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# The Amount of Melanophore–Stimulating Hormone in Single Pituitary Glands of *Xenopus laevis* Kept under Various Conditions

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The MSH (melanophore–stimulating hormone) content of single anterior and intermediate/posterior pituitary lobes of *Xenopus laevis* was determined using skin pieces of *Anolis carolinensis* as test objects. Significant changes in the MSH content of single intermediate/posterior lobes of *Xenopus* were found after adaptation during a 48 hour period to a black or to a white background. The ratios of the pigment–stimulating potencies of anterior to intermediate/posterior pituitary lobes are about 1:38 for white background–adapted *Xenopus*, and about 1:9 for black background–adapted animals. The results of the experiments indicate that under the given adaptation conditions the release of MSH is inhibited in white background–adapted *Xenopus* and the hormone stored in the pituitary. The hormone is continuously released in black background–adapted animals. Exposure to complete darkness does not deprive the pituitary of *Xenopus laevis* or that of *Rana temporaria* of its MSH.

## INTRODUCTION

It is generally assumed that the color change in amphibians is chiefly regulated by MSH. To elucidate the mechanism of this phenomenon, several investigators have studied the MSH content of pituitaries from amphibians which were kept in light or in complete darkness. The results of these experiments, however, were not always consistent. For instance, Koller and Rodewald (1933) and Rodewald (1935) found that the MSH content of the pituitaries of *Rana temporaria* rapidly decreases and finally disappears when the animals are kept in darkness, and that light is required for the production of this hormone. Masselin (1939) and Stoppani (1942), on the other hand, showed that in the pituitaries of *Bufo arenarum* the amount of MSH decreases in light but increases in darkness; Kleinholz and Rahn (1940), however, found no significant differences in the MSH content of pituitaries of *Rana pipiens* kept in darkness or in light.

The effect of white, neutral, and black background–adaptation on the MSH content of pituitaries of *Rana pipiens* was studied by Ortman (1954, 1956), who found striking histological differences in the intermediate lobes of the pituitaries of frogs kept on various backgrounds. Bioassay of pooled pituitaries from animals of different experimental groups, however, revealed no significant differences in MSH content.

Among amphibians, the South African clawed toad *Xenopus laevis* shows a great ability to change its color, being yellowish on a white background, pitch–black on a black one, and grayish in complete darkness (Hogben and Slome, 1931; Landgrebe *et al.*, 1944). For this reason, *Xenopus* was chosen as the experimental animal for studying the effect of various backgrounds on the MSH content of single pituitary lobes.

## MATERIAL AND METHODS

The specimens of *Xenopus laevis* (bodyweight 11.2–21.7 gm) used in these experiments were bred in our laboratory.

Background adaptation was achieved by keep-

ing the animals, during various periods, in black or white jars containing 500 ml tap water and illuminated by a 100 watt bulb suspended 40 cm above the water level. The various adaptation periods are indicated as follows: 48 B, animals kept for 48 hours in black jars in light; 48 W, animals kept for the same period in white jars in light, and 48 D, animals kept for 48 hours in complete darkness.

The water in all jars was changed between the successive adaptation periods. At the end of each period the melanophore index (M.I.) of the toads was determined according to the method of Hogben and Slome (1931). At the end of the final adaptation period each animal was weighed and decapitated. The pituitary gland was then excised, its anterior and intermediate/posterior lobes separated, and each part transferred into a 10 ml beaker containing 2 ml *Xenopus* physiological saline solution (prepared according to Burgers and van Oordt, 1956). Finally, the glandular material was mixed with a small quantity of glass powder and thoroughly ground. The beakers were placed on a black plastic sheet and illuminated by a 100 watt bulb suspended 50 cm above the beakers.

The biological assay for melanophore-stimulating activity was carried out with skin pieces of the lizard *Anolis carolinensis* as test objects (Burgers, 1961). These skin pieces show a remarkable color change; when for instance, bright green pieces are in contact with a physiological saline solution containing MSH, they become black-brown in a few minutes. If such a black-brown skin piece is transferred to a physiological saline solution without hormone, it regains its original color. The skin pieces were obtained in the following manner: a bright green lizard was decapitated, its tail and extremities cut off, and a medio-ventral incision made in its body; the skin was then peeled off and transferred to a Petri dish containing physiological saline solution. Owing to the hydrophilic properties of the ventral side of the lizard skin, it stretched itself and floated on the surface of the saline solution. About one and one-half hours later, the bright green skin which had covered the dorso-lateral sides of the animal was cut into about seventy small pieces. To determine the melanophore-stimulating potencies of the extracts, one of these skin pieces was transferred from the Petri dish to each beaker containing pituitary extract. When the color of the skin piece changed from bright green to brown, the sample was diluted with physiological saline solution and a fresh piece of lizard skin added. If this piece also became brown, the procedure was repeated until the concentration of the hormone in the solution induced a color change in the lizard skin from a bright green to a definite brownish green within 30 min.

