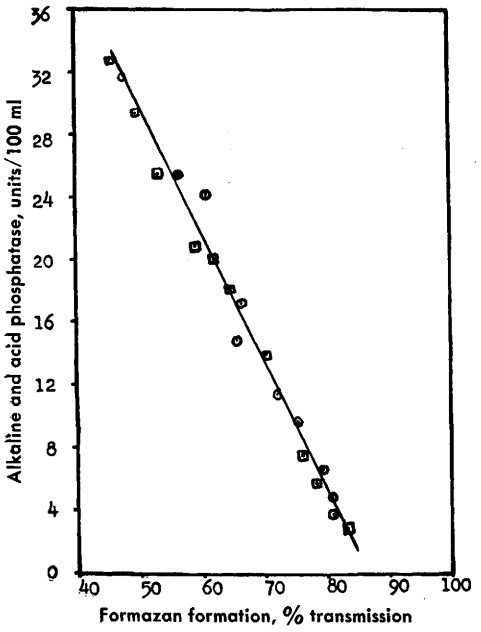

FIG. 1. Phosphatase activity vs. formazan formation. % transmission at 485 mμ. Alkaline phosphatase in Bodansky Units *(dotted square)*. Acid phosphatase in King-Armstrong Units *(dotted circle)*.

prostate and osteogenic sarcoma, neoplasms with high serum phosphatase activity.

This led us to investigate phosphatase activity. Fig. 1 shows the correlation between phosphatase activity of serum and the formation of formazan from triphenyltetrazolium.

Summary. The reduction of tetrazolium by serum is not a specific test for malignancy. It seems however to be correlated with the serum alkaline and acid phosphatase activity.

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Received July 12, 1954. P.S.E.B.M., 1954, v87.

Factors Controlling Color Change in the Tree Frog, *Hyla versicolor* Wied. (21272)

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Control of color change in amphibians has been studied by many workers; material published prior to 1943 was summarized by Parker(1). According to Parker's summary, frogs respond to cool dark environments by release of melanophorotropic hormone and darkening of integument, whereas light and warmth result in melanophore contraction and light colors through retention or non-secretion of hormones by the pituitary. Many chemical factors also have melanophorotropic actions and produce darkening of integument, either by stimulating release of melanophorotropic hormone by action on pituitary, or by direct action upon melanophores(2). Recently the study of factors which control color changes in various species has become of considerable practical importance. Johnsson and Högberg(3), Sulman(4) and Thing(5) reported melanophorotropic effects of ACTH

TABLE I. Progressive Color Changes in *Hyla versicolor* and Numerical Assignments.

Color	No.
Green	1
Olive	2
Mottled	3
Beige	4
Brown	5

in amphibians. These studies, based upon hypophysectomized *Rana*(3,5) and light-adapted Amphibia(6,7,8), or untreated Hylid frogs(9) provide simple techniques for the assay of adrenocorticotropic substances.

In searching for suitable Nearctic frogs for this assay, we have studied environmental factors controlling color change of *Hyla versicolor* and *Hyla cinerea*(9).

Materials and methods. Frogs were maintained in groups of 5-8 in 600 ml beakers, with about 100 ml of water. Light was provided by a 100 watt frosted bulb placed exactly one foot from near side of beaker, and for darkness, beakers were covered with a double layer of cloth toweling. Experiments were carried on in a darkened cold-room (3-5°C) or at normal laboratory temperatures (20-24°C). Color changes are regular in occurrence, passing from a light green to a dark brown; they have been arbitrarily divided into 5 stages. For quantitative analyses each stage has been assigned a numerical value (Table I). Responses have been summarized by using simple mean values termed the "color index" (CI). This correlates closely with the melanophore index of Hogben and Slome(10).

Results. Effects of constant light: In preliminary experiments frogs were placed into constant light for 2 weeks. All frogs were then beige or brown (CI = 4.7). After 72 hours under normal diel conditions they were again subjected to continuous light for one week; the color index remained 4.7. Controls for the following experiments were maintained in constant light; color indices remained around 4.7 throughout.

