both the inertia of the locomotory appendages and the resistance to their movement in a viscous medium are power functions of the velocity of limb movement. Such nonlinear mechanical constraints are presumably especially significant at higher velocities of locomotion. Third, under natural circumstances the optokinetic control system can probably be overridden by other sensory and central influences (*7*). Finally, the gain of the optokinetic control system, defined as the ratio of the output velocity (the speed of attempted locomotion) to the input velocity (the treadmill speed) and computed from the data presented in Fig. 2, is less than unity (*8*). Under such circumstances the output of a positive-feedback control system is stable (*4*).

We have demonstrated a new class of optokinetic responses, distinguished from previously known optokinetic responses by their utilization of positive rather than negative visual feedback. Our data show that within a given species the responses involve more than one motor system and are sufficiently strong to play a major role in sustaining and reinforcing normal locomotory behavior. Our studies on several species of arthropods, as well as suggestive findings of other investigators (*9*), indicate that positive-feedback optokinetic responses are of widespread occurrence in the animal kingdom.

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8. The overall gain of the optokinetic control system over the entire range of input velocities in Fig. 2 is 0.387. To obtain this value we assumed that the retraction velocity of a walking leg of a restrained lobster is equivalent to the locomotory (output) velocity. The average output velocity corresponding to each treadmill speed (input velocity) could then be computed directly from the data in Fig. 2. The mean gain (output velocity/input velocity) was then determined for each input velocity, and these were averaged to obtain the overall gain.
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23 March 1972; revised 17 May 1972

Daily Variation in Concentration of Cortisol in Plasma in Intact and Hypophysectomized Gulf Killifish

Abstract. *A daily variation in concentration of cortisol in plasma is synchronized by a 12-hour daily photoperiod in intact as well as in hypophysectomized fish. The daily rhythm in concentration of the adrenal steroid does not depend on a daily rhythm in the concentration of pituitary adrenocorticotropic hormone.*

The adrenocortical (interrenal) tissue of teleost fish is stimulated by adrenocorticotropic hormone (ACTH) secreted by the anterior lobe of the pituitary gland. Hypophysectomy results in a decrease in the plasma concentration of cortisol, the major corticosteroid in poeciliid fishes (*1*, *2*), and a regression of the adrenocortical tissue which can be alleviated or prevented by administration of mammalian ACTH (*3*). Daily rhythms in the concentration of plasma glucocorticoids have been reported for teleosts (*4*), as they have been for mammals (*5*) and birds (*6*). Although it was not investigated in teleosts, many of the daily rhythms of adrenal steroids in mammals are synchronized by the photoperiod (*5*). The rhythms in concentration of the glucocorticoids in plasma are thought to be direct consequences of daily rhythms of release of ACTH. If this assumption is correct for teleosts, hypophysectomy should not only cause a reduction in overall concentrations of cortisol in plasma, but it should also result in a loss of the daily rhythm. We demonstrate here that the presence of the pituitary is not required for the maintenance of a daily rhythm in the concentration of cortisol in plasma, nor for the photoperiodic synchronization of the rhythm in a teleost fish.

We studied intact and hypophysectomized male Gulf killifish *Fundulus grandis* (*7*). One group of intact fish and one group of hypophysectomized fish were maintained on a 12-hour daily photoperiod with light beginning at 0800 (series 1), whereas another group of intact fish and a group of hypophysectomized fish (series 2) were subjected to an inverted photoperiod of 12 hours, with light beginning at 2000 on 2 November 1971. After 15 days of acclimatization, the fish were killed at one of several times of day (4, 8, and 12 hours after the onset of light). The times were chosen on the basis of direct (*8*) and indirect evidence (*9*), which

Table 1. Concentration of cortisol in plasma of intact and hypophysectomized adult male *F. grandis*. Results are expressed as means ± standard error. Numbers in parentheses indicate number of fish studied. The quadratic curve of results for each of the four groups of fish of the two series follows a similar temporal pattern, whether a comparison is made between intact and hypophysectomized fish of series 1 versus intact and hypophysectomized fish of series 2, or between intact versus hypophysectomized fish in each series.

