THE PIGMENTARY RESPONSE IN PHOXINUS LAEVIS

THE EFFECT OF BLOOD FROM A PATIENT WITH MELANOSARCOMA

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INTRODUCTION

The mechanism controlling the color changes which take place in a large variety of cold-blooded animals has been a subject of investigation for years, but only recently has the dominance of the pituitary in regulating these changes been recognized.

Smith (1) in 1916 and Allen (2) in 1917 showed that the removal of the pituitaries of tadpoles resulted in a permanent state of contraction of the melanophores so that "albino" animals were produced. The dark coloration of these animals could be restored by the implantation of pituitary glands. Swingle (3) in 1921 found that similar darkening could be produced by implanting only the intermediate lobe of the pituitary. Hogben and Winton (4) observed marked melanophore expansion following the injection of posterior pituitary extracts in frogs and claimed that this was a specific response to a pituitary substance. They showed that the active substance was not identical with the pressor factor but that it resembled the oxytocic principle. Subsequent work by Fenn (5), Dreyer and Clark (6), Hogben and Gordon (7), Rowe (8), and Stehle (9) has shown that the melanophore-expanding substance is a specific one differing from both oxytocic and pressor factors.

This substance produces darkening in reptiles and amphibias by expansion of the melanophores. In fish this is not the case. Spaeth (10) has shown that in the fish studied by him the action was one of melanophore contraction.

The response of melanophores in frogs has been shown to be non-specific since substances other than pituitary extracts will cause melanophore expansion. Spaeth found, in the study of isolated fish scales, that changes in the pH of the medium and alterations in the concentrations of certain ions have a decided influence on the melanophores. It has been found, also, that such drugs as quinine, yohimbin, curare, and choline are very active in causing melanophore expansion. In view of the limitations of the melanophore response, Zondek and Krohn (11) proposed employing the nuptial coloration of the minnow Phoxinus laevis as a test for the pituitary substance. This species of minnow presents a very distinct seasonal variation in color. During the spawning season (May and June) there develops a striking color change, the so-called "wedding dress"; the dorsal surface of the fish, usually a dark green marked with black bands, becomes perceptibly paler, while the abdomen changes from a pearly white into a crimson red. This red coloration begins at the bases of the ventral and pectoral fins and spreads so as to
cover the whole abdomen, extending into the fins themselves and to parts of the head. Zondek and Krohn (11) showed that this red coloration could be promptly produced by pituitary extracts, and they named the active pituitary substance intermedin. They stated that it could be found in the pituitary gland and the wall of the third ventricle but nowhere else in the body, and that it was present in highest concentration in the intermediate lobe of the pituitary. No other substance was found which would give this reaction.

The identity of Zondek's intermedin with the melanophore-expanding principle has been questioned by Sulzberger (12), who found that there was no correlation between the minnow potency of an extract and its efficacy in causing melanophore expansion. In the specificity of its response to this pituitary substance Phoxinus laevis appears to be unique among fish. Thus Saphir (13), working with Chrosomus erythrogaster, a fish very similar to the minnow, found that yohimbine hydrochloride would give the chromatophore reaction; this and other similar drugs have been shown to be active in producing the nuptial response in such species as Rhodeus amarus.

Although the pituitaries of higher animals have been found to contain large amounts of chromatophore-active substance, there has not as yet been established any function of this substance in the mammal. Sulzberger (14) reported a marked antidiuretic effect of Zondek's intermedin preparation in two cases of diabetes insipidus, but inconstant results have been obtained by others (15). Bushke (16) attempted to show an effect of this substance on the adaptability of the human eye to darkness, but without success.

Because of the discordant results which have been reported, the present study was undertaken in an attempt to establish the specificity of the minnow response and to investigate the possibility of its application to human clinical material.

Speculation upon the relation of the pituitary to human pigmentation has engaged the attention of several workers. Sugiura (17) investigated the effect of Zondek's intermedin upon the growth rate of mouse melanoma, and found a slight but distinct acceleration of tumor growth following injections of intermedin. He found, also, that extracts of mouse melanoma tissue gave a positive minnow response while extracts of other animal tumors did not. In view of these findings, it was decided to investigate human cases of melanosarcoma.

**METHODS**

A supply of Phoxinus laevis was obtained through the New York Aquarium. The fish were kept in large glass aquaria in running tap water to which environment they are well suited. During the tests they were placed in individual glass jars fed by the overflow water from the main tanks. The material to be tested was injected subcutaneously in aqueous solution with a fine hypodermic needle. The needle was inserted close to the caudal fin near the midline of the back and run in subcutaneously for about 1 cm. The volume injected varied, but 0.05 c.c. was found to be the most satisfactory amount, as it was well tolerated and did not leak out after withdrawal of the needle.
PLATE I

FIG. 1a. LATERAL VIEW OF PILOXINUS LAEVIS IN ITS NORMAL WINTER COLORATION
FIG. 1b. VENTRAL VIEW OF NORMAL WINTER COLORATION

