rough handling, was described by several authors (VON WITTICH, 1854; KROPP, 1927; PARKER and SCATTERTY, 1937; a.o.). In addition, many other reactions like changes in blood circulation, micturition or skin gland secretion can be observed when amphibians are suddenly disturbed. We consider all these phenomena as expressions of the alarm-reaction[1].

The pigment migrations in the melanophores of amphibians are not only influenced by intermedine but also by adrenaline. Already in 1906 LIEBEN showed that adrenaline has a concentrating effect on the pigment granules of the melanophores of *Rana temporaria*. As adrenaline is thought to play an important role in the alarm reaction, it is not improbable that it is the cause of the excitement colour reaction, i.e. in *Xenopus* the excitement darkening reaction.

[1] This reaction was defined by SELYE (1949, p. 837) as "the sum of all non-specific systemic phenomena elicited by sudden exposure to stimuli to which the organism is quantitatively or qualitatively not adapted".

Our interest in the hormonal regulation of the melanophores was aroused several years ago by two facts, viz. 1, the presence of black-brown pigmented cells in or on internal organs of amphibians, as e.g. on the testes, in the pericard or in the peritoneum of frogs and toads, and 2, by the so-called KONSULOFF-reaction, a pregnancy diagnosis test, in which the pigment dispersal in the melanophores of hypophysectomized frogs plays a dominant role.

As regards the first the question arose whether these internal cells react in the same way as and simultaneously with dermal melanophores.

It appeared that the reactions of these internal pigment cells differed fundamentally from those of the dermal melanophores. We found for instance (BURGERS, BOSCHMAN and VAN DE KAMER, unpublished; cf. BURGERS 1956, p. 32) that intermedine, causing a maximal pigment dispersion in dermal *Xenopus*-melanophores had hardly any effect on the black pigmented cells present in the wall of the intestine, even when it was administered in much higher concentrations than those giving a maximal reaction in dermal melanophores. Consequently these internal pigment cells are fundamentally different from the dermal melanophores; their physiological signification, however, is still obscure.

In 1934 KONSULOFF introduced the test, which later was named after him. He then described his experiments with hypophysectomized frogs, in the dorsal lymphsac of which human urine was injected. As only the frogs darkened in case the urine originated from pregnant women, and even from women in the first weeks of pregnancy, KONSULOFF concluded that in pregnancy urine a substance is present which acts on the pigment dispersion of frogs' melanophores.

After the war the KONSULOFF-reaction was re-introduced as a pregnancy test by DE BOURGRAAF and DINGEMANSE (1946) and independently by SERVANTIE, CAMBAR, MORETTI and BONNAL (1947). During an investigation, partly carried out in our laboratory, MEIJHORST, STOLTE, DE ROO and CREUTZBERG (1949) found that the KONSULOFF-reaction is a reliable pregnancy test, if certain precautions have been taken. These workers were then of opinion that intermedine was the active substance in the pregnancy urine. Later STOLTE, BAKKER, VERBOOM and DAUVILLIER (1953) assumed that the KONSULOFF-reaction is caused by chorionic gonadotropin and that there are at least two substances with melanophore hormone activity in the pituitary gland: adrenalcorticotropic hormone (ACTH) and luteinisation hormone (LH) and(or) lactogen.

In 1951 we decided to study the pigment dispersion and concentration in amphibian melanophores in detail. For these investigations the South-African clawed toad, *Xenopus laevis*, was chosen.

In preliminary experiments it had been shown that *Xenopus* is admirably suited for investigations into the regulation of melanophores. In the first place *Xenopus*, like *Rana*, can adapt itself to a white and to a black background: on a white background these animals become pale, whereas on a black background they darken. Moreover, in *Xenopus* only melanophores are found, which, contrary to the melanophores and other chromatophores of *Rana*, do not overlap each other, a phenomenon which enables us to determine the melanophore reactions more accurately. Finally, the webs between the first and second, and between the second and third digits of the hindlegs are ideally suited for studying the melanophores *in vivo*.

In the following pages the changes of the melanophores will be indicated with the aid of HOGBEN and SLOME's melanophore index (1931; M.I.), in which the different stages of pigment dispersal in the melanophores are indicated by the figures 1–5, 1 relating to a melanophore in which the melanin granules are concentrated around the nucleus, and 5 to the stage of maximal dispersion of these granules (fig. 1).

