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Occurrence of Leucophores on the Caudal Fin of
the Fish, *Oryzias latipes*, following Administration of
Androgenic Steroids

With 1 Text-figure

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Okada and Yamashita (1944) have pointed out that, in the male of the fresh water teleost, *Oryzias latipes*, many leucophores appear along the distal border of the caudal fin during the breeding season, while in the female, the caudal fin is usually devoid of leucophores. The sexual difference in the degree of development of the caudal-fin leucophores²⁾ has been confirmed by several investigators. However, no experiments have been carried out to clarify the hormonal conditions governing the development of these leucophores. A preliminary experiment demonstrated that treatments with minute amounts of androgen provoked the appearance of leucophores on the caudal fin of the fishes in sexually inactive seasons. This stimulated the authors to conduct series of experiments in order to analyse the relation between the leucophores and androgenic substances in detail.

In the present paper, observations on leucophores in normal fish and those under various experimental conditions, and the results of studies on the effect of several hormonal steroids on these cells are briefly dealt with.

Before going further, we wish to express our hearty thanks to Prof. Kiyoshi Takewaki for his valuable criticism and encouragement.

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2) These leucophores were called guanophores by Okada and Yamashita (1944). But, since the chemical nature of the granules in these cells has not yet been determined, they are referred to as leucophores in this paper.

OBSERVATIONS AND EXPERIMENTS

Observations on normal fish The number of leucophores along the distal boader of the caudal fin was counted in normal specimens, 14-36 mm in standard length, from two different stock colonies of the red variety of *Oryzias latipes*, which had been kept in large aquaria under standard conditions. The counts were made in anesthetized fish by means of a dissecting microscope. The first examination was done on September 21 and 22, 1960, and the second on May 24, 1961.

From the results of the observations given in Figure 1 and Table 1, it seems evident that, (1) although the number of leucophores varies considerably among different individuals, there is a marked sexual difference in the number of the

