THE ACTIVITY OF THE MELANOPHORES OF AN AMPHIBIAN, RANA CLAMITANS, WITH SPECIAL REFERENCE TO THE EFFECT OF INJECTION OF ADRENALIN IN RELATION TO BODY WEIGHT

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ONE TEXT FIGURE AND TWO PLATES (FIVE FIGURES)

INTRODUCTION

In a recent paper (Pierce, '41) it was shown that the melanophores in the skin of certain teleosts and lizards concentrate their pigment in response to injections of adrenalin. It was also demonstrated that there existed a positive correlation between the body weight of the animal and the duration of the period of concentrated pigment within the melanophores. The following experiments were undertaken primarily to determine if a similar relation existed in an amphibian. The response of the melanophores to light, cold, and anesthetics was also tested.

The experiments were carried out during July and August at the University of Michigan Biological Station. I wish to express my appreciation to Dr. A. H. Stockard, director, for the use of laboratory facilities and for the general co-operation which I received. I wish also to thank my colleague, Prof. Rudolf T. Kempton, for assistance with the photomicrography.

MATERIALS AND METHODS

The animal used in these experiments was Rana clamitans (Latreille) the common green frog. These frogs were collected from a pond near Wilderness Park, Emmet County, Michigan.

Most of the time the stock animals were kept in the aquarium, but for a day or so they were often retained in large screened cages in the laboratory. These cages, provided with damp moss, decayed wood, and a large pan of water, proved very satisfactory for temporary housing. Since the frogs were collected at frequent intervals it was not considered necessary to feed them. They were liberated at the close of the experiments.

To test the response of the melanophores to black and white backgrounds, the frogs were placed in boxes ($30 \times 20 \times 12.5$ cm.) lined with black or white oilcloth. A small dish of water was placed with the frogs. These boxes were the type commonly used as cages for small amphibians, reptiles, or mammals. They were made with screened holes at each end and a sliding glass top. For illumination a 60-watt bulb was suspended 8 inches above the boxes. The temperature was 24° to 26°C.

Injections were made as in previous experiments (Pierce, '41). The volume used was 0.25 cc. adrenalin chloride of concentration 1:1000 (Parke Davis Co.). The standard used for determining the end point of the dark and pale state was the condition of the melanophores in the web of the foot. With the toes spread apart, the transparent integument could be easily and quickly examined under a binocular dissecting microscope.

EXPERIMENTS WITH SIMPLE BACKGROUND

If a frog is placed on an illuminated black background, a slow gradual change in the shade of the integument occurs, until by the end of 4½ to 5 hours the animal becomes dark (fig. 2). This time for the period of darkening was established only after many trials. Since the process of darkening was so gradual, the end point could not be determined merely by gross observation of the skin of the animal. At appropriate intervals, the web of the foot was examined under a microscope, and careful record kept of the condition of the melanophores at that moment. The symbols used were adopted

from Hogben and Slome ('31, p. 12), the letters P, S, R, representing the punctate, stellate, and reticulate conditions of the melanophores, with proper combinations of PS and SR to indicate intermediate stages. When the melanophores had reached the reticulate stage, that is with their processes intermingling to form a dense network, the animal was considered dark (figs. 5 and 6). Even then the exact moment of the appearance of the end point was difficult to determine.

If a frog is placed on an illuminated white background, the response of the melanophores is variable. Usually the integument becomes pale in the course of 10 hours; occasionally the pale state does not occur for a day or two. The time for blanching proved very variable and uncertain, with a minimum of 10 to 12 hours. The melanophores scarcely ever reached the completely punctate stage. When they reached the punctate stellate stage the frogs were considered pale (figs. 3 and 4). The end point was extremely difficult to determine with precision.

Similar behavior of melanophores has been noted for Rana pipiens by Parker and Scatterty ('37) who concluded that the chromatophore system of that frog was relatively sluggish in comparison to other animals. The present observations on the melanophores of Rana clamitans definitely support this conclusion.