FIG. 2a. MELANOPHORE EXPANSION PRODUCED BY EXPOSURE TO A 1:1,000,000 SOLUTION OF YOHIMBIN HYDROCHLORIDE
FIG. 2b. MELANOPHORE CONTRACTION PRODUCED BY INJECTION OF 1/40 C.C. OF 1:1,000 ADRENALIN CHLORIDE SOLUTION

FIG. 3a. INTERMEDIIN REACTION, LATERAL VIEW
FIG. 3b. INTERMEDIIN REACTION, VENTRAL VIEW
Tissues were extracted by mincing finely and boiling for five minutes with five volumes of 0.25 per cent acetic acid, according to the method of Zondek and Krohn. Blood was treated with five volumes of acetone and the precipitate washed with acetone and dried. The dry powder was then extracted with 0.25 per cent acetic acid. The filtrates from the acid digests were partially neutralized before injection; they may be concentrated by evaporation in vacuo. Complete neutralization of the acid extracts results in the formation of a heavy precipitate which carries down the active substance, which can be quantitatively recovered by redissolving the precipitate in dilute acid.

Although the same fish could be used many times it was found that certain fish developed an immunity after a number of weeks of injection so that they could not be made to respond again even to potent extracts.

Results

A number of endocrine substances were tested for their effects; ketohydroxyestrin, amniotin (Squibb), synthetic progesterone (proluton Schering), pregnancy urine extract (follutein Squibb), and testosterone gave no response in the doses tolerated. Acid extracts of normal tissues, brain, liver, kidney and muscle of normal human blood and urine gave negative results. Drugs known to effect the melanophores of other animals were tested and although some of these caused marked changes in the color of the dorsal region of the fish, none had any potency in evoking a reddening of the ventral surface. Thus yohimbin hydrochloride caused a prompt and very marked darkening of the head and dorsal regions, later extending downward toward the ventral surface and outward into the fins. This reaction could be produced by exposing the fish to a one in one million solution in the aquarium water. The reverse effect was produced by the injection of adrenalin, the resulting pallor becoming so marked that the fish was distinctly translucent (Figs. 2a and 2b, Plate I).

Commercial pituitary extracts were also tested. The anterior lobe preparations were much less active than were the posterior lobe extracts. Surgical and obstetrical pituitrin, in addition to evoking the specific response, caused a marked pallor of the dorsal region. These posterior lobe preparations gave the specific response in higher dilutions than the anterior lobe preparations. Nevertheless, injections of 0.05 c.c. of the following anterior lobe preparations gave a positive response: anterior-pituitary liquid (Squibb), phyone (Wilson), and prolactin (Squibb).

Blood from the patient whose history appears below gave a positive minnow response when the equivalent of 0.01 c.c. was injected (Figs. 3a and 3b, Plate I). Attempts to demonstrate the active substance in the urine yielded negative results.

Case History

The patient was a white female aged thirty-two. A little over three years ago a small nodule was burned off the right thigh with the electric needle. The lesion recurred promptly and was excised. The microscopic examination showed a malignant pigmented mole. Following this an excision of the entire area was performed and the inguinal lymph nodes were
Fig. 4. Recurrent Melanosarcoma of the Thigh in a Woman of Thirty-Two

Fig. 5. Photomicrograph of the Tumor Shown in Fig. 4
resected. This was in 1932. There was no further trouble until about November 1935, when the scar, which had been gradually thickening, showed pink elevated areas. These areas showed, on microscopic study, recurrence of the original growth. Following excision, there was local recurrence, as well as recurrence in the inguinal region, progressing rapidly. The right leg became swollen and painful, due to the venous blockage, and the recurrence at the site of the scars broke down and ulcerated.

The accompanying photographs (Figs. 4 and 5) show the condition of the leg at the time of admission to the hospital and the microscopic structure of the tumor. In the next few months, although irradiation was given, the tumor continued to spread over the surface of the thigh and to extend beneath Poupart's ligament into the abdominal cavity. In spite of the rapid increase in size of the masses above and below Poupart's ligament, the patient remained free of any signs of distant metastases and suffered only from pain and discomfort produced by mechanical pressure of the tumor on the vessels of the leg and the intrapelvic organs.

**Summary and Conclusions**

1. The effects of a number of drugs, endocrine preparations, and tissue extracts upon the coloration of the minnow (*Phoxinus laevis*) have been investigated.

2. Extracts of the pituitary resulted in the development of the nuptial coloration, as did the blood from a patient with melanosarcoma, but no other substance tested gave the response.

3. It would appear that the minnow response is specific for a hormone of the intermediate lobe of the pituitary, and that this hormone was present in the blood of a case of melanosarcoma.

4. Further study of a number of similar cases is necessary before the significance of these findings can be established.

**Note:** In the Report of Activities during 1935 of the International Cancer Research Foundation, Ferguson and his co-workers refer to the distribution of intermedin in normal and neoplastic blood and tissues, stating that they are continuing work based on Zondek's experiments with the middle lobe of the pituitary. They state that intermedin has been found in large amounts in melanoma bloods and has been of value in diagnosis in disputed cases. However, no specific cases or data are cited.

**Bibliography**


