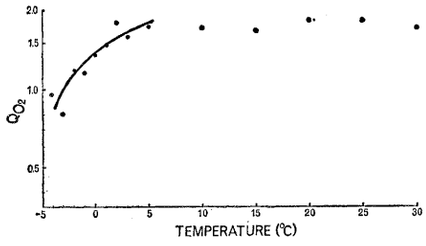


Fig. 1. Oxygen consumption in vitro of brain slices of *Trematomus bernacchii*.

vitro (Fig. 1) show an interesting relation to the lethal-temperature data. The oxygen consumption values were determined manometrically (*8*) with a medium described by Ekberg (*9*). After a fish had been killed by severing its spinal column immediately posterior to the brain, the roof of the skull was cut away, and the entire brain was removed and placed in chilled Ringer solution. After the meninges had been removed, the brain (excluding the hypophysis) was sliced into thin sections with a scissors. The tissues were immediately added to the manometer flasks that contained chilled medium. After a 15-minute period of temperature equilibration, readings were taken at a single temperature at 10-minute intervals for 3 hours. The metabolic rate. $Q_{O_2}$, based on the 1st hour of oxygen uptake, is expressed as microliters of $O_2$ per milligram of tissue (dry weight) per hour.

While it may be somewhat inaccurate to use data on brain metabolism in vitro as an index of the brain's ability to function adequately in vivo, the data (Fig. 1) nevertheless suggest that some type of breakdown occurred in the brain tissue at temperatures near the upper incipient lethal temperature. Extensive tissue disintegration in the Warburg flasks was also observed at temperatures of 5°C and higher. Studies of the metabolism in vitro of brain tissue of other fish species have yielded similar results (*10*). Wohlschlag (*5*) observed a decrease in the whole organism metabolism of *T. bernacchii* at temperatures of 1° to 3°C.

The metabolism in vitro of gill tissue of *T. bernacchii* did not exhibit this sharp sensitivity to temperature (*11*). This suggests that the thermal sensitivity of the central nervous system may be of primary importance in determining the thermal tolerance of the whole organism.

The site of thermal injury within the brain tissue cannot be conclusively determined from our data. However, the different sensitivities of brain and gill tissues to thermal injury and the low temperature at which impairment of brain metabolism occurred suggest that thermal death was not due to extensive enzyme denaturation. If direct thermal inactivation of one or more of the important cellular enzymes had been the result of exposure to the upper inicipient lethal temperature, an unlikely possibility at 5°C, then a similar pattern of inactivation would probably have occurred in both tissues. The rate of reaction of an isolated enzyme system from *T. bernacchii* actually increased linearly with temperature up to approximately 30°C (*11*). However, one cannot exclude the possibility that the brain possessed particularly thermolabile enzymes.

Nonenzymatic loci of thermal injury have been proposed. Heilbrunn (*12*) hypothesized that thermally caused changes in the biophysical state of the cellular lipids can lead to death. Study of the lipid composition of goldfish brain as a function of the acclimation temperature (*13*) supports Heilbrunn's hypothesis; it appears that the chemical properties of the brain lipids are adjusted during temperature acclimation to permit the maintenance of a constant "liquid crystalline" state of the cellular membranes (*13*). The temperatures at which this requisite biophysical state is disrupted may be considerably less extreme than the temperatures at which enzyme denaturation occurs. Thus, thermal death of the *Trematomus* fishes at 6°C may have resulted from subtle biophysical changes in the cellular lipids, perhaps the membrane lipids, rather than from direct thermal destruction of enzyme function.

GEORGE N. SOMERO
ARTHUR L. DEVRIES
*Department of Biological Sciences,*
*Stanford University,*
*Stanford, California 94305*

