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A New Method of Assay of Chromatophorotropic Hormone by Means of Excised Lizard Skin.

CORINNE KEATY AND ALLAN J. STANLEY. (Introduced by Albert E. Casey.)

From the Zoology Department, Louisiana State University.

The principle of the method to be described for testing pituitary chromatophorotropic substances is based on the fact that the dermal melanophores of excised skin of *Anolis carolinensis* respond to pituitary chromatophorotropin[1] by dispersion of the melanosomes.

The use of hypophysectomized frogs and lizards and light adapted frogs involves several undesirable conditions. They are all time-consuming, and in the case of operated reptiles, endocrine imbalance, especially thyroid effects, are involved. The use of excised skin of *Anolis* as a test object for measuring intensity of effect, is very rapid, entails no difficult operative procedure, requires only small amounts of material, and eliminates the influences of other possible endocrine or neural disturbances. The test has been found to be reliable. Uniform responses are produced on skin of well-fed animals 100% of the time without difficulty. The complete dispersion of melanosomes is easily detected since the color change from green to brown is very striking.

Acetone-dried pituitaries of several vertebrates (man, beef and dog) have been tested and complete assay of pituitaries of *Rana catesbeiana* has been made by this method. It has been found that frog material is best employed in dilutions of 1 mg in 20 cc cold-blood Ringer's solution. Stronger concentrations react upon the skin too rapidly for accurate observations to be made. The solution

TABLE I.

Melanosome Dispersion-rate of Excised Skin of *Anolis carolinensis* in 0.25 cc of Solution of 1 mg of Frog Pituitaries in 20 cc Frog Ringer's, at Room Temperature. Two hundred twenty-eight observations. The time is in minutes.

Material	Fresh, unfiltered	Fresh, filtered	24 hr refrig. unfiltered	24 hr refrig. filtered	24 hr room temp. unfiltered	24 hr room temp. filtered
			Skin of Back and Thigh.			
Summer pituitary	6:12.7	6:1.0	6:12.8	6:50.1	12:41.2	12:34.7
Spring pituitary	3: 8.3	3:5.4	2:41.9	2:27.1	11:32.6	11:48.0
			Skin of Belly.			
Summer pituitary	8:20.5	8:5.0	8:18.7	8:21.1	15:41.3	14:52.1
Spring pituitary	4:27.8	4:0.2	4:14.2	4: 0.9	10:29.4	10:16.6

[1] Hadley, C. E., *J. Exp. Zool.*, 1931, **58**, 221.

is refrigerated 24 hours and then filtered. The filtrate is saved for the test. It has been found that the unfiltered solution shows partial inactivation of the hormone after 24 hours at room temperature. Complete inactivation is accomplished after 48 hours.

Lizards used for such tests should be well-fed and watered daily, since variations in reactions or complete unresponsiveness results when the animals are not fed and watered regularly. Animals kept in the laboratory throughout the winter have been used successfully.

It has been found that skin from the back or top of the thigh is best for the test, since the scales in these regions possess greater numbers of melanophores and are of uniform size. Skin from the belly reacts more slowly. After removal, the skin is placed on the surface of cold-blood Ringer's solution, and cut with sharp scissors into small pieces. Pieces from 2 to 4 mm square are large enough. After 20 minutes in Ringer's, the skin becomes uniformly green. Scales along the cut edges, or scales injured by manipulation with instruments generally remain brown and will not react to other stimulation.

After complete melanosome condensation has been effected, a single piece of the skin is placed on the surface of 0.25 cc of the filtrate on a clean depression slide. A hypodermic syringe is used for measuring the amount. The color change to brown is best observed with a binocular dissecting microscope. The speed of the reaction is conveniently used to measure potencies of solutions prepared as described above. Table I shows the melanosome dispersion rates obtained by this method for pituitaries of *Rana catesbeiana* during early spring and late summer at room temperature.

The *Anolis* skin has been found to react to acetone-dried pituitaries of man (negro and white), cattle and dog.

Preliminary tests have been made of temperature variation on the rate of the reaction. The indications are that the K_{10} is applicable to the reaction.