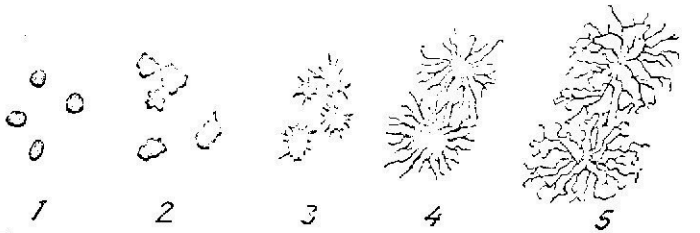

Fig. 1. Melanophore index (M.I.) after HOGBEN and SLOME (1931).

In a preliminary study (BURGERS, BOSCHMAN and VAN DE KAMER, 1953) it was shown that among the amphibians *Xenopus* is an exception with reference to its melanophore reactions, following excitement stimuli and adrenaline injections. As to the excitement stimuli we found that light-adapted adult specimens of *Xenopus* react to stimuli with a distinctly perceptible excitement darkening, whereas most other adult amphibians react to such stimuli with an excitement pallor. Only SIEDLECKI (1909) has shown that in the amphibian *Polypedates reinwardtii* excitement stimuli also produce skin darkening. KETTERER and REMILTON have confirmed our observations in 1954.

Further experiments showed that hypophysectomized specimens of *Xenopus* also show an excitement darkening reaction. Hence, it was concluded that in *Xenopus* the excitement darkening reaction is not induced by a hormone secreted by the pituitary gland. Consequently the question had to be answered by what agent this excitement darkening is caused.

As it appeared that rough handling of *Xenopus* induces not only an excitement darkening but simultaneously an abundant release of skin secretion, we thought it worth while to investigate whether a relationship exists between these two phenomena.

In the skin of *Xenopus* a number of macroscopically visible glands is present, most of them being found in the dermal layer. Each gland is connected to the exterior by a narrow duct. Two kinds of glands can be distinguished: large ones, filled with short microscopical needle-like crystals, consisting of a protein-like substance, and small ones, containing mucus. According to SPANNHOF (1954) the mucous glands release their contents continuously, whereas the sticky substance of the protein glands is only secreted, following certain stimuli. By a simple procedure, i.e. by massaging the skin of normal adult *Xenopus*, we easily could collect this secretion. It appeared to consist chiefly of the product of the protein glands. Injected extracts of this skin secretion induced pigment dispersion in *Xenopus*, adapted to a white background. As it acts also on the melanophores of isolated *Xenopus*-webs—which possess no skin glands—it was shown that this is a direct action.

We assumed, therefore, that in the skin secretion of *Xenopus* a substance is present, which may be responsible for the excitement reaction.

Already in 1930 GUNN supposed that in *Xenopus* skin secretion a sympathomimetic substance, similar to but not identical with adrenaline, is the main active principle. Later, one of us (BURGERS, 1956) confirmed that this secretion possesses an adrenaline-like action.

We have already mentioned that amongst the amphibians *Xenopus* is an exception with regard to its melanophore reactions following adrenaline injection. We observed that melanophores with dispersed pigment granules react to adrenaline with a pigment concentration, like those of other amphibians, whereas melanophores of pale animals react to the same hormone with a pigment dispersion, contrary to those of other amphibians (BURGERS, BOSCHMAN and VAN DE KAMER, 1953, p. 75)[1].

As there is no appreciable difference in melanophore reaction to adrenaline in normal *Xenopus*, adapted to a white background and in hypophysectomized toads, this pigment dispersion cannot be the result of an action of intermedine secreted by the pituitary gland under the influence of injected adrenaline. This view is supported by the results of *in vitro* experiments in which isolated *Xenopus*-webs were immersed in adrenaline solutions and in saline. From fig. 2 it appears that the M.I.'s of the melanophores of isolated webs from normal light adapted

[1] In the present paper we will focus our attention especially on the pigment dispersion caused by adrenaline in pale animals.

animals increase distinctly under the influence of adrenaline, whereas the M.I.'s of the control webs, immersed in saline, slightly decrease.