The melanophore-stimulating potency of the original sample is indicated by the degree of dilution which is required to induce the last mentioned color change reaction, the color of the skin pieces in the beakers always being compared with that of skin pieces in a beaker containing physiological saline only. For convenience, the quantity of a substance which induces a color change from bright green to a definite brownish green in a piece of *Anolis* skin within 30 min is called an *Anolis unit* (A.U.).

As a rule, the pituitaries were excised between 9 and 11 A.M. The experiments were carried out during November and December under standardized conditions of light and at a temperature of 20–24°C.

## RESULTS

### 1. MSH content of pituitaries of black background-adapted *Xenopus* (48 B).

In our first experimental series the melanophore-stimulating activities of anterior and of intermediate/posterior pituitary lobes of six animals were studied. The M.I. of these animals was 4.9–5.0 at the end of the adaptation period 48 B. It was found that the quantity of MSH in the anterior lobes varied from 4–8 A.U. (average 6) and in the intermediate/posterior lobes from 10–50 A.U. (average 27.5). These results show that the intermediate/posterior pituitary lobe contains about 4–5 times as much MSH as the anterior lobe of these animals (Fig. 1).

### 2. Comparison of the MSH content of pituitaries of white background-adapted (group 48 B 48 W) and black background-adapted (group 48 B 48 B) *Xenopus*.

To determine the amounts of MSH in pituitaries of animals kept in various environments, the following experiment was carried out. To obtain uniformly adapted animals, twelve specimens were placed in black jars during 48 hours (48 B). These animals were then divided into two groups (W and B) of six animals each. Those of

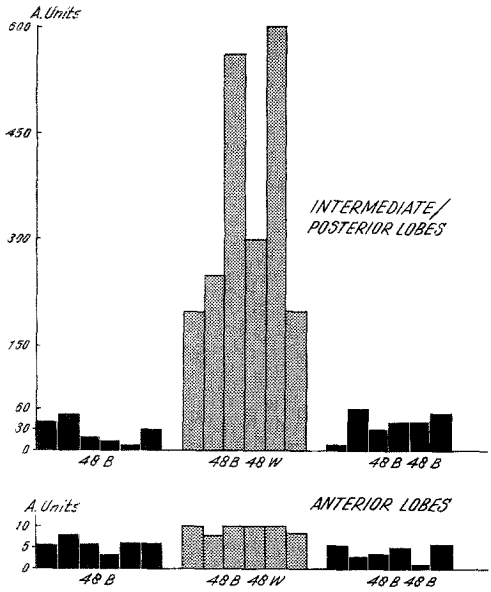


FIG. 1. The amount of melanophore-stimulating hormone present in single intermediate/posterior pituitary lobes and anterior pituitary lobes of *Xenopus* following *a*, adaptation during 48 hours to a black background (48 B); *b*, adaptation during 48 hours to a black and thereupon during the same period to a white background (48 B 48 W); *c*, adaptation during two successive periods of 48 hours each to a black background (48 B 48 B). Note the difference in scale used in the upper and lower portions of the figure.

group W were transferred from black to white jars, and those of group B from black to other black jars. Both adaptation periods lasted 48 hours.

At the moment of transfer the M.I. of the animals was 4.8–5.0. At the end of the second adaptation period, the M.I. of those in the white jars was about 2.8 and the M.I. of those in the black 5.0.

Determination of the MSH content of the pituitaries of these animals revealed that in the intermediate/posterior lobes of the white background-adapted animals it was 200–600 A.U. (average 350), and in those of the black background-adapted animals only 10–60 A.U. (average 38.3). The melanophore-stimulating activities of the anterior pituitary lobes of white and black background-adapted animals, however, did not show such large differences, being 8–10 A.U. (average 9.3) and 2–6 A.U. (average 4.3) respectively (see Fig. 1).

These results show that the MSH content of the intermediate/posterior pituitary lobes of white background-adapted *Xenopus* is about nine times higher than that of black background-adapted animals, whereas the average amount of melanophore-stimulating activity of the anterior lobes of white background-adapted animals is only about twice as high as that of black background-adapted ones.

From the results obtained in experimental series 1 and 2 it can be concluded that the MSH content of the intermediate/posterior pituitary lobe increases considerably within 48 hours by transferring *Xenopus* from a black to a white jar. Moreover, these results indicate that in animals having a low M.I. (2.8) caused by a white background (48 B 48 W), the release of MSH by the pituitary is inhibited and the hormone is stored in the gland. In those having a high M.I. (4.8) caused by a black background (48 B 48 B), the MSH is continuously released.

3. MSH content of *Xenopus* which had been first adapted to white and black backgrounds and subsequently transferred to a jar placed in complete darkness.

In the introduction it was mentioned that Koller and Rodewald (1933) and Rodewald (1935) found that the MSH content of the pituitaries of frogs rapidly decreases and finally disappears when the animals are placed in darkness. To establish whether this phenomenon can also be observed in *Xenopus* the following experiment was carried out. Four animals were adapted for 48 hours to a black background and then for a further 48 hours to a white background (48 B 48 W); four other animals were adapted during two successive periods of 48 hours each to a black background (48 B 48 B). The animals of both groups were then placed in complete darkness for 48 hours. At the end of the last adaptation period, the MSH content of the pituitary lobes of these ani-

mals was determined. It was found that melanophore–stimulating activity was present in the intermediate/posterior as well as in the anterior pituitary lobe of all animals. The amount of MSH in the intermediate posterior lobes of the animals of group 48 B 48 W 48 D was considerably higher than that of group 48 B 48 B 48 D. These results contradict those of Koller and Rodewald (1933) and Rodewald (1935), who, however, used *Rana temporaria* as experimental animal.