**References and Notes**

1. J. R. Brett, *Quart. Rev. Biol.* **31**, 75 (1956).
2. F. E. J. Fry, in *Handbook of Physiology*, Sec. 4, *Adaptation to the Environment*, D. B. Dill, Ed. (American Physiological Society, Washington, D.C., 1964), pp. 715–728.
3. J. R. Norman, in *Discovery Reports* (Cambridge Univ., London, 1940), vol. 18, pp. 1–104).
4. J. L. Littlepage, in *Biology of the Antarctic Seas*, M. O. Lee, Ed. (American Geophysical Union, Washington, D.C., 1965), vol. 2, p. 6.
5. D. E. Wohlschlag, in *Biology of the Antarctic Seas*, M. O. Lee, Ed. (American Geophysical Union, Washington, D.C., 1964), vol. 1, pp. 33–62.
6. F. E. J. Fry, *Publ. Ontario Fish. Res. Lab.* **68**, 1 (1947).
7. P. F. Scholander, L. van Dam, J. Kanwisher, T. Hammel, M. S. Gordon, *J. Cell. Comp. Physiol.* **49**, 5 (1957).
8. W. W. Umbreit, R. H. Burris, J. F. Stauffer, *Manometric Techniques* (Burgess, Minneapolis, 1964), pp. 1–17.
9. D. R. Ekberg, *Biol. Bull.* **114**, 308 (1958).
10. J. A. Freeman, *ibid.* **99**, 416 (1950); F. A. Fuhrman, N. Hollinger, J. M. Crismon, J. Field II, F. W. Weymouth, *Physiol. Zool.* **17**, 42 (1944).
11. G. N. Somero, unpublished.
12. L. V. Heilbrunn, *An Outline of General Physiology* (Saunders, Philadelphia, 1952), pp. 488–491.
13. P. V. Johnston and B. I. Roots, *Comp. Biochem. Physiol.* **11**, 303 (1964).
14. We thank members of the U.S. Navy Task Force 43, of the U.S. Naval Air Development Squadron Six, and of the U.S. Antarctic Research Program for their support and cooperation. This work was supported by NSF grants GA-45 and GA-537, administered by Drs. Donald E. Wohlschlag and Arthur C. Giese, respectively.

18 November 1966; 27 February 1967

## Maternal and Environmental Influences on the Adrenocortical Response to Stress in Weanling Rats

Abstract. *Handling rat pups either for 10 or 20 days after birth resulted in a reduction of adrenocortical steroids in the plasma at weaning after the pups were exposed to novel stimuli as compared with controls that were not handled. Nonhandled offspring of mothers which had been handled in infancy also show a reduction in plasma steroids in response to novel stimuli when compared to nonhandled weanling rats of nonhandled mothers. Handling of the offspring when they are infants appears to counteract the influence of the experience of the mother during her infancy.*

Stimulation of an infant rat markedly affects the adrenocortical response to novel stimuli. Rats handled in their infancy from days 1 to 21 exhibit a significantly reduced steroid response after they are exposed to novel conditions in adulthood (*1*). Denenberg and Whimbey (*2*) report that, in rats, the offspring of mothers that had been handled in infancy were heavier at weaning as compared to offspring of mothers that had not been handled. Further, the experience of the mother during her infancy resulted in differential open-field behavior of the pups when they reached adulthood. The two experiments reported here were designed to investigate the question whether handling during infancy would affect the steroid response of pre-

pubertal weanling rats after they were exposed to novel stimuli, and second, to study the interaction between the experience of the infant pup and the experience of the mother in infancy on changes in plasma corticosteroids induced by novel stimuli.

For the first experiment 81 rats (Long-Evans) were used. The animals were 21 days old at the time of testing; they were from litters containing 8 to 10 animals. There were 42 males and 39 females.

The rats were reared in a light- and temperature-controlled colony room on solid-bottom breeding cages which were enclosed except for the cage top. Food and water were delivered externally. Litters were assigned randomly to a group handled for 10 days, a group handled for 20 days, or a group that was not handled. The handling procedure consisted of removing the entire litter from the mother, placing the litter in a plastic cage for 3 minutes, and then replacing it in the nest. On day 21 approximately half the animals in each group were rapidly removed from the home cage and quickly decapitated. The remaining animals within each group were placed in an Akromills stock cabinet, model JD, with compartments 3.8 cm high, 6.82 cm wide, and 13.64 cm long. The animals were retained for 15 minutes at which time they were decapitated and a blood sample was obtained. The stock cabinet has proved effective in eliciting an elevation of adrenal corticosteroids in mice (*3*). The blood was collected in heparinized beakers and plasma corticosteroids of individual animals were determined by the method of Glick, Redlich, and Levine (*4*).

There were no apparent differences in the steroid response as a function of sex, therefore the data presented in Table 1 are values of male and female weanlings. Table 1 summarizes the plasma corticosterone concentrations of both the control and novelty (stress) conditions for the various experimental groups. For all groups there was a marked and highly significant elevation of plasma corticosterone after they were exposed to the novel situation. However, there were differences among the groups in the magnitude of this response. Although there were no significant differences between the manipulated groups that had been handled for 10 days as opposed to those that had been handled for 20, both groups differed significantly from the controls that had not been handled

Table 1. Plasma corticosterone concentration (mean and standard error of the mean) for control animals and animals exposed to novel stimuli for 15 minutes. The number of animals per mean is given in parentheses.

| Handling (days) | Plasma corticosterone ($\mu$g/100 ml) | |
|---|---|---|
| | Control | Experimental |
| None | $20.8 \pm 2.0$ (14) | $38.7 \pm 1.3$ (14) |
| 10 | $18.1 \pm 1.2$ (14) | $31.0 \pm 0.94$ (10) |
| 20 | $14.8 \mp 0.99$ (15) | $29.6 \mp 1.5$ (14) |

($t = 4.27$, $P < .005$, $t = 4.45$, $P < .005$). In the case of the nonhandled control animals, however, there was also a significant difference in the basal concentrations of plasma corticosterone. This difference, however, was only significant when compared to the basal concentrations of plasma corticosterone of the animals handled for 20 days. The difference in concentrations of plasma corticosterone between the rats handled for 10 days and the control rats not handled was not significant.