EXPERIMENTS WITH ADRENALIN

After the normal time for blanching and darkening had been determined for this frog, numerous series of animals varying from 5 to 70 gm. in weight were injected and studied. Time for blanching and darkening was again determined. It was found in these experimental animals, as in the normal ones, that the changes from dark to pale and the reverse were slow and gradual. However, after injection, the time for blanching was substantially reduced, from 10 to $1\frac{1}{2}$ to 2 hours. This was constant for all sizes of frogs. After injection, the period necessary for darkening was of course increased. Even though the frogs were kept on black backgrounds, the melano-

phores remained with concentrated pigment for a period longer than that of the normal. Small frogs of 6 to 7 gm. remained pale for 9 to 10 hours instead of the usual 4½ to 5 hours; while large ones of 60 to 70 gm. showed little if any increase in the period over which the pigment remained concentrated. In brief, the pigment remained concentrated longer in the smaller animals (fig. 1).

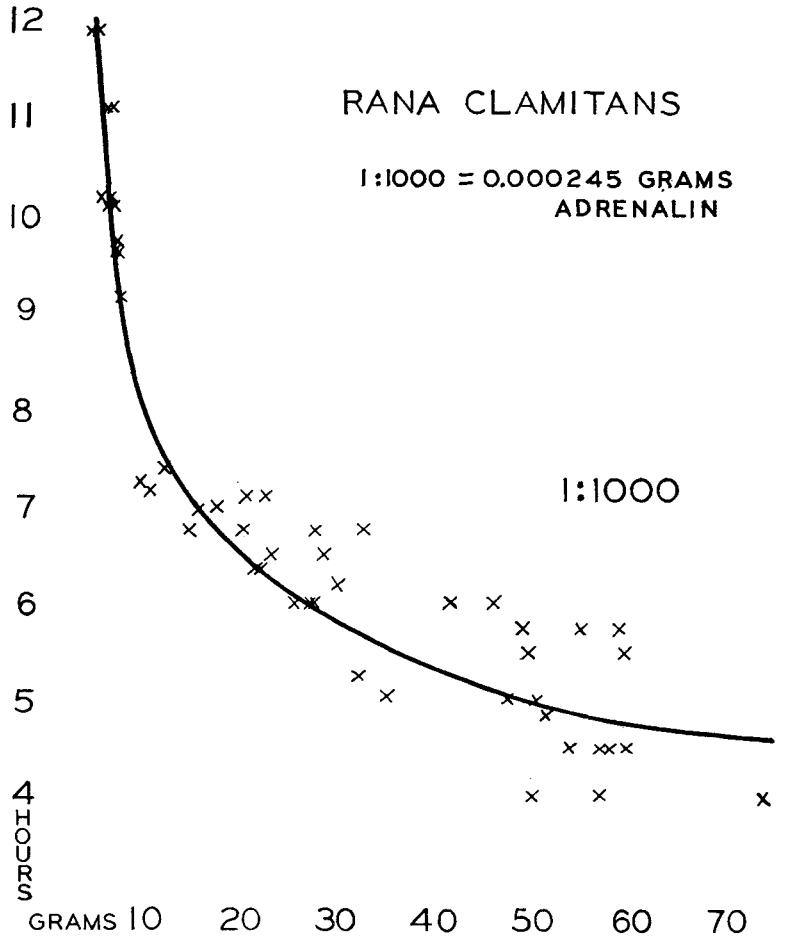

Fig. 1 Graph showing relationship between body weight of animal and the concentrated period of melanophore pigment, after injection of adrenalin.

The experiments with different concentrations of adrenalin, 1:5000, 1:10,000, and 1:50,000, such as had been tried on fishes and lizards (Pierce, '41), were not performed. It was discovered early in the work that the volume 0.25 cc. adrenalin of concentration 1:1000 was the smallest that would affect the 30 to 60 gm. frogs without killing the 6 to 10 gm. ones. Furthermore since the reaction of frog melanophores is vague and indecisive, the frog was not considered the most valuable material for extensive study in this direction.

A study of the graph (fig. 1) reveals a curious grouping of the animals into classes of small, medium, and large weights. Throughout the experiments this characteristic bunching was evident. In fact it was impossible to collect frogs of the intermediate weights. Such observations indicate that within this frog population there were present at least three definite age groups.