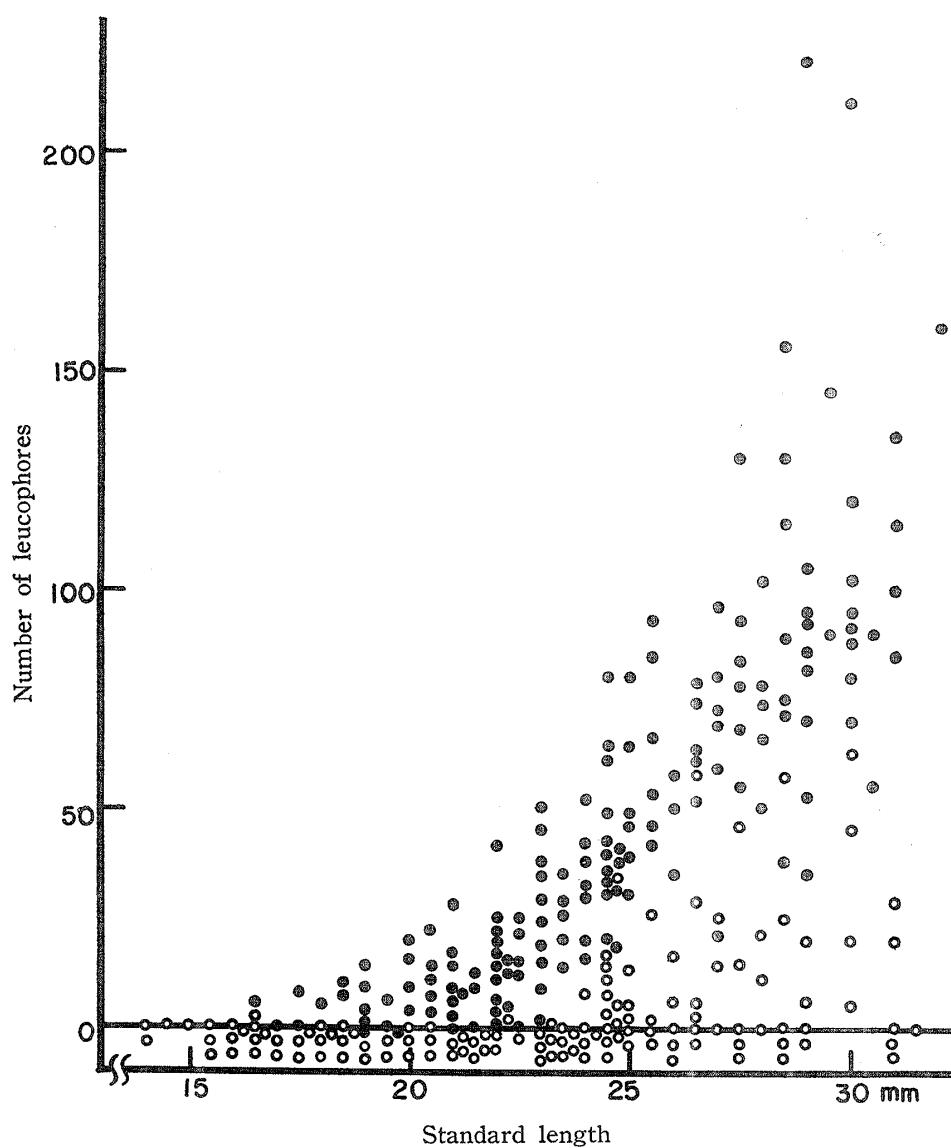


Fig. 1. Sexual difference in number of leucophores on the caudal fin in intact fish of various body lengths. solid circles: males; empty circles: females.

Table 1

Mean number of leucophores on the caudal fin of female fish of varying lengths in the breeding season

Standard length (mm)	Male	Female
27.0 — 28.9	89.0 (1)	0.0 (3)
29.0 — 30.9	128.6 (5)	6.0 (2)
31.0 — 32.9	157.4 (17)	13.8 (9)
33.0 — 34.9	130.7 (6)	20.3 (36)
35.0 — 36.9	98.0 (1)	25.5 (18)

Figures in parentheses denote the number of fishes examined.

cells, the males exhibiting more numerous leucophores than the females of comparable size, (2) in the males, the number of leucophores is increased with the size of the fish, and (3) leucophores may occur in considerable number on the caudal fin in some large females. In order to ascertain seasonal changes in the number of leucophores, the caudal fins of 20 males and 20 females from the same colony, 25–30 mm in standard length, were examined in January, March, April, July, August, October and December, 1960. Table 2, summarizing the results, shows that in winter leucophores disappear almost completely, while in summer they are maximally increased in number.

Table 2

Seasonal changes in the number of leucophores on the caudal fin of normal fishes

Month	Mean No. of leucophores	
	male	female
January	0.0	0.6
March	8.3	0.0
April	44.7	3.8
July	86.4	6.9
August	72.5	11.3
October	52.0	8.4
December	8.3	0.2

Castration in the males In the spring of 1960, a small number of males having a well-developed group of leucophores on the caudal fin were castrated. In 2 individuals surviving for a period of about one month, the leucophores disappeared almost completely.

On May 18 and 19, 1961, another group of males 25–32 mm in standard length, having many leucophores on the caudal fin were castrated. Four of 8

castrated males which survived until August 9 had no leucophores on the caudal fin. One of the 4 fishes was carefully dissected, but no fragment of testicular tissue was found in the body cavity. In the remaining 4 fishes showing varying number of leucophores (55–216) on the caudal fin, fragments of testicular tissue were found in their body cavity. From this result, it seems likely that leucophores on the caudal fin of the males are maintained by the testis.

In addition, a number of females having leucophores on the caudal fin were ovariectomized, but the results were inconsistent as will be reported elsewhere.

Treatment of castrated males with testosterone In this series of experiments, androgen-containing water was made by adding desired amounts of a propylene glycol solution of testosterone (1 mg per ml) to tap water.

On August 9, 2 castrated males showing no leucophores on the caudal fin were transferred into water containing 500 μg of testosterone per liter. On August 15, examination of the caudal fin of each of the fish revealed 29 and 30 leucophores, respectively.

Treatment of female fish with testosterone On April 14, 6 groups of 3 female fish, 28–30 mm in standard length, were placed in water containing 500, 62.5, 7.8, 2.0, 0.5 and 0.3 μg of testosterone per liter, respectively. The method of application of androgen to the fish was the same as that in the preceding experiment. A group of 3 female fish kept in ordinary tap water served as control. Temperature during the experimental period was 26–27°C. At the commencement of the experiments, no leucophores were found on the caudal fin in all of the fish.

