Radioimmunoassay for Alpha Melanocyte Stimulating Hormone. (31447)

G. T. Ross, W. D. Odell,* and T. Goodfriend† (Introduced by R. Hertz)
Endocrinology and Metabolism Branch, Reproduction Program, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Md.

Methods currently available for quantifying melanocyte stimulating hormone (MSH) in biologic fluids, while sensitive, lack precision and specificity. The production of antibodies to α MSH(1) and the availability of highly purified α MSH provided the necessary components for a radioimmunoassay. Such an assay has been developed and some of its characteristics are reported here.

Materials and methods. Details of the method of preparation of antisera to α MSH have been reported(1). Five antisera were screened and one was found to be suitable for use in the assay to be described. The limits of detection with this antiserum ranged from 0.25 to 0.5 mμg of the standard hormone preparation.

Purified porcine α MSH and β MSH were the generous gift of Dr. Andrew Schally.‡ Bioassay of α MSH by a method previously described(2) revealed 0.125 mμg of this material to be a minimal effective dose in hypophysectomized frogs. Purified ACTH was obtained from commercial sources.§

Pituitary glands of male rats (Holtzman strain) weighing 150-200 g were removed immediately following sacrifice by a lethal dose of barbiturate given intraperitoneally. Intermediate lobes were separated with blunt dissection from anterior lobes and homogenized in 0.01 M phosphate buffer pH 7.8 containing 0.15 M NaCl. The homogenate was centrifuged to remove particulate matter and clear supernatant diluted appropriately with more buffer.

Radioiodination of α MSH by the method of Greenwood *et al*(3) resulted in preparations with specific activities of 100-500 μc per μg.

For all components in the reaction mixture, 0.01 M phosphate buffer pH 7.8, containing 0.15 M NaCl, 2.5% normal rabbit serum and 0.1% sodium azide was used as a solvent. In the assay procedure, reactants were added to 15 $\times$ 1 cm glass test tubes as follows:

1. Buffer, as described, in a quantity sufficient to make a final volume of 1 ml when all reactants had been added.
2. 0.1 ml 0.1 M EDTA.
3. 25 to 400 μl of standard or unknown preparation to be assayed.
4. 0.1 ml of a solution containing 0.05-0.1 mμg of ^{131}I α MSH in buffer (15,000 to 25,000 CPM).
5. 0.1 ml of the antisera to α MSH diluted so that 50 to 75% of the ^{131}I of α MSH was antibody bound in tubes containing no unlabelled α MSH. The final dilution was usually 1:300.

After the final additions had been made, tubes were shaken in a Vortex shaker to ensure adequate mixing and thereafter incubated at 4°C for 5 days.

Bound and free ^{131}I α MSH were separated by the method of Feinberg(4) as modified by Morgen *et al*(5). To this end, on the fifth day of incubation at 4°C, an excess (usually .150 ml) of sheep anti-rabbit gamma globulin was added to each tube in the cold. After an additional 24 hours of incubation at 4°C, tubes were centrifuged at 500 $\times$ *g* for 20 minutes and the supernatant aspirated by continuous suction.

In each assay tubes containing no anti α MSH antiserum and tubes containing no added unlabelled α MSH were prepared. The former were used to correct for mechanical trapping of counts in the precipitate. The latter were used to measure maximal number of counts precipitable under the condition of

* Present address: Dept. of Medicine, School of Med., Univ. of California, Los Angeles.

† Depts. of Pharmacology and Medicine, Univ. of Wisconsin School of Med., Madison.

‡ Veterans Admin. Hosp., New Orleans, La.

§ Mann Research Laboratories, New York, Adrenocorticotropic hormone, porcine pituitary, 200 I.U./mg.

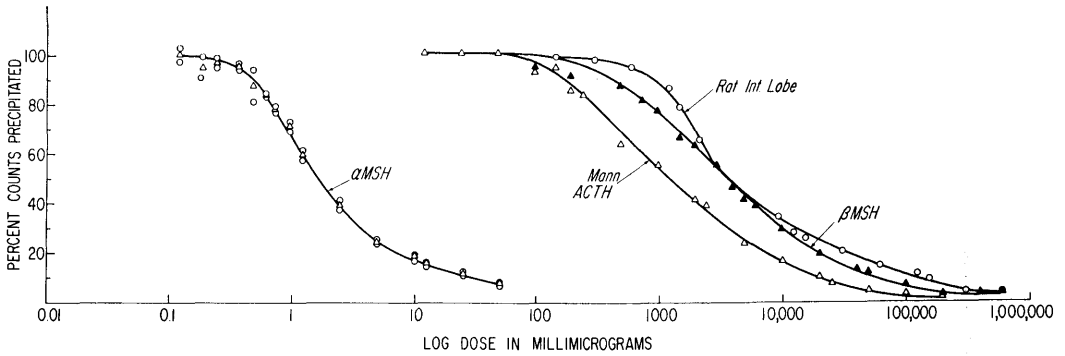

FIG. 1. Log dose response curve for alpha MSH, porcine ACTH, porcine β MSH and intermediate lobe of rats' pituitary. On the ordinate percent counts precipitated and on the abscissa log dose of material assayed in millimicrograms are depicted.

each assay. This number of counts was arbitrarily defined as 100% counts precipitable.