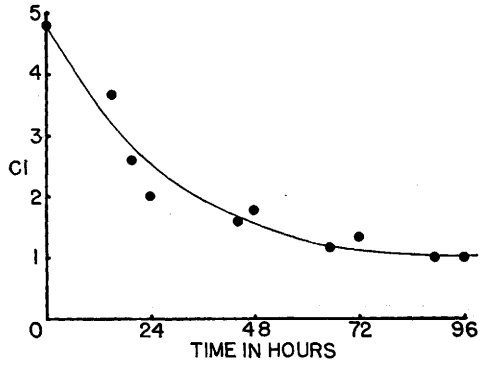

FIG. 1. Decrease of color index (CI) with increasing period of dark-adaptation. The curve was fitted visually.

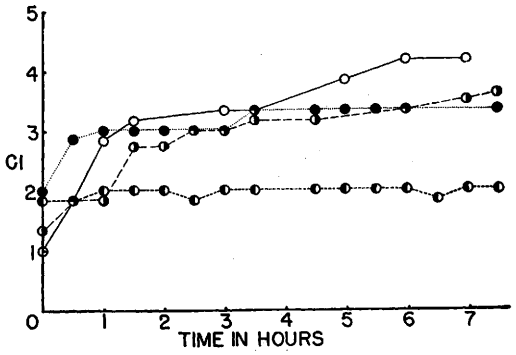

FIG. 2. Light effects after varying periods of dark-adaptation. Solid circles and dotted lines: 24 hr; half circles open to right and short dashed line: 48 hr; half circles open to left and long dashed line: 72 hr; hollow circles and solid line: 96 hr; ¾ circles; common point of 2 or more lines.

Effects of constant darkness: Constant darkness for varying periods of time resulted in lowered color indices. To determine the relationship between time and color index, groups of 6 frogs were darkened for varying periods. The decrease of color index with extended periods of darkness is shown in Fig. 1. Although about 48 hours of darkness was enough to cause a complete response (green color) in most frogs, over 72 hours were necessary to turn all frogs green.

Light-adaptation after varying periods of darkness: At the completion of above experiments the frogs were returned to constant light. No consistent relation between dark-adaptation time and change toward light-adapted condition was found. CI values after 24, 48, 72 and 96 hours of dark-adaptation have been plotted in Fig. 2, and are typical of responses obtained. The 72 and 96 hour groups showed more complete, though less rapid, returns toward control condition, than the 48 hour frogs which showed essentially no reaction.

Effects of temperature: The above data suggested that light tended to darken frogs and darkness to lighten them. As cold causes darkening in most frogs, 8 light-adapted frogs were refrigerated at 3-5° C, and 5 were darkened at regular laboratory temperatures (Fig. 3). Cold had no obvious effect upon already expanded melanophores, while controls at nor-

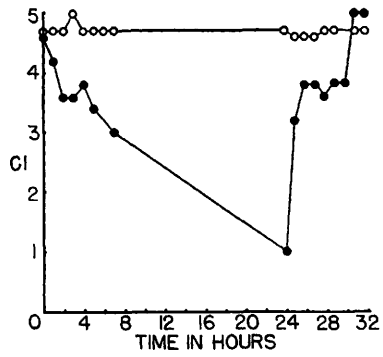

FIG. 3. Effects of darkness and temperature on light-adapted frogs. Group 1 (hollow circles) placed in darkened cold room; Group 2 (solid circles) darkened at room temperature. Both groups returned to light at room temperature at 24 hr.

mal laboratory temperatures showed decrease of CI. Return to constant light and normal laboratory temperatures had no effect upon cold-dark-adapted frogs, whereas warm-dark-adapted frogs showed return toward control conditions. Two groups of 6 frogs each dark-adapted for 72 hours, were then placed in refrigerator for 24 hours. Five frogs died during experiment; color index values for the remainder have been plotted in Fig. 4, together with controls, maintained in the dark at normal laboratory temperatures. Thus low temperatures also cause integumentary darkening.

Discussion. These data indicate that *Hyla versicolor* responds to darkened laboratory environments by contraction of integumentary melanophores and to light or low temperatures by the expansion of these cells. Expansion of the melanophores in frogs is controlled by pituitary hormone(1) known variously as intermedin, melanophore expanding hormone, chromatophore hormone, etc. It has been suggested that this hormone is a member of an adrenocorticotropic hormone complex(11), although separation of the factors is possible under certain conditions(12).

Data presented here suggest that release (or secretion) of melanophorotropic hormone is largely under the control of light, which acts through the eyes of the frogs to stimulate the pituitary (see number 1 on effects of blinding). In darkness, hormone is retained and melanophores contract. Low temperatures also darken the integument, presumably by stimulating hormone release.