In the second experiment 92 weanling rats of both sexes were used to investigate the effects of the experience of the mother during her infancy on elevations in plasma adrenal steroids induced by novel stimuli. The mothers used in this study had been bred in this laboratory. Half the females within each group had previously been handled from days 1 through 20. The other half were controls that had not been handled. The pups of either the handled or nonhandled mothers were divided into two groups. The first group was handled from days 1 through 20; the second received no treatment during infancy. When the rats were 21 days old, the procedures used in the first experiment were followed identically. In this experiment weanlings from at least three litters of handled and three litters of nonhandled mothers were studied under each condition (Table 2).

Table 2. Plasma corticosterone concentration (mean and standard error of the mean) for the different groups. The number of animals per mean is given in parentheses. Abbreviations: M, mother; P, pups; N, nonhandled; H, handled.

| Infant experience | | Plasma corticosterone ($\mu$g/100 ml) | |
|---|---|---|---|
| M | P | Control | Experimental |
| N | N | $21.1 \pm 2.28$ (8) | $45.67 \pm 2.32$ (8) |
| N | H | $11.39 \pm 0.96$ (8) | $34.34 \pm 3.27$ (8) |
| H | N | $12.84 \pm 1.22$ (9) | $25.31 \pm 3.24$ (9) |
| H | H | $19.23 \pm 1.88$ (9) | $28.68 \pm 3.29$ (9) |

The analysis of variance for the second experiment indicated no significant main effect attributable to the experience of the mother in her infancy; however, there was a significant difference ($f = 100.7$, df 1, 64, $P < .001$) as a function of exposure to the novel situation and a significant effect of manipulation of the infant offspring ($f = 15.32$, df 1, 64, $P < .001$). However, the interaction between the experience of the mother during her infancy and handling of the pups was significant ($f = 20.90$, df 1, 64, $P < .001$) (Table 2).

Thus, nonhandled weanling rats reared by mothers that had been handled in their infancy showed a reduced steroid response to novel stimuli as compared to controls reared by mothers that were not handled in infancy. However, handling the pups before they were weaned tended to obliterate the effects of the experience of the mother in infancy.

These studies indicate that the experience of the offspring during infancy and the experiences of the mother during her infancy both affect the weanling's adrenocortical response induced by exposure to novel stimuli. The results of the first experiment bear directly on the question of the maturation of the capacity of the organism to show "emotional" responses to novel environments. Candland and Campbell (*5*) report that weanling rats show minimal fear responses as indicated by the lack of defecation after the rats are exposed to a 2.1-m open field. They interpret these data as indicating that any type of novel stimulation would provide less stress for the infant rat than for more mature animals. However, the data from this study and other data obtained by use of the open field (*6*) demonstrate that the weanling rat responds with a marked increase in adrenal steroids—similar to that observed in the adult rat—after they are exposed to novel stimuli. It should be noted that while Candland and Campbell were concerned with, as they define it, "emotional behavior," my study was concerned primarily with one of the physiological responses associated with "emotional" states. Thus it is possible that the maturational sequence necessary for the mature expression of fear behavior is different from the maturation of neuroendocrine function involved in the response of the hypothalamo-hypophyseal adrenal system. The developmental pattern of the

steroid response to stress has been described (*7*).

The results of the second experiment establish that the experiences of the mother during her infancy markedly affect the steroid response of her pups. Thus the offspring of handled mothers showed a reduced steroid response to novel conditions when compared to offspring of nonhandled mothers. However, if the offspring are themselves handled, these differences are abolished. These data can be interpreted in either of two ways. First, direct stimulation of the pups is so profound that it overrides the maternal influence. Second, handling the infant results in changed maternal behavioral and physiological processes, and disturbance of the mother as a function of handling the infant tends to counteract the influence of the experience of the mother during her infancy. Work in our laboratory suggests that maternal stress or treatment of the mother with adrenocorticotropic hormone during nursing significantly affects neuroendocrine maturation and later behavior (*8*).

There is sufficient experimental evidence (*9*) to indicate the importance of maternal influences on subsequent performance both in terms of physiological functions and behavior of the offspring.