Precipitates were counted in an automatic gamma spectrometer.‖ Results were expressed in terms of percent of counts precipitated when compared to counts precipitated in tubes containing no unlabelled α MSH after correction for mechanical trapping incident to precipitation.

Results and discussion. Dose response curves for α MSH, ACTH, β MSH and intermediate lobes of rats' pituitary glands in a typical assay are shown in Fig. 1. It can be seen that in the assay depicted, accurate detection extended from 0.4 to 5 mμg of the standard α MSH preparation, or approximately a 10-fold dose range. Within these limits, reproducibility in duplicate and replicate assays was $\pm$ 10% of a determined value, a precision difficult to achieve by biological assay.

A minimal detectable quantity in the assay was similar to a minimal effective dose in the biological assay system using hypophysectomized frogs(2). The extinction point of the assay then offered no advantage over biological assay systems.

It will be noted in Fig. 1 that approximately 1000 times more ACTH and approximately 8000 times more β MSH on a weight basis were required to produce curves similar to those obtained with α MSH. On a wet weight basis, intermediate lobe of rats' pituitary was comparable to the purified β MSH preparation. Neither the ACTH nor the β MSH was thought to be free of contamination with α MSH, and, indeed, contamination to the extent of 0.1% would account for these results. Under these circumstances, testing does not permit discrimination between contamination and cross-reacting antigens. However, the fact that the dose response curves for β MSH and ACTH, and rats' intermediate lobe, were slightly different in shape to that of α MSH suggests that cross-reaction is a likely explanation of these results.

Attempts to measure α MSH in the blood of normal rats and frogs have been unsuccessful. Two major problems exist: 1) the sensitivity is apparently insufficient, since comparable volumes of plasma have no detectable biologic activity in an equally sensitive assay using hypophysectomized frogs(2). This indicates α MSH must circulate in amounts less than about 0.4 mμg per ml—a value less than the level described for luteinizing hormone(6), growth hormone(7) or glucagon(8) and thyrotropin(9). 2) Plasma from hypophysectomized rats, humans, or frogs interferes with this assay, even though the technique utilized in separation of antibody bound from free hormone permits quantitation of insulin(11), growth hormone(7), thyrotropin(9) and luteinizing hormone by radioimmunoassay(6). The reasons for this interference are not clear at this time. Quantitation of α MSH in tissue extracts or in buffer may be performed with excellent precision.

The assay has been used in measuring the plasma half-life of α MSH injected into

‖ Picker X-ray automatic gamma spectrometer.

hypophysectomized rats(10). Further studies are in process to attempt to improve the sensitivity by developing better antisera.

The assay devised seems to be a sensitive, specific, and precise assay permitting assays of large numbers of samples with relative ease.

Summary. A radioimmunoassay has been developed for alpha melanocyte stimulating hormone (α MSH). The assay compares favorably with biologic assays in terms of sensitivity and appears to be better in terms of precision and specificity.

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Bone Marrow Composition of Cholesterol-Fed Guinea Pigs.* (31448)

ROSEMARIE OSTWALD, OLFAT DARWISH, DIANA IRWIN, AND RUTH OKEY
Department of Nutritional Sciences, University of California, Berkeley

Dietary cholesterol produces a hemolytic anemia in guinea pigs, which may be either normocytic or macrocytic. The condition is accompanied by enlarged and fatty livers, enlarged spleens and hyperplastic bone marrow (1). The mechanism of this response is unknown. One of the unanswered questions is whether defective RBC are released from the bone marrow of anemic animals or whether the blood cells, as formed, are essentially normal but are damaged by a factor or factors in the peripheral circulation. Pinter and Bailey (2) studied a cholesterol-induced anemia in rabbits. Results from cross-transfusion studies led to the conclusions that much of the trauma to the red cells had occurred during their production and, therefore, that dietary cholesterol had altered the function of the erythropoietic tissue. Our studies (unpublished) with anemic guinea pigs have led us to similar conclusions. A role of lipids not only in the structure but also in the function of cells and subcellular fractions has been recognized(3). It appears plausible that changes in lipid composition may cause disturbances in the cellular metabolism of bone marrow, with subsequent production of damaged red blood cells. We are presenting the results of a study of changes in the lipid composition of bone marrow of guinea pigs in response to dietary cholesterol.

Materials and methods. Groups of young male guinea pigs were fed a semi-synthetic diet containing 30% of casein, 6% of cottonseed oil and amounts of vitamins and minerals adequate for normal growth, with or without addition of 1% cholesterol (Table I). Development of the anemia was monitored by blood counts. When the RBC count had fallen to less than 3 mill/mm^3, the animals and a group of controls were sacrificed. The ends of the tibias and femurs were cut off and preserved for the preparation of marrow sections. Bone marrow smears were obtained from the heads

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