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Temperature, stress and the distribution of leukocytes in red-spotted newts, *Notophthalmus viridescens*

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Summary. Adult newts, *Notophthalmus viridescens*, were maintained in constant darkness and at a constant temperature of 3.5 °C or of 11.0 °C or of 21.0 °C. Animals of each group were sacrificed after an 8-day sojourn in their constant environments, at which time blood smears for each newt were prepared. Differential counts of leukocytes of the animals maintained at 3.5 °C and of those that lived at 21.0 °C were compared with the differential counts of the newts that lived at 11.0 °C. In the blood of animals maintained at 3.5 °C and at 21.0 °C, there were more neutrophils and fewer lymphocytes than the same types of cells in the blood of newts that lived at the intermediate temperature, 11.0 °C (Table 1). Those differences, statistically significant at $P<0.001$, may indicate that the constant high and low temperatures constitute physiological stress for this species.

Introduction

For many years, red-spotted newts, *Notophthalmus* (*Triturus*) *viridescens,* common inhabitants of ponds and streams of eastern United States, have been known to zoologists as subjects of several important types of laboratory investigations. Their reproductive maturation and its endocrinology, as well as their physiology of molting and its hormones, were elucidated by Adams and her students (Adams 1930, 1938, 1940, 1946, 1950; Adams et al. 1932). Morgan and Fales (1940) and Morgan and Johnson (1942) described the histology of the thyroid of newts through various seasons of the year, while Reinke and Chadwick (1940) analyzed the hormonal controls of the well-known water drive of these amphibians. In our laboratories, the anterior pituitary-adrenal axis and circadian cycles of red-spotted newts have been studied (Bennett et al. 1972; Bennett and Johnson 1973; Bennett 1976). Schotté (e.g. 1961) and more recently Singer (e.g. 1978), Singer and his associates (e.g. 1976) and others (for a review, see Wallace 1981) have contributed extremely valuable and basic information about regeneration based on their work with this important experimental animal. During the course of all the investigations cited, newts were maintained under conditions of light intensity, photoperiod, and temperature which differ from those typical of their natural environments.

To what degree have the physical factors of the laboratory regimens affected the results of the studies involving *Notophthalmus*? That question has been posed as a result of our finding that exposure to constant light (LL) for as few as 8 consecutive days, a period of LL appreciably shorter than that often employed in analyses of circadian phenomena (Aschoff 1981) or regeneration (Maier and Singer 1977), evoked changes in the differential blood counts of newts which are characteristic responses to physiological stress (Bennett and Reap 1978). More specifically, increases in the relative number of neutrophils and decreases in the relative number of lymphocytes, changes associated with Selye's General Adaptation Syndrome (G.A.S.) (Selye 1950), were found in *Notophthalmus* living under LL of 60 lux. Neutrophilia and lymphopenia have also been documented for newts stressed osmotically (Bennett and Johnson 1973), treated with hydrocortisone acetate (Bennett et al. 1972), or injected with ACTH (Bennett and Johnson 1973). ACTH and some adrenal steroids are among the hormones implicated as mediators of the G.A.S., and are among those known to have a variety of profound effects on vertebrate metabolism (Norris 1980), and perhaps, also, on *Notophthalmus* regeneration (Wallace 1981).

Here, we report that an 8-day exposure to constant temperature of 3.5 °C or one of 21.0 °C, conditions under which *Notophthalmus* have lived in laboratories (Adams 1946; Maier and Singer 1981; Morgan and Johnson 1942), also results in neutrophilia and lymphopenia. Consequently, it is suggested that temperature regimes such as those may result in physiological stress which stimulates increases in endogenous secretions of ACTH and adrenal steroids, and that those increased secretions may, in turn, mediate or affect results of experiments with this species.

Materials and methods

Adult male and female *Notophthalmus viridescens*, shipped periodically to Maine from Tennessee, were studied during October and November, 1980, and February, April and May, 1981. As soon as the newts arrived in the laboratory, they were divided into groups of 20 to 30 individuals, and placed in containers of well-aerated pond water. One of the groups lived in an environmental chamber in constant darkness and at a constant temperature of 3.5 ± 0.5 °C for 8 days; a second group lived in a similar chamber in constant darkness and at a constant temperature of 11.0 ± 0.5 °C for 8 days; the third group was maintained in an environmental chamber in constant darkness and at a constant temperature of 21.0 ± 0.5 °C for 8 days. The groups that lived at 11.0 °C in the dark for 8-day periods are considered as the normal controls for the experiments, since it has been found in many earlier studies (Bennett 1976) that the average differential blood counts of *Notophthalmus* living under those conditions are similar to the blood counts determined for newts within 24 h of their being removed from their natural environments.

