Use of *Hyla cinerea* in Assay of Melanophorotropic Potency of ACTH. (20839)

RICHARD A. EDGREN. (Introduced by F. J. Saunders.)

From the Division of Biological Research, G. D. Searle & Co., Chicago, Ill.

The nature of the relationships between ACTH and melanophorotropic hormone has not yet been satisfactorily settled(1). Either, as claimed by Sulman(2), the two represent fractions of a corticotropic complex of hormones or, as Reinhardt, *et al.*(3) believe, the melanophorotropic action of ACTH preparations is the result of contamination by melanophorotropin. Should the two hormones be shown to be identical, assays employing melanophore reactions will become increasingly important; should they be demonstrated to be separate hormones it will become increasingly important to know the melanophorotropic potency of ACTH preparations as a possible undesirable side effect in ACTH therapy(4).

Although assay methods for melanophorotropin have been published by many workers, the simplicity of Sulman's(5) technic has much to recommend it. In a search for an American frog which would fulfill the requirements of this assay, *Hyla cinerea* has been studied in this laboratory.

Materials and methods. Tree frogs (*Hyla cinerea* Schneider) were maintained in 1500 ml beakers covered with watch glasses. No more than 6 frogs were placed in a single beaker. Each beaker contained about 2 cm of tap water. Frogs were irregularly fed on *Drosophila*. Injections were made directly into the dorsal lymph sac; dosages were contained in 0.1 ml of distilled water. As gross color changes seemed to give as accurate an estimate of melanophore condition as microscopic examination, they were used exclusively. After ACTH injection the green frogs went through a regular series of color changes: green to light olive to dark olive to blackish-brown. This continuum was divided into 4 arbitrary stages, and each stage was assigned a numerical value (Table I). In statistical treatments the numerical values for all frogs at a given dose level were averaged; the resulting mean was termed the color index (CI). As a result of the rapidity of the response, time-response curves were based on readings taken at 5-minute intervals. No change in sensitivity has been noted with repeated use of the frogs, although generally 48 hours were allowed to elapse between tests. A single sample of ACTH was employed; this sample assayed at 1.3 IU/mg.

TABLE I. Numerical Values Assigned to Colors of *Hyla cinerea*.

Color	Numerical assignment
Green	1
Light-olive	2
Dark-olive	3
Brown	4

Experimental. Since no study of environmental control of color change in *Hyla cinerea* had ever been published, and such information was prerequisite to the interpretation of the assay, a preliminary analysis was carried out in this laboratory. Details of methods used will appear elsewhere(6). Light (and its absence), temperature and humidity have been shown to be the most important environmental stimuli for color change(7). In constant light *Hyla cinerea* showed no alteration of the green color; in constant darkness they tend to blanch slightly, becoming yellowish after 24-48 hours. Low temperatures (4-5°C) stimulated darkening in only ⅓ of the frogs after about 30 minutes. By keeping all frogs in beakers containing similar amounts of water, relative humidities were maintained at constant levels throughout.

Effects of ACTH on color change: ACTH was administered in doses from 0.1 to 50.0 μg/frog. Both the magnitude of the CI and the time required for the index to return to control levels were related to dose (Fig. 1). Distilled water, which was used as a control, produced a slight initial increase in CI which fell to normal in 30 minutes to 1 hour. Inspection of the curves of Fig. 1 indicates that the 30-minute response best differentiated the

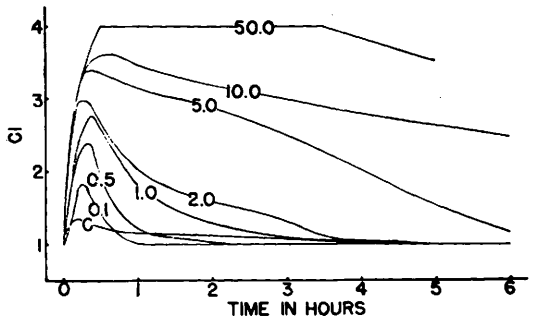

FIG. 1. Temporal responses in color change of *Hyla cinerea* following μg injections of ACTH at 0 hr. CI = color index (see text); C = distilled water controls.

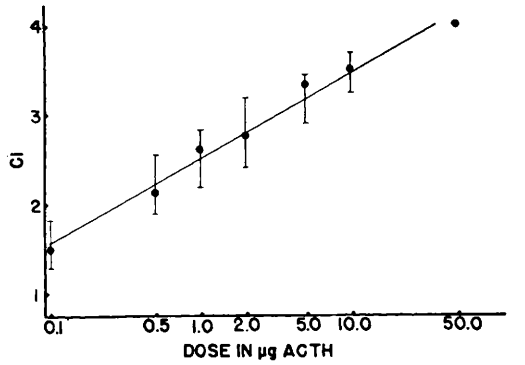

FIG. 2. Regression of color index (CI) on log dose ACTH 30 min. after injection. CI = 1.56 + 0.95 log (10 dose), by method of "least squares." Vertical lines indicate 95% confidence limits.

various levels of ACTH. The half-hour responses were directly related to log dose (Fig. 2). In a series of experiments, known doses of ACTH were administered to groups of 4 frogs each; the investigator reading the frogs was in ignorance of the sequence of the doses. In all cases it was possible to make correct determinations of administered doses.

Discussion. It is apparent from the experiments summarized on the normal control that under conventional laboratory conditions of temperature (20-24°C) and light there is little or no color change. Thus frogs may be maintained in the laboratory with no special care, obviating the difficulties of hypophysectomy necessary with Ranid frogs and the time and space necessary for light-conditioning in *Hyla arborea* or *Xenopus* tadpoles(5,8). The rapidity of the response of these frogs to ACTH preparations allows the assay of materials in less than 2 hours. The response observed with the injection of distilled water may be interpreted as the result of the release of endogenous melanophorotropic hormone or perhaps epinephrine as a result of handling, the excitement darkening of Burgers, *et al.* (9). Since this reaction is neither as great in magnitude nor in duration as that obtained with low ACTH doses employed, it does not interfere with the assay.

Summary. The lack of integumentary color changes in *Hyla cinerea* under normal laboratory conditions makes it a suitable organism for the assay of the melanophorotropic potency of ACTH. Intensity of darkening is directly related to the log of the dose.

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