SEYMOUR LEVINE

*Department of Psychiatry, Stanford University School of Medicine, Palo Alto, California 94304*

**References and Notes**

1. S. Levine, G. C. Haltmeyer, G. C. Karas, V. Denenberg, *Physiol. Behav.* **2**, 55 (1967).
2. V. H. Denenberg and A. E. Whimbey, *Science* **142**, 1192 (1963).
3. S. Levine and D. Treiman, *Endocrinology* **75**, 1 (1964).
4. D. Glick, D. von Redlich, S. Levine, *ibid.* **74**, 653 (1964).
5. D. K. Candland and B. A. Campbell, *J. Comp. Physiol. Psychol.* **55**, 593 (1962).
6. S. Levine and J. Hewitt, in preparation.
7. S. Levine, D. Glick, P. Nakane, *Endocrinology*, in press; M. X. Zarrow, G. C. Haltmeyer, V. H. Denenberg, J. Thatcher, *ibid.* **79**, 3 (1966).
8. S. Levine, *1st Int. Symp. Biorhythms Clin. Exp. Endocrinol.* (abstract), (1966).
9. R. H. Ressler, *Science* **137**, 129 (1962); *J. Comp. Physiol. Psychol.* **56**, 882 (1962); *ibid.* **61**, 264 (196 → V. H. Denenberg, D. R. Ottinger, M. W. Stephens, *Child Develop.* **33**, 65 (1962); D. R. Ottinger, V. H. Denenberg, M. W. Stephens, *J. Comp. Physiol. Psychol.* **56**, 313 (1963).
10. Supported by research grant NIH MH 07435, the Leslie Fund, and by research career development award 5-K3-MH-19,936. I thank J. Hewitt and C. Silver for technical assistance.

27 February 1967

## Internal Behavior in Fish Schools

Abstract. *Structural changes within fish schools correlate with declines in environmental oxygen. The changes may result from the responses of individual fish to the environmental consequences of group metabolism. Individual behaviors are adaptive to the school in that they tend to maintain stability between school members and their environment.*

During late fall in North America striped mullet, *Mugil cephalus*, form dense reproductive schools and migrate from bays of the Gulf Coast and southern portions of the eastern seaboard into the open sea. Since the mullet are large (20 to 40 cm), often school at the surface, and usually must migrate through passes into the ocean, extended observation of school structure is possible. We have accumulated data on types of schools and their alterations. When viewed from above the schools resemble geometric figures, such as circles, discs, ellipses, triangles, wedges, crescents, and lines. Internal structure is often modified within seconds or minutes, causing school shape to change in a kaleidoscopic fashion. Normally the schools are composed of a large proportion of polarized individuals. Change in school shape may or may not involve disruption of this parallel orientation, but if it does, disruptions are transient or localized within areas of the school. Individual members of a school continually exchange positions through slight alterations of swimming speed or direction even if school shape is not altered. Similar behaviors have been noted in other schooling species (see *1–5*).

Several factors may cause school structure to change. Nonenvironmental factors, such as variation in individual tendencies to associate or disassociate, could result in the described group behaviors. The importance of these and other innate tendencies are difficult to assess. Environmental factors that might operate include temperature, light, salinity, water currents and waves, predation, feeding, bottom topography, and water chemistry. Of these factors we believe that levels of temperature, light, salinity, and water movement are too homogeneous in marine environments to be responsible for the continuous changes observed in behavior. School structure changes in response to acute predation but it is usually recognizable as a radiating eruption of portions of the school from the water surface (*2*). It bears no resemblance to the slower and continuous modifications of school structure described here. The constant shifting in position of individuals within mullet schools, however, could function in feeding. Positional shifts would place fish in a forward and, presumably, a favorable position for feeding even if the position were maintained for only short periods. But it seems unlikely that all of the school shapes and transitions observed can be solely ascribed to feeding behavior. Examination of the stomach contents of 20 mullet taken from migrating schools in November 1966 revealed that these fish had not been actively feeding for some time before being caught. In general, striped mullet feed in loosely associated groups and not just before spawning (*3*).

The most obvious chemical factors that may be involved include soluble gases and dissolved organic substances of inter- or intraspecific origin. Of these factors only respiratory gases (oxygen and carbon dioxide), dissolved substances such as organic wastes, and perhaps specific organic secretions (pheromones) of intraspecific origin seem likely candidates. Only the respiratory gases, however, can be easily and rapidly analyzed in the field.

If we assume that respiratory gases may effect certain changes or characteristic "postures" in school structure, then the following sequence of events may take place. Reduction of dissolved oxygen and increase of carbon dioxide from school metabolism may be sensed by individual fish. This detection of altered environmental-gas concentration could induce modified behaviors such as changes in direction (orientation), spacing, and swimming velocity. The overall result would be the observed tendency toward continuous variability in group behavior. This hypothesis implies that individual physiological and behavioral response is transferred into social behavior in such a manner that it shortens the exposure of individual fish to less favorable conditions (low oxygen, high carbon dioxide and low *p*H, or both). In-