Hyla versicolor is highly unusual, responding to light in a manner opposite to other species. Kohler(13) has suggested that color changes in lower vertebrates may be explicable on the basis of Selye's concept of stress(14). The stressor stimulates the release of pituitary ACTH which then results in melanophore expansion. If this explanation is valid, light must act as a stressor in *Hyla versicolor* but not in a congeneric form, *Hyla arborea*, which turns green in the light(6). Dampness, a necessity for amphibian life, would also be a stressor(1). Though Selye's GAS and color-changes may be related phenomena, the relationship is certainly not as simple as Kohler implies.

The unexpected control of melanophorotropin release in *Hyla versicolor* may be the result of selection for darkly colored frogs during the day when the frogs are inactive and take refuge in recesses in bark and other such niches. In such places a green frog would be highly conspicuous and perhaps subject to predation. Thus selection may have favored the darker daytime color, and hormone release in response to light.

Summary. *Hyla versicolor* responds to light by melanophore expansion, and to darkness by contraction. The completeness of contraction depends upon duration of the period of dark-adaptation, generally more than 72 hours are required for all frogs to turn green. The change to brown after dark-adaptation is vari-

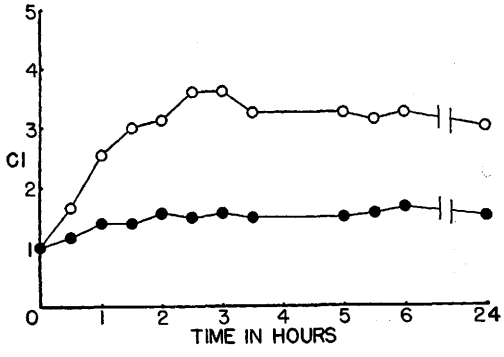

FIG. 4. Effects of cold after 72 hr dark adaptation at room temperature. Solid circles: frogs maintained in darkness; hollow circles: frogs transferred to cold-room.

able and no consistent relationship has been found between length of darkness and length of recovery period. Temperatures of 3-5° C darken frogs as does light. Light and cold appear to be stimuli which cause release of melanophorotropic hormone (ACTH?) from the pituitary gland. Light-induced changes, opposite to those shown by other frogs, seem explicable on the basis of natural selection during the diurnal period when these frogs are inactive.

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Received July 14, 1954. P.S.E.B.M., 1954, v87.

Effect of Immunity on Resistance to Infection in Irradiated Mice and Rats.* (21273)

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Bacteria which are common inhabitants of the intestinal tract are frequently found in blood and various organs of lethally irradiated mice(1-3). The efficacy of antibiotics in reducing mortality(4,5) and the effect of certain of the organisms on survival time(2,6) show that these infections cause or contribute toward death in the irradiated animal. Irradiation reduces the defenses against infection, in part, by decreasing the number of circulating granulocytes and by interrupting the production of antibodies. Granulocyte count has recently been shown to be quantitatively related to survival after midlethal radiation(7). In the present experiments the effect of immunity acquired prior to irradiation on ability to withstand bacterial challenge and on survival following lethal radiation are examined.

Procedures. Male N.I.H. littermate mice and Sprague-Dawley rats from the N.I.H. animal colony, maintained on a diet of Purina Laboratory Chow, were used in these studies. The animals were caged individually from

TABLE I. Effect of Immunization on Survival of Animals Experimentally Infected with *Proteus* after LD_5 Irradiation.

Exp.	Immunizing antigen	No. of animals	% surviving	P†	Days from chal. to death, ± S.E.
	Mice: 6 immunizing inj., challenge 5 days after irradiation				
1	0	47	17		3.3 ± .5
	Prot. Kf 7	50	66	<.001	10.3 ±1.2
2	0	40	2		1.1 ± .1
	Prot. AM	39	31	<.01	5.2 ± .6
3*	0	39	0		1.2 ± .3
	Prot. AM	40	63	<.001	6.5 ± .7
	Rats: 6 to 7 immunizing inj., challenge 4 days after irradiation				
A	0	43	42		2.2 ± .4
	Prot. Kf 7	60	90	<.001	9.8 ±3.4

* Challenge organism was *Proteus* Kf 7 in this experiment.

† Significance of differences in survival of Exp. and Control groups by the method of χ^2.

* Supported in part by the Department of Defense.