Quantitative Assay of Melanophore-Expanding Property of Preparations of ACTH and Corticotropin B. (20535)

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In the past the melanophore-expanding property of anterior pituitary and median lobe preparations has been estimated in frogs or on excised frog skin and was attributed to a hormone termed "intermedin"(1,2). Recent studies have cast doubt upon the existence of "intermedin" as a separate entity and it has been suggested that ACTH and "intermedin" are identical(3,4). The present experiments demonstrate that the melanophore-expanding activity of preparations of ACTH can be quantitatively assayed on the amputated tadpole tail. Among the several preparations used in studying this procedure was Corticotropin B, previously shown to exert potent melanotropic and ascorbic acid depleting activity(5).

Materials and methods. Tadpoles (*R. clamitans*; 4 mm hind limb stage) were obtained from a commercial source and maintained in spring water in white enameled pans. To assure maximal contraction of the tail skin chromatophores, the tadpoles were exposed to artificial white light for 9 hours per day for a period of at least 3 days. A diet of boiled spinach was supplied daily.

Weighed samples of the ACTH preparations to be assayed were diluted to the appropriate concentration in amphibian saline (0.7% NaCl) and immediately placed in small covered Petri dishes. The Provisional USP Corticotropin Reference Standard was used as a standard for melanophore-expanding activity and was arbitrarily assigned a melanophore-expanding potency of 0.005 unit per mg. All results have been expressed in terms of this arbitrary unit. The USP Corticotropin Standard was diluted to contain 0.08 to 0.0009 melanophore-expanding unit per ml. Within this concentration range the log dose-response curve was essentially linear.

The tadpole tails were amputated and immediately immersed in the test solutions for 30-40 minutes. They were then removed, gently blotted and scored under the microscope (100x) for the degree of melanophore-expansion. The scoring procedure was patterned after that of Langrebe and Waring (6,7). In this system the unexpanded melanophore was assigned a score of 1; the fully expanded, multipolar structure was assigned a score of 5. Intermediate scores (2, 2½, 3, 3½, etc.) were given to the partially expanded, asteroid melanophores depending upon their degree of extension. This procedure is one of reasonably satisfactory precision under the conditions employed. According to Thing(8) measurements of melanophoric expansion obtained with a photoelectric cell yielded no better results than subjective scoring.

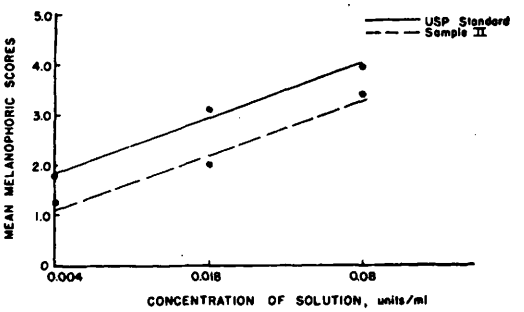

FIG. 1. Dose response curves; USP corticotropin Standard and Sample II (Tables I and III).

Results. Fig. 1 shows the log dose response curves plotted from the data (melanophore indices) recorded in Table I. The Analysis of Variance (Table II) indicates that these 2 dose response curves are essentially parallel and that the slight curvature is not significant. The potency of the unknown relative to the standard, calculated by the procedure given by Bliss(9), was 58 ± 12 units per mg.

The results of 6 assays performed by this method are shown in Table III.

Summary. Skin melanophores of freshly amputated tadpole tails expand when placed in solutions of ACTH. An assay for rapid estimation of this melanophore-expanding activity of ACTH preparations has been de-

TABLE I. Melanophoric Indices Observed in Isolated Tadpole Tails; 35 Min. of Immersion (Avg of 2 Readings per Tail).

Conc., units/ml	Standard	Unknown
.08	3.75	3.50
	4.00	3.25
	4.00	4.00
	4.00	2.75
	4.00	3.50
	$S_H = 19.75$	$U_H = 17.00$
.018	3.50	2.00
	3.25	2.00
	2.75	1.75
	3.00	2.75
	3.00	1.50
	$S_M = 15.50$	$U_M = 10.00$
.004	2.25	1.50
	2.25	1.00
	2.00	1.00
	1.50	1.75
	1.00	1.00
	$S_L = 9.00$	$U_L = 6.25$

TABLE II.
Analysis of Variance (Melanophoric Indices).

Variation due to:	df	ms
Samples	1	4.03*
Slope	1	23.11†
Parallelism	1	—
Curvature	2	.26
Error	24	.16

* Highly significant source of variation.
† Very highly significant source of variation.

veloped, using USP corticotropin as the Reference Standard. All ACTH preparations tested showed this activity. Corticotropin B, the most highly purified preparation available, showed the highest degree of melanophore-expanding activity and was also the most potent in causing adrenal ascorbic acid depletion.

TABLE III. Melanophoric Potencies of 5 Corticotropin Preparations (Units/mg).

Sample	Melanophoric potency	Ascorbic acid depletion potency
I	9 ± 5	5
II	58 ± 12	160
III	58 ± 12	60
IV	21 ± 5	60
V	10 ± 3	5
VI*	269 ± 48	163 ± 141

* Sample of Corticotropin-B.

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Effect of Adrenal Cortical Extract and Steroids upon Glucose Tolerance of Eviscerated Rats. (20536)

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The data of this study support the conclusions of an earlier report(1) that adrenal cortical extract inhibits the tolerance of the eviscerated rat for intravenously administered glucose. Cortisone and hydrocortisone are less effective.

Methods. General details have been published(1,2). Male rats were eviscerated at 250 ± 2 g weight. Each rat received by continuous intravenous injection 20 cc per 24 hours of a solution containing glucose, 4 units of regular insulin, 10,000 units of penicillin