TEMPERATURE

The usual temperature of the cages was 24° to 26°C. To test the response of the melanophores to cool temperatures, the frogs were placed in large jars which were packed in crushed ice. These jars were painted externally black or white, and illuminated as usual from above. The temperature could be maintained at 4°C. for many hours. At this low temperature the animals became very sluggish, in fact were practically anesthetized. In each experiment four animals were used, two pale and two dark, and the procedure was repeated many times. The following table shows the results.

TEMPERATURE °C.	FROG	BACKGROUND	
		White	Black
4	dark	dark	dark
4	pale	half dark	half dark

The change from the pale to the half dark stage required 9 hours, and no further change occurred although the experimental conditions were maintained for several hours longer.

To test the response of the melanophores to heat, the frogs were placed in boxes on a very hot day. Above 32°C. the frogs died. Between 26° to 32°C. the response of the melano-

phores was not uniform and no conclusions were reached. From general observations of the frogs in nature and in the laboratory, it appeared that heat had no striking effect upon melanophore response.

Hogben and Slome ('31) working on Xenopus, concluded that between 12° and 33°C. (which is the lethal limit) the temperature had little if any effect upon the color change; however, below 10°C. a definite darkening took place. The behavior of Rana clamitans is in accord with these results. Field ('31) showed that in Necturus both extremes, 2° and 30°C. brought full expansion of the melanophores, while intermediate temperatures resulted in moderate expansion only.

From these data it is concluded that in several amphibia, at least, low temperatures cause a dispersion of the pigment within the melanophores. Intermediate temperatures appear to have little if any effect.

In Rana clamitans the response of the melanophores to cold appears to be more effective than the response to light.

ABSENCE OF LIGHT

The reactions of the frog melanophores to absence of light (darkness) was next determined. Four pale and four dark frogs were placed in a photographic dark room at 25°C., and the same number in a refrigerator at 4°C. The results were most gratifying because of their uniformity. Dark animals remained dark and pale animals became dark. To make this change the pale animals required 36 hours at room temperature and slightly longer, 48 hours, at 4°C.

Reactions of the frog melanophores to heat were not attempted.

The results of the present experiments are at variance with those of Kropp ('27, p. 311) who reported that frogs behaved as did Fundulus, namely exhibiting a pale state in the absence of light. The results are more nearly in agreement with several other workers on Amphibia who report a half dark or intermediate phase under such conditions (Field, '31; Hogben and Slome, '31, p. 17; Parker, Brown and Odiorne,

'35, p. 453). More recently Parker and Scatterty ('37, p. 302) reported that the dark phase of Rana pipiens could be approximated by placing the frog in complete darkness. This reaction of the melanophores is in agreement with the responses observed in Rana clamitans. Since most of the data presented admit a partial if not maximum darkening of the amphibian integument in the absence of light, it may be concluded that under such conditions the melanophores tend to disperse their pigment in degrees varying from an intermediate to a completely dispersed state.

ENUCLEATED FROGS

Three frogs were enucleated. This operation can be easily and quickly performed when cracked ice is used as the anesthetic. The animals were permitted to recover slowly. After a day or two in a large cage in which they hopped about quite normally, these eyeless individuals were placed on black and on white backgrounds. Frogs of intermediate shade placed on a black illuminated background reached their darkest shade in a minimum of 8 hours. Occasionally they required longer. Two frogs showed a reticulate stage and one a stellate stage of the melanophores. This response was definite, if slower, than the normal time. These same frogs were then placed upon an illuminated white background. The melanophores reached the stellate or slightly stellate punctate stage after an exposure of 24 hours, and longer exposure brought about no further concentration of the pigment.

These results agree substantially with Kropp ('27). He concludes that blinded animals undergo color changes, but the range of change is small compared to the normal animal. He also maintains that the chromatic change is often accomplished with a lag which is hard to measure. The present results, although based on only three animals, agree also with the findings of Parker, Brown and Odiorne ('35), who concluded that extreme fluctuations of enucleated frogs were far short of normal ones on either a black or a white background; and with Hogben and Slome ('31) who report that eyeless

animals display neither maximum pale nor dark conditions of the skin.