From these experiments we concluded that in *Xenopus* adrenaline has a direct pigment dispersing action on melanophores with concentrated pigment granules.

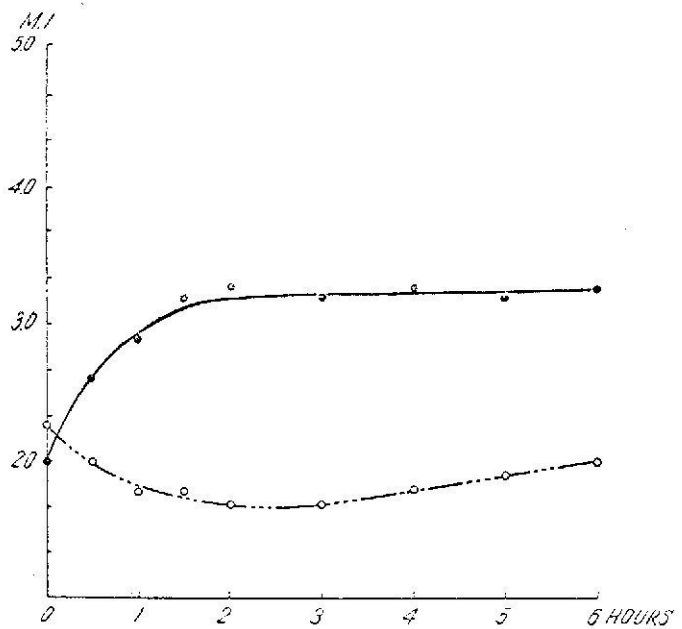

Fig. 2. Graph, showing M.I.-curves of melanophores in isolated webs of normal light adapted *Xenopus* and immersed in adrenaline (solid line) or in saline (broken line).

In a further series of experiments we found (BURGERS, 1956; BURGERS, 1957; BURGERS and VAN OORDT, 1956) that in isolated webs, immersed in *Xenopus* skin secretion extract, in human pregnancy urine extract, or in solutions of adrenalcorticotropic hormone (ACTH), of intermedine and of a large number of phenylalkylamines (adrenaline included), a distinct pigment dispersion in the melanophores was evoked. In control webs, however, which were immersed in saline solution, no pigment dispersion was observed.

Interesting with regard to the phenylalkylamines is the relation of the chemical structure of these compounds and their biological activity. As a matter of fact it could be demonstrated that the presence of two OH-groups in the 3,4 positions at the phenylnucleus of these substances is essential to obtain pigment dispersion.

In view of the melanophore reactions of isolated *Xenopus* webs, caused by these extracts and compounds, we are of opinion, that

ACTH and intermedine on the one hand, and extracts of *Xenopus* skin secretion or of human pregnancy urine and the phenylalkylamines on the other, evoke two different melanophore patterns in isolated webs. In control webs, which were immersed in saline only, no pigment dispersion, of course, was observed (fig. 3).

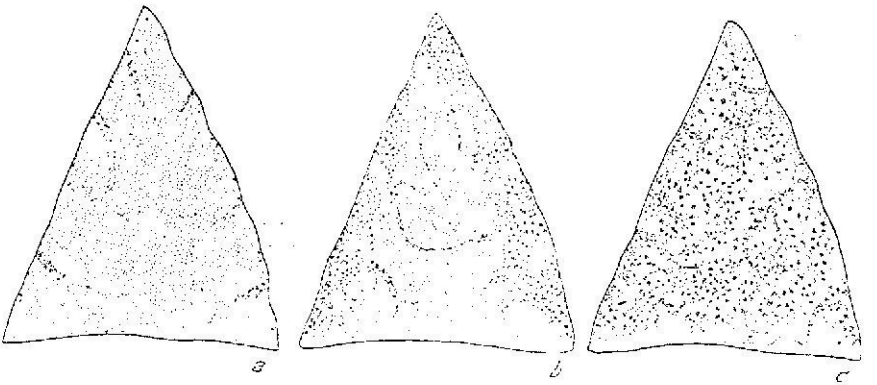

Fig. 3. Drawings of *Xenopus*-webs to illustrate the differences in melanophore pattern after immersion in *a.* control saline solution, *b.* ACTH or intermedine, and *c. Xenopus* skin extract, pregnancy urine extract or phenylalkylamines. After BURGERS (1956).