Group	Cortisol in plasma (micrograms per 100 ml) (hours after onset of light)		
	4	8	12
	Series 1; light from 0800 to 2000		
Intact	7.43 ± 2.23 (7)	19.50 ± 5.88 (6)	8.70 ± 3.32 (5)
Hypophysectomized	5.19 ± 1.21 (8)	14.29 ± 4.06 (7)	8.14 ± 3.32 (7)
	Series 2; light from 2000 to 0800		
Intact	19.42 ± 3.85 (6)	49.17 ± 8.04 (6)	21.08 ± 4.66 (6)
Hypophysectomized	5.0 ± 1.41 (6)	13.20 ± 2.42 (5)	7.75 ± 2.78 (4)

indicate that the daily increase in concentration of cortisol in plasma in male *F. grandis*, maintained on daily photoperiods of about 12 hours, occurs about 8 hours after the onset of light. The concentration of cortisol in plasma was determined by the corticosteroid-binding globulin (CBG) method (*10*).

There was a distinct variation in the concentration of cortisol in plasma in the intact fish (Fig. 1 and Table 1). High concentrations were found at 8 hours, and low concentrations at 4 and at 12 hours after "dawn." Because the temporal patterns in cortisol concentrations were similar in the groups maintained on the "normal" 12-hour photoperiod (0800 to 2000), and on the inverted photoperiod (2000 to 0800), it is probable that the daily variation in cortisol concentrations is synchronized by the daily photoperiod.

Although we verified the completeness of hypophysectomy (*11*), daily variations in concentrations of cortisol in plasma were present in the hypophysectomized fish (Fig. 1 and Table 1). Moreover, the high concentrations occurred 8 hours after the onset of light, as they did in the intact fish. The similarity of temporal pattern in series 1 (light from 0800 to 2000) and series 2 (light from 2000 to 0800) indicates that the variation in cortisol concentrations is also synchronized by the daily photoperiod in hypophysectomized fish. The differences and variations cited are highly significant ($P < .01$), according to an analysis of variance.

Our interpretations are based on the assumption that cortisol is the major circulating corticosteroid in *F. grandis*, as was reported for *Fundulus heteroclitus* by Liversage and co-workers (*2*). They compared the CBG method with fluorometric and double-isotope derivative methods in *F. heteroclitus*, and found that the three methods produced comparable results. A possible interference by sex steroids (*10*) would seem to be unlikely in hypophysectomized fish.

As in *F. heteroclitus* (*2*), the concentrations of cortisol in plasma are lower in hypophysectomized *F. grandis* than they are in intact fish, but the reduction is only partial. Because the target organs of the hypophysis regress slowly in poikilotherms after hypophysectomy, ACTH is probably necessary for the long-term maintenance of adrenal function. The retention of adrenal function in the hypophysectomized fish over the short term has the advantage of making it possible to demonstrate the existence

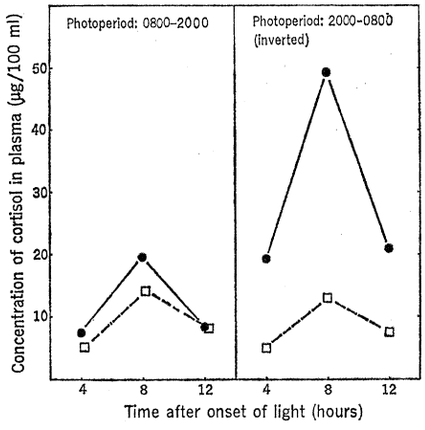

Fig. 1. Daily variation in concentration of cortisol in plasma of intact (circles) and hypophysectomized (squares) *F. grandis* at two 12-hour daily photoperiods (light from 0800 to 2000, and from 2000 to 0800).

of another factor in the control of the daily rhythm of cortisol in plasma that is not dependent on the hypophysis.