Table 3
Mean number of leucophores produced following
treatment with testosterone

Concentration ($\mu\text{g/l}$)	Date of observation	
	April 19	May 2
500.0	84.3	107.6
62.5	80.0	71.7
7.8	71.3	57.7
2.0	42.0	50.7
0.5	29.7	42.3
0.13	3.3	24.0
0.0	23.3	27.7

On April 19 and May 2, the caudal fins were carefully examined for leucophores. The results summarized in Table 3 indicated that testosterone at the concentrations higher than 0.5 μg per liter initiated the occurrence of leucophores on the caudal fin and that the leucophores were increased in number with the concentrations of testosterone. Leucophores occurred on the caudal fin earlier

than did papillary processes³⁾ on the rays of the anal fin of the same fish. Moreover, the threshold concentration of testosterone for inducing the occurrence of leucophores was lower than that for causing the production of the papillary processes.

Treatment of females with steroids other than testosterone In August of 1960, 6 groups of 5 females, 32–35 mm in standard length, were transferred to water containing 100 μg and 500 μg of cholesterol, 100 μg and 500 μg of estrone, and 100 μg and 500 μg of progesterone per liter, respectively. The experiments were carried out at room temperature. The method was similar to that in the preceding experiments. When examined at the end of a period of 2 weeks, the fish showed neither papillary processes on the anal fin nor leucophores on the caudal fin.

Table 4a

Mean number of leucophores produced following treatment with testosterone, testosterone propionate, dehydroisoandrosterone or methylandrostenediol

Steroid	Concentration ($\mu\text{g}/\text{l}$)				
	6400	1600	400	100	0
methylandrostenediol	220.0	124.5	150.7	69.0	—
testosterone	—	105.3	65.0	38.9	—
testosterone propionate	—	96.5	26.5	27.0	—
dehydroisoandrosterone	31.3	5.3	11.2	9.3	—
none	—	—	—	—	6.2

Table 4b

Mean number of papillary processes produced following treatment with testosterone, testosterone propionate, dehydroisoandrosterone or methylandrostenediol

Steroid	Concentration ($\mu\text{g}/\text{l}$)				
	6400	1600	400	100	0
methylandrostenediol	25.5	46.3	51.0	40.5	—
testosterone	—	26.8	0.0	0.0	—
testosterone propionate	—	8.0	0.0	0.0	—
dehydroisoandrosterone	7.3	18.0	0.0	0.0	—
none	—	—	—	—	0.0

3) It is well established that the papillary processes are normally present on the anal-fin rays in adult male specimens of *Oryzias latipes* but not in females. They are androgen-dependent male sexual characters (Oka, 1931; Egami, 1954).

In May and June of 1961, 4 other groups of 4 female fishes, 33-35 mm in standard length, were placed in water containing 10 μ g and 100 μ g of desoxycorticosterone acetate, and 10 μ g and 100 μ g of cortisone acetate, respectively. After a 3-week period, it was found that the steroids had no effect on the caudal fin.

On August 19, 1961, 14 groups of 4 female fishes were transferred to 50 ml of water containing either testosterone, testosterone propionate, dehydroisoandrosterone or methylandrostenediol, at different concentrations (Table 4). A group of 4 female fish placed in ordinary tap water served as control. All the groups were kept at room temperature. On August 24, the number of leucophores produced on the caudal fin, and on September 6, that of papillary processes on the rays of the anal fin were counted in all of the specimens.

The results, presented in Table 4, indicate that (1) both the leucophores and papillary processes occurred following treatment with any of these androgenic steroids, (2) leucophores on the caudal fin were increased in number with the concentration of the steroids, (3) in stimulating the occurrence of leucophores, methylandrostenediol is the most effective of the 4 steroids tested, and dehydroisoandrosterone the least, and (4) the doses necessary to produce papillary processes on the anal fin are larger than those needed for producing leucophores on the caudal fin.

Another series of experiments carried out between September 13 and 18, 1961, with females of *Oryzias latipes*, yielded similar results (Table 5). In this series, the fish exhibited a much higher mortality than in the preceding series but the results of the two series were in good agreement with each other, showing that methylandrostenediol is the most effective of the steroids tested in inducing leucophores on the caudal fin.

Table 5

Mean number of leucophores and papillary processes in female fishes produced following treatment with testosterone or methylandrostenediol

Character	Concentration of methylandrostenediol (μ g/l)		Concentration of testosterone (μ g/l)	
	100	1.6	1000	100
Leucophores	96.0	94.0	8.0	0.0
Processes	32.5	20.0	0.0	0.0

DISCUSSION

Among the secondary sexual characters of *Oryzias latipes* hitherto reported, the occurrence of leucophores on the caudal fin is one of unique nature: (1) the leucophores occur not only on the fin of adult males but also on that of large-sized females, and (2) the cells are maximally developed in summer but completely obliterated in winter.