During the morning of the day following the 8-day treatments, the newts were sacrificed, and smears of their blood were prepared with Wright's Blood stain. A differential count, based on 100 leukocytes, was determined for each individual. The results of all 5 series have been combined for analyses and comparisons, since the results of the different series were generally the same.

Results and discussion

In Table 1 are presented the average per cent distributions of 5 types of white blood cells in the animals maintained in each of the 3 temperature regimens. The numbers of eosinophils, basophils and monocytes were small, and their variations among the three groups of newts were slight and insignificant. Comparable distributions of these rarer types of leukocytes, and very slight differences in them from one group of *Notophthalmus* to another were typical for the newts observed in our earlier investigations (Bennett et al. 1972; Bennett and Johnson 1973; Bennett and Reap 1978; Rose and Bennett 1978). Finding lymphocytes the most numerous type of the leukocytes and neutrophils the second most numerous type of white blood cells in all groups reported here (Table 1) was also similar to the observations made during those previous studies. In addition, the average per cent distributions of lymphocytes, 63.5%, and neutrophils, 24.3%, (Table 1) in the control newts, i.e., those living in constant darkness and 11.0 °C, were within the ranges found for the distribution of those 2 types of cells in *Notophthalmus* observed under identical conditions in our laboratories during investigations of the last 10 years.

Table 1. Average % distribution of leukocytes of the newts maintained at each of the three temperatures

Temperature	Number of animals	Type of Cell				
		Lymphocyte	Neutrophil	Eosinophil	Basophil	Monocyte
11.0 °C	120	63.5	24.3	6.2	3.2	2.8
3.5 °C	133	58.4	30.7	3.5	5.4	2.0
21.0 °C	123	53.2	40.0	4.3	1.5	1.0

When compared to the average per cent distributions of lymphocytes and neutrophils in the control animals, the distributions of cells in the newts living at 3.5 °C and of the animals living at 21.0 °C show similar differences. In both the latter groups, there was an increase in the relative number of neutrophils and a decrease in the relative number of lymphocytes (Table 1) compared to the counts for *Notophthalmus* that lived at 11.0 °C. However, the degree of those differences varied: in the animals removed from the 3.5 °C temperature, the lymphocytes decreased 8.0% and the neutrophils increased 26.3% compared to those of the controls; in newts maintained at 21.0 °C, the lymphocytes decreased 16.3% and the neutrophils increased 64.6% compared to the averages for those 2 types of leukocytes in the controls. Nevertheless, results of the Student's t-test showed that all the differences between control and experimental counts are statistically significant ($P < 0.001$).

The changes in the differential blood counts evoked by the 3.5 °C and 21.0 °C treatments are very much the same as the significant changes effected in *Notophthalmus* by hydrocortisone acetate (Bennett et al. 1972), by ACTH (Bennett and Johnson 1973), by immersion in a NaCl solution hyperosmotic to their body fluids (Bennett and Johnson 1973), and by constant light of 60 lux for 8 days (Bennett and Reap 1978). Comparisons of the results of all those earlier studies and those of the investigation reported here emphasize a point made by Bennett and Reap in 1978, viz, the amplitudes of the increases in relative numbers of neutrophils and of the decreases in relative numbers of lymphocytes compared to control counts vary

Table 2. % changes in the relative numbers of neutrophils and lymphocytes in experimental animals compared to control newts in four different studies

Treatment	% increase in neutrophils	% decrease in lymphocytes	Reference
Hydrocortisone acetate	95	37	Bennett et al. 1972
ACTH	41	34	Bennett and Johnson 1973
Immersion in 2% NaCl	82	54	Bennett and Johnson 1973
LL (60 lux)	65	16	Bennett and Reap 1978

from one experimental manipulation to another (Table 2). Precise explanations for those varying degrees of change are not possible at present. Bennett and Reap (1978) suggested that the differences merely indicate that the different experimental treatments vary in their effects on differential blood counts.

More important are the basically similar effects of osmotic stress, of constant light, and of maintenance at 3.5 °C and at 21.0 °C. Those conditions have been found to cause changes in the differential blood counts of *Notophthalmus* that are the same as the general changes stimulated by ACTH and hydrocortisone acetate. Consequently, particular temperatures as well as photoperiods may be considered stressful to red-spotted newts, and are factors which should be taken into account as experiments with these animals are planned, and when the results of studies with these animals are analyzed and evaluated.

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