The concentrations of cortisol in plasma in the intact fish were higher in series 2, in which the photoperiod was inverted (light from 2000 to 0800) as compared to those in series 1 (light from 0800 to 2000). The readjustment of an animal to an inverted photoperiod might constitute a stress that is expressed as a prolonged elevation in the concentration of cortisol in plasma. This reaction did not occur in the hypophysectomized fish on an inverted photoperiod. Donaldson and McBride (*12*) reported that stress produces an increase in the concentrations of cortisol in plasma in the intact, but not in the hypophysectomized, *Salmo gairdnerii*. These results point out the importance of not disturbing the fish before taking the blood for analysis, although the disturbance of handling had only a small effect in raising the concentrations of cortisol in plasma in intact *F. grandis* (*7*). Because the plasma concentrations of cortisol were only slightly lower in the hypophysectomized fish as compared to the intact fish in series 1, we believe that our methods of taking blood did not result in artificially high concentrations of cortisol in plasma as a consequence of stress.

Although the stress response seems to depend on the presence of the pituitary, the daily rhythm in plasma concentration of cortisol does not. The phases of the daily variations with respect to the photoperiod are similar in the intact and in the hypophysectomized fish, and the rhythms in both are synchronized by the photoperiod. The daily variation in concentration of cortisol in plasma apparently does not depend on a driving rhythm of pituitary ACTH, even in the intact fish.

The daily rhythm in concentrations of adrenal steroids in plasma, it appears, is important in the regulation of many physiological events in vertebrates, including the control of fat storage and reproduction in diverse vertebrate species, the timing of developmental stages in amphibians, and the control of seasonal conditions in a migratory sparrow (*13*). In *F. grandis* maintained in continuous light, injections of cortisol entrain daily rhythms of fattening responses to prolactin (*14*). In some temporal patterns, administration of the two hormones causes rapid increases in body fat stores, whereas administration in other temporal patterns causes losses of body fat. Inasmuch as a daily photoperiod also entrains a daily rhythm of fattening response to prolactin in fish (*9, 15*), Meier and co-workers (*14*) proposed that the adrenocortical (interrenal) hormones are important mediators of the synchronizing system that is set by the photoperiod. Because a daily rhythm in concentration of cortisol in plasma is present in hypophysectomized *F. grandis*, the persistence of a daily rhythm of fattening responses to prolactin in hypophysectomized fish maintained on a daily photoperiod (*15*) cannot be considered evidence that the adrenal steroids are not involved in setting these rhythms. Whether or not the persistence of daily rhythms in any hypophysectomized vertebrate constitutes proof that the adrenal cortex does not occupy an important position in the organization of daily rhythms is yet to be determined.

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Rouge, Louisiana. The fish were maintained on natural photoperiods indoors for 2 weeks in storage tanks containing 10 percent synthetic seawater (Instant Ocean). They were fed an overabundance of dried fish feed (Tetramin) supplemented by freeze-dried brine shrimp several times before and during the experimental period. The water was filtered continually and was maintained at 24° ± 2°C. From 26 October to 2 November 1971, the fish were weighed, measured, and hypophysectomized by a technique described by G. E. Pickford [*Bull. Bingham Oceanogr. Collect.* **14**, 5 (1953)]. On 2 November, the hypophysectomized fish were divided into two groups coupled with two groups of intact fish. In experimental series 1, intact and hypophysectomized fish were kept in six aquariums on a 12-hour photoperiod, with light beginning at 0800. In series 2, intact and hypophysectomized fish were placed on a 12-hour photoperiod with light beginning at 2000. The light was supplied by "daylight" fluorescence (250 lumen/m^2 at the water surface). After 4 days, some of the hypophysectomized fish showed evidence of osmotic failure; therefore, the concentration of the water was increased to full strength "seawater" for all the fish. On 17 November, each fish was anesthetized with tricaine methane sulfonate (MS-222), and the blood was collected from the caudal artery in heparinized microhematocrit tubes, was centrifuged, and was then frozen. Care was taken to disturb the fish as little as possible before removing the blood. L. E. Garcia (unpublished data) has found that our procedure has only minimal effect in raising plasma concentrations of cortisol in intact *F. grandis*, probably by less than 0.5 μg/100 ml during the time it takes to obtain the blood sample. The carcasses were used for measurement of body and testes weights.