The results given in the present paper make it probable that androgen is

responsible for the occurrence and maintenance of the leucophores. Masuda (1952, 1953) has already reported the disappearance of the caudal-fin leucophores in castrated males and the appearance of the cells in spayed females bearing testicular transplants. Therefore, it seems likely that the occurrence of leucophores on the caudal fin in large females is also caused by some androgenic substance secreted from the ovary and/or the adrenocortical tissues of the females. Recently we have obtained an extract from the loach testes which is active in inducing the occurrence of leucophores on the caudal fin in the females of *Oryzias latipes*.

It is interesting to notice that methylandrostenediol which is much less androgenic than testosterone when tested on warm blooded vertebrates is decidedly more potent in causing the occurrence of leucophores on the caudal fin of the females. Since 1.6 μg per liter water of methylandrostenediol is more active than 1600 μg per liter of testosterone, the former is at least 1000 times more potent than the latter in the effect in question.

Several workers have tested the activity of different androgenic steroids in fish. Turner (1942) reported that methyltestosterone was about twice as effective as ethinyltestosterone on the production of the gonopodium-like structure in *Gambusia*. Tsuneyoshi (1959, 1960) also showed that in the females of *Oryzias latipes*, papillary processes were produced on the rays of the anal fin if they were kept in water containing either methyltestosterone, ethinyltestosterone or testosterone. According to this author, of the 3 steroids, methyltestosterone was the most active in this respect, and testosterone the least. The results reported by Eversole (1941), Régnier (1941) and Turner (1941) make it likely that ethinyltestosterone which is less androgenic than testosterone propionate in higher vertebrates, much more potent than testosterone propionate in fish.

Although several investigators have obtained extracts from teleostean testes which are slightly effective in stimulating the growth of comb in capon of chick (Hazleton and Goodrich, 1937; Potter and Hoar, 1954), the nature of the active substance has not yet been worked out (Potter and Hoar, 1954). On the other hand, administration of testicular extracts from fishes sometimes yielded only ambiguous results in mouse, rat and chick (see Pickford and Atz, 1957). All these results seem to suggest that androgen elaborated by the fish testes is different from that produced by the testes of birds and mammals.

SUMMARY

1. In the adult males of the teleostean fish, *Oryzias latipes*, many leucophores occur along the distal border of the caudal fin during the breeding season, extending from April to September. The females usually lack the leucophores, although large-sized females may occasionally exhibit them during the summer months. In winter the leucophores disappear almost completely in both males and females.

2. In the male, the leucophores disappeared following castration and reappeared following treatment with testosterone.

3. Cholesterol, progesterone, estrone, cortisone acetate and desoxycorticos-

terone acetate were ineffective in increasing leucophores on the caudal fin in female fish. Testosterone, testosterone propionate, dehydroisoandrosterone and methylandrostenediol caused a marked increase in number of the leucophores. Methylandrostenediol was at least 1,000 times more active than testosterone in producing leucophores.

REFERENCES

- Egami, N. 1954 Annot. Zool. Japon., **27**, 122.
Eversole, W. J. 1941 C. R. Acad. Sci., Paris, **215**, 537.
Hazleton, L. W. and F. J. Goodrich 1937 J. Am. Pharmacol. Assoc., **26**, 420.
Masuda, A. 1952 Res. Rep. Kōchi Univ., **1** (7), 1.
——— 1953 Bull. Fac. Educ. Kōchi Univ., **1** (3), 49.
Oka, T. B. 1931 J. Fac. Sci., Imp. Univ. Tokyo., IV, **2**, 209.
Okada, T. and H. Yamashita 1944 J. Fac. Sci., Tokyo Univ., IV, **6**, 383.
Pickford, G. E. and J. W. Atz 1957 The physiology of the pituitary gland of fishes. New York.
Potter, G. D. and W. S. Hoar 1954 J. Fish. Res. Board Canada, **11**, 63.
Régner, M. T. 1941 C. R. Acad. Sci. Paris, **215**, 537.
Tsuneyoshi, M. 1959 J. Fac. Educ. Kagoshima Univ., **11**, 35.
——— 1960 *ibid.*, **12**, 53.
Turner, C. L. 1941 Biol. Bull., **80**, 371.
——— 1942 Physiol. Zool., **15**, 263.