It is not yet known which pigment dispersion agent is present in either *Xenopus* secretion or in human pregnancy urine. On the basis, however, of a comparative study of these melanophore patterns, we assume that the active principles present in *Xenopus* skin secretion and in human pregnancy urine are also compounds, possessing a phenyl-nucleus with 2 OH-groups in the 3,4 positions (BURGERS and MIGHORST, 1957). We hope to solve this problem in the biochemical department of our laboratory.

In the last ten years the psychic actions of the drug d-lysergic acid diethylamide [LSD-25[1], delysid (SANDOZ Ltd., Basle)] have been extensively studied in man and other mammals. Little work, however, has been done on its action in lower Vertebrates. It was found that in fishes the influence of LSD manifests itself in both behaviour- and colour-changes. Besides characteristic changes in behaviour a distinct darkening of the teleost fish *Betta splendens* was obtained with LSD by ABRAMSON and EVANS (1954) and by EVANS *et al.* (1956). Moreover, CERLETTI and BERDE (1955) and BERDE and CERLETTI (1956, 1957) demonstrated that LSD causes a marked pigment dispersion in the melanophores of the female guppy, *Lebistes* (= *Poecilia*) *reticulatus*, *in*

[1] In the following pages only the initials LSD will be used.

vivo as well as *in vitro*. Hence, the last mentioned authors assumed that LSD acts directly on the dermal melanophores of this fish.

In recent experiments we have examined the action of LSD on *Xenopus*-melanophores. We found that LSD, solved in the water of aquaria, in which specimens of *Xenopus*, adapted to a black background are present, or injected in doses of 1 μg into the dorsal lymphsacs of these animals, induces a marked pigment concentration in the melanophores which lasts many hours. In *Xenopus*, adapted to a white background, we observed a further slight pigment concentration, following administration of this drug.

We also noticed that injection of ACTH (intermedine) in LSD-treated, as well as in normal pale *Xenopus* causes a similar pigment dispersion reaction (fig. 4). Moreover, we found that LSD has no effect on the melanophores of isolated webs, in which a pigment dispersion had been induced by immersing them first in an intermedine solution. Finally we showed that a pretreatment of the webs with LSD does not

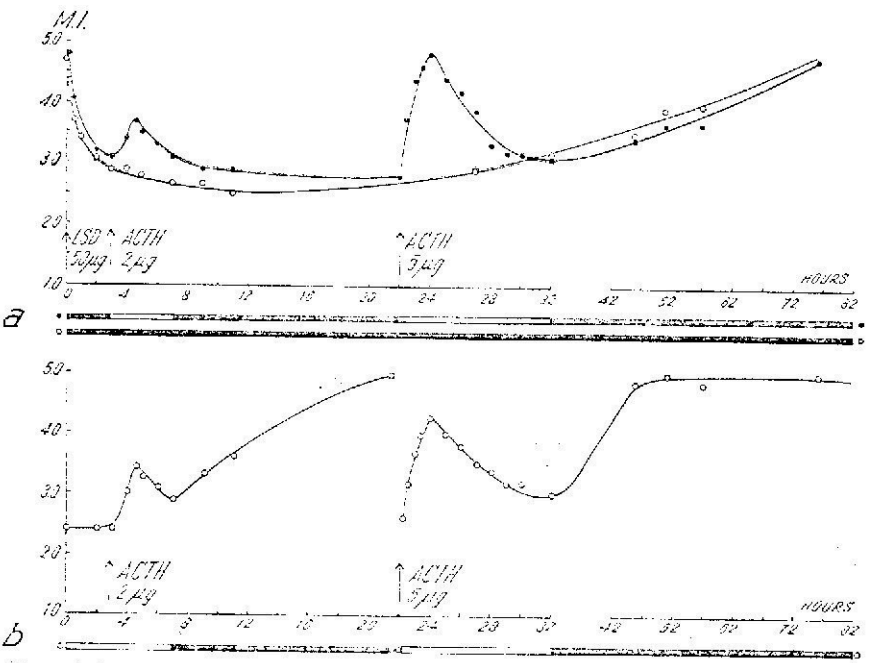

Fig. 4. LSD- and ACTH (intermedine)-effects in *Xenopus*. *a.*: effect of LSD on the melanophores of two groups of *Xenopus*-specimens, both adapted to a black background and the increase in M.I., caused by injection of ACTH in one group; *b.*: effect of ACTH (intermedine), injected into 2 groups of non-LSD-treated *Xenopus*-specimens, adapted to a white background. (The time spent by the animals in white or in black jars is indicated by the white or black stripes under the figures.) After BURGERS, LEEMREIS, DOMINICZAK and VAN OORDT, 1958.

influence the pigment dispersion, induced in the melanophores by immersing these webs into an intermedine solution.