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9 March 1972; revised 19 April 1972

Fetal Liver Erythropoiesis and Yolk Sac Cells

Marks and Rifkind, in their thought-provoking article on control of erythrocyte protein synthesis during fetal and postfetal development (*1*) referred (their reference 8) to a paper by Moore and Metcalf (*2*) as supporting the following statement: "Neither in vivo nor in vitro is there substantive evidence that yolk sac cells seed fetal liver erythropoiesis (*8–10*)." This is opposite to the view that Moore and Metcalf in fact took, stating (in their summary): "Organ cultures of . . . embryos or yolk sacs after separation have shown . . . the dependence of intra-embryonic haemopoiesis, particularly in embryonic liver, on colonization by yolk sac haemopoietic cells" (*2*).

They were led to this view by a series of elegant transplantation and culture experiments, including 2-day organ cultures after which 7-day yolk sacs and embryos or yolk sacs alone each contained 55 to 81 in vitro colony-forming cells. Embryos alone contained none. Two lethally irradiated mice which survived for 30 days after injections of 8×10^6 chromosomally marked yolk sac cells showed 89 to 100 percent donor-type mitoses in bone marrow, spleen, thymus, and mesenteric lymph nodes (*2*).

The question of whether or not immature, primitive (yolk sac) cells are the precursors of the fetal liver and adult erythroid cell line is of fundamental importance in understanding the process of determination involved (*3*) (when the hemoglobin changes from fetal to adult type), and much current research is directed to this area (*3*). Even Marks and Rifkind admitted that the possibility has not been excluded "that the yolk sac contains cells which are direct precursors of fetal liver erythropoiesis" (*1*). Their contribution to one side of this question should not have misrepresented the contribution of Moore and Metcalf to the other.

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24 March 1972

Although the work of Moore and Metcalf (*1*) cited by Harrison and Russell indeed claims to demonstrate ". . . the dependence of intra-embryonic haemopoiesis, particularly in embryonic liver, on colonization by yolk sac haemopoietic cells," in fact these studies do not document any contribution of yolk sac cells to later fetal erythropoiesis. The data cited concerning in vitro colony-forming cells (CFC) are irrelevant to this question since such cells have thus far been only shown to be granulocytic leukocyte precursors incapable of erythrocytic differentiation (*2*). The "elegant transplantation" studies cited do not provide any evidence of fetal hepatic erythropoiesis in explanted 7-day fetuses, whether or not the yolk sac is left attached, after 2 days of culture. These studies quite simply show that the yolk sac contains in vitro CFC, which may circulate into the embryo when the vasculature is developed. Furthermore, in vitro CFC are first detected in the intact fetal liver on day 10. Since the 7-day explants disintegrate after 2 days in culture, it is not possible to conclude that yolk sac in vitro CFC have colonized the liver or any other fetal organ.

Harrison and Russell further adduce experiments with chromosomally marked donor yolk sac cells injected into lethally irradiated adult recipients as evidence in support of the contribution of yolk sac in vivo CFC to later embryonic hemopoiesis. The donor cells in these experiments were from 11-day embryos. By the 11th gestational day the fetal liver is already contributing hemopoietic precursors to the embryonic circulation (*3*). There is no reason to assume, therefore, that the observed colony-forming activity in these experiments is of yolk sac origin. The simplest explanation of the data presented by Moore and Metcalf (*1*, figure 6) concerning in vivo CFC is that such hemopoietic precursors may be found in all hemopoietically active tissues and that the question of colonization remains as yet unsupported by substantive experimental data, as stated in our article (*4*).

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