ANESTHETICS

As a final experiment the reaction of the melanophores to chloroform, ether, and chloretone was tested. Even small quantities of chloroform proved fatal to the frogs which died in a pale state. This observation appears to support Biedermann's statement (1892) that chloroform may paralyze the melanophores and thus prevent a dispersion of pigment. A few drops of ether in the jar anesthetized four pale and four dark animals slowly. The pigment of the melanophores was dispersed in 20 minutes, and, although the animal was removed to tap water, the pigment remained dispersed for an hour. This reaction time is much slower than that given by Kropp ('27, p. 301). It may be that in the present experiments, the melanophores were partly paralyzed, for although the pale animals became dark, the melanophores never reached the maximum stage of dispersion of pigment. Chloretone gave by far the most uniform and satisfactory results. In a 0.3% solution the melanophores dispersed their pigment fully in the course of 1½ to 2 hours and remained in this condition for 2 or more hours. This darkening process continued after the animals were placed in tap water, and on a white background. Long after recovery from the anesthetic the animals were dark. For Rana clamitans chloretone gave the most uniform and clear-cut results, yet Kropp ('27) reports no reaction of the melanophores of adult frogs to this drug. He did, however, observe complete dispersion within tadpole melanophores.

Noxious stimuli upon the frogs, such as teasing, irritating with a needle, or shock from an electric current, were not tried. No pallor from handling was observed.

SUMMARY

1. When a frog is placed upon an illuminated white background, the melanophores of the integument usually concentrate their pigment within 10 to 12 hours.

2. When a frog is placed upon an illuminated black background, the melanophores disperse their pigment within 5 hours.

3. The reactions of frog melanophores lack the rapidity and precision of those of the teleost and the lizard.

4. After an injection of adrenalin, the reaction time for concentration of pigment is reduced to 1½ hours.

5. To the same dosage of adrenalin, the pigment of the smaller frog remains concentrated for a longer period than the pigment of the larger frog.

6. At a low temperature, 4°C., the pigment within the melanophores is dispersed. Dark frogs remain dark regardless of background. Pale frogs tend to assume an intermediate stage, regardless of background.

7. In absence of light, the pigment of melanophores is dispersed.

8. In enucleated frogs, the melanophores exhibit concentration and dispersion of the pigment with change in background, but fail to reach the maximum in either direction.

9. Upon immersion in a weak solution of ether the pigment of the melanophores becomes dispersed within 20 minutes, and remains so for about an hour.

10. Upon immersion in a 0.3% solution of chloretone, the pigment of the melanophores becomes fully dispersed within 1½ hours and remains so for 2 or more hours.

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PLATE 1

EXPLANATION OF FIGURE

2 Two specimens of Rana clamitans to show the dark and pale condition of the frog in response to black and white backgrounds. × ¾.

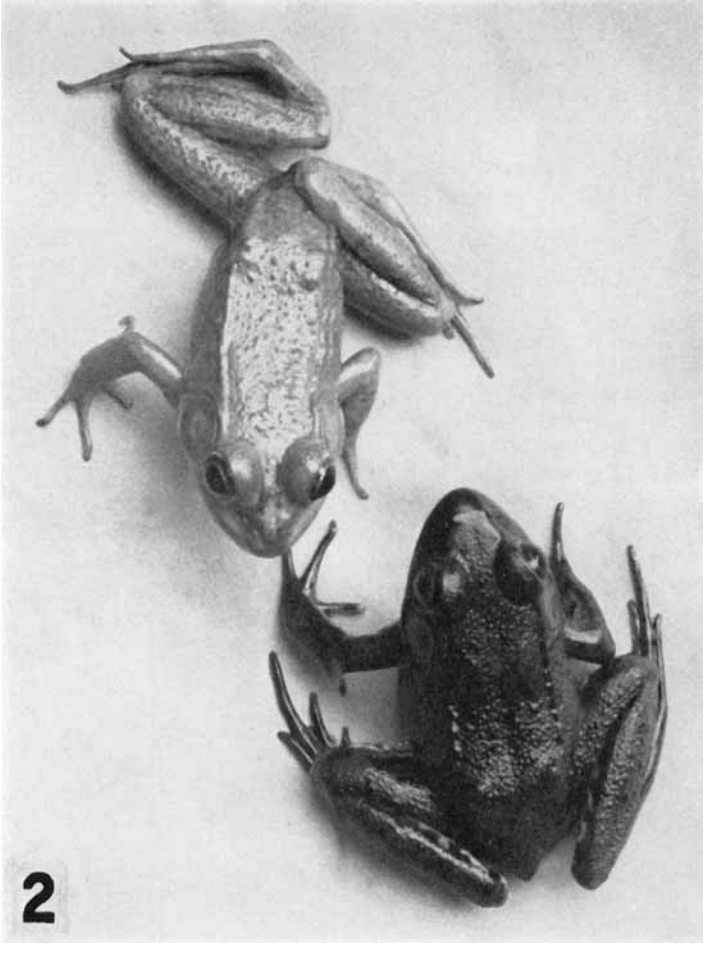
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PLATE 2