It was therefore decided to repeat the experiments with *Xenopus* as well as with *Rana temporaria*. In these experiments the procedures prescribed by Rodewald (1935) were followed. Thus the animals were placed in darkness for 30 min. after which they were killed, their pituitaries excised, and whole pituitary extracts prepared. These procedures were carried out in weak red light and during the same season (winter) as that in which Koller and Rodewald performed their experiments. It was found that all extracts caused a strong pigment–dispersing reaction when tested on pieces of *Anolis* skin. Based on these findings it was concluded that darkness does not destroy the MSH present in either the pituitaries of *Xenopus laevis* or in those of *Rana temporaria*.

## DISCUSSION

Earlier quantitative investigations into the melanophore–stimulating potencies of different parts of the pituitary have revealed that the ratio of these potencies in the anterior and intermediate/posterior lobes is 1:7 for *Bufo arenarum* (Houssay, 1949) and 1:60 for *Rana pipiens* (Reinhard *et al.*, 1952). In our experiments this ratio was about 1:38 (9.3:350; see Section 2) for white background-adapted *Xenopus* (48 B 48 W), and about 1:9 (4.3:38.3; see Section 2 for black background–adapted ones (48 B 48 B). As neither Houssay nor Reinhardt *et al.* mentioned the adaptation conditions of their animals, it is impossible to compare their results with ours in detail.

Study of the melanophore–stimulating potency of highly purified MSH preparations has shown that one A.U. is about equal to 0.0001 $\mu$g of highly purified $\beta$–seryl–MSH prepared from beef glands (Li and Burgers, unpublished data). If it then be assumed that the melanophores of *Anolis* are equally sensitive to the MSH present in the pituitary of *Xenopus* as to $\beta$–seryl–MSH, it may thus be possible to calculate the total amount of this hormone in a single intermediate/posterior lobe of *Xenopus*. From the finding (see Section 2) that a single intermediate/posterior lobe of a white background–adapted *Xenopus* contains about 350 A.U., and that of a black background–adapted animal about 38 A.U., it may be concluded that in terms of order of magnitude, the MSH contents of the intermediate/posterior lobes of such animals are respectively 0.035 $\mu$g ($350 \times 0.0001$ $\mu$g) and 0.0038 $\mu$g ($38 \times 0.0001$ $\mu$g). In connection with this, it is interesting to mention that Ortman (1956) calculated the maximum amount of MSH in the intermediate/posterior pituitary lobe of a *Rana pipiens* (body weight about 45 gm) as being about 0.1 $\mu$g. If the body weight of our animals (11.2–21.7 gm) is taken into account, our results with white background–adapted *Xenopus* are in agreement with those of Ortman.

Since the investigations of Li (1958), it has been known that ACTH possesses intrinsic melanophore–stimulating properties. Moreover, the presence of ACTH in lower vertebrates, i.e., frogs, has been demonstrated by Geschwind *et al.* (1952) and Reinhardt *et al.* (1952). It is therefore interesting that our investigations demonstrate the presence of a small amount of a melanophore–stimulating substance in the anterior pituitary lobe of *Xenopus*. This phenomenon may be due to either contamination of the anterior pituitary lobe with a small quantity of tissue from the intermediate/posterior lobe or to the presence of ACTH.

As the melanophore–stimulating potency of the anterior pituitary lobe is not much affected by the background response of the animals, and as there is generally no correlation of the melano-

phore–stimulating potencies of the anterior and intermediate/posterior lobes, it is unlikely that the melanophore–stimulating activity of the anterior pituitary lobe of *Xenopus* is caused by contamination with MSH. Therefore, it is probable that the melanophore–stimulating activity of the anterior pituitary lobes is due to the presence of ACTH.

The discrepancy between the results of Koller and Rodewald (1933) and of Rodewald (1935), on the one hand, and those of Masselin (1939), Kleinholz and Rahn (1940), Stoppani (1942) and ourselves, may be due to species differences of the animals used, or to variations in the experimental procedures employed by these investigators. In one of our experiments, however, *Rana temporaria* was used, and the experimental animals were killed and their pituitaries prepared as prescribed by Rodewald (1935); even so, we were unable to confirm her results. In connection with this, it must be noted that for the determination of the MSH content of the pituitary extracts, Rodewald used frogs (*Rana temporaria*), while we used skin pieces of a lizard (*Anolis carolinensis*). If we assume that this fact accounts for the contradiction mentioned above, this implies that in extracts of pituitaries of *Rana temporaria* adapted to darkness, a substance is present which inhibits the pigment–dispersing reaction in the melanophores of *Rana temporaria* but not in those of *Anolis* skin. Whether or not this assumption is correct must be established by further research.

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