The results of these *in vivo* and *in vitro* experiments indicate that in *Xenopus* LSD acts centrally. Hence it was concluded (BURGERS, LEEMREIS, DOMINICZAK and VAN OORDT, 1958) that LSD inhibits the production and/or the release of intermedine in *Xenopus*.

In connection with these findings, experiments relating to the important question whether or not serotonine (5-hydroxytryptamine or enteramine)—the action of which is thought to inhibit that of LSD (cf. BERDE and CERLETTI 1956, 1957) affects the colour change reaction induced by LSD in *Xenopus*, are now in progress in our laboratory.

In addition to the hormones and drugs, acting directly or indirectly on the pigment migrations in the melanophores of *Xenopus*, there is still another factor which also affects the melanophores, i.e. light.

Already in 1905 BLESS observed that in young *Xenopus*-larvae the pigment granules in the dermal melanophores of the head disperse in light, whereas in darkness the pigment is concentrated. The melanophores of the tailfin, however, show reverse reactions: in light the melanin granules are concentrated and in darkness dispersed.

BAGNARA found in 1957 that light has a direct effect on the pigment migrations in the melanophores of *Xenopus*-tadpoles; this can very easily be demonstrated in the tailfin melanophores. Isolated tails of these animals placed in darkness for at least 30 minutes become extremely dark; when transferred to bright light they again become transparent in 5–6 minutes.

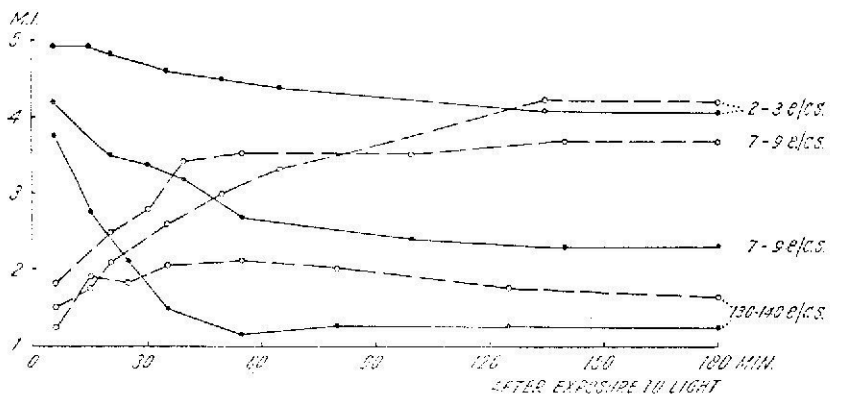

Fig. 5. M.I.-curves, showing the effect of light of different intensities (expressed in ergs/cm².sec.) on the melanophores of isolated tails of *Xenopus*-tadpoles, which had been exposed to absolute darkness (solid lines) or to bright light (broken lines) during 0.5 h.

These findings were confirmed by VAN DER LEK, DE HEER, BURGERS and VAN OORDT (1958), who in addition studied the effect of light of different intensities on the melanophores of tails, isolated from tadpoles which had been adapted for various periods to absolute darkness or to bright light. Some of the results of these experiments are shown in the graph, fig. 5.

In this graph it is visible that a relation exists between the intensity of the light to which the melanophores are exposed and their reaction. Moreover, it was found that the melanophore activity in isolated tails is effected by the length of the period during which the intact tadpoles were kept in absolute darkness or in bright light.

To elucidate the physiological processes in the tailfin melanophores by light, it is planned to analyse the biophysical background of this phenomenon in the near future. In addition we hope to collect more data on the changes in sensitivity of the melanophores to light and to certain hormones during the development of the *Xenopus*-tadpoles.

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