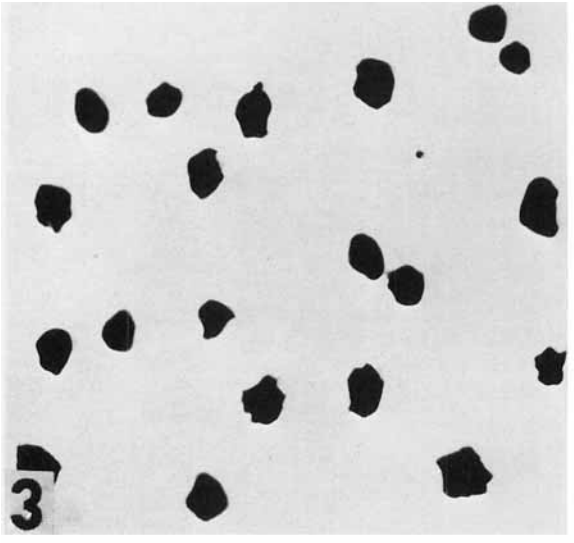
EXPLANATION OF FIGURES

3 Photomicrograph of the concentrated pigment of the melanophores of the integument, to show the response to white background. This piece of integument was removed from the web of the foot. × 130.

4 Same as figure 3. × 1020.

5 Photomicrograph of the dispersed pigment of the melanophores of the integument, to show the response to black background. This piece of integument was removed from the web of the foot. × 130.

6 Same as figure 5. × 1020.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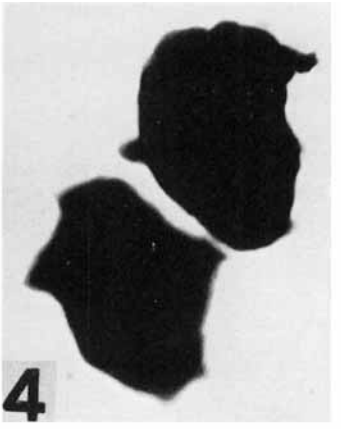
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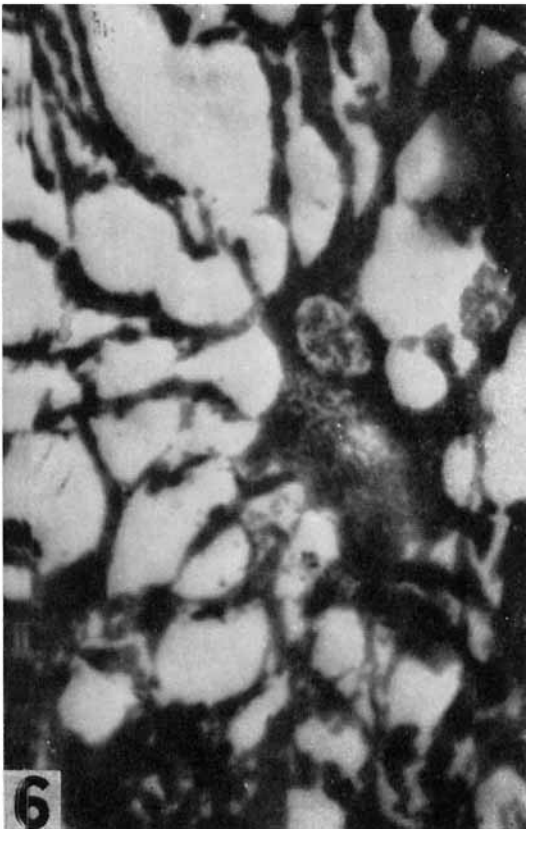
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