MSH-like Substance from Human Placenta.* (24716)

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A melanocyte stimulating hormone (MSH) from pituitary which is of functional importance in lower animals has been known for several years(1-4). Interest in this hormone was renewed in 1954 after Lerner and coworkers showed that MSH had a role in human physiology(5). This group also reported that blood and urine levels of this homone were elevated during pregnancy(6). This paper describes isolation of MSH-like substance from human placenta.

Isolation procedure is a modification of that employed by Lerner *et al.* for preparation of pituitary MSH(5), which was an adaptation of E. B. Astwood's method of extracting corticotropins from pituitary(7,8). Application of their technics to placenta will be described but details of directions will not be repeated. Full term placentas, average weight 550 g, were collected from Chicago Lying-In Hospital soon after delivery, and then extracted individually. Umbilical cord and membranes were cut and discarded. Remaining placenta together with some maternal blood was cut into squares 5 x 5 cm and placed in 1000 ml of glacial acetic acid and kept at room temperature. The entire procedure was completed within 1 hour after delivery. After 3 days, the solid material was processed with Waring blender and additional 500 ml of glacial acetic acid was added. Total mixture was then heated to 70° and cooled, and solid material discarded. To the supernatant, acetone and 5 M aqueous sodium chloride were added as described by others(5). The resulting precipitate was discarded. An equal volume of ether was added to supernatant and the precipitate was washed thoroughly with acetone, transferred to desiccator, and dried over NaOH. A tan powder was formed, 21 g in average weight/placenta. This was then treated with 20 g of oxycellulose and eluted with 0.1 N hydrochloric acid. The solution was neutralized with NaOH, subjected to brief dialysis, and lyophilized. An average of 350 mg of this material was collected from each placenta. To decrease ACTH potency, this powder was dissolved in H_2O, adjusted to pH 12.8 with NaOH, and heated 5 to 10 minutes at 90-95°(9). The solution was cooled, neutralized, and lyophilized. The final product averaged 120 mg/placenta. The assay employed was similar to *in vitro* frog skin method(10). However, the skin of *Anolis carolinensis* (chameleon) was substituted because of certain advantages(11). Selected lots of hog pituitary MSH from research labs. of Armour and Co., were used as standards.

Properties. The material extracted was a tan powder readily soluble in water. The activity decreased greatly within a matter of weeks when stored at room temperature. As a result the material was assayed soon after preparation. Loss of activity was slowed down by refrigeration. The substance caused darkening of *Anolis carolinensis* skin similar to that caused by known hog pituitary MSH. When the skin is placed in a solution containing the material, there is continual darkening for 20 minutes after which some paling takes place. The darkening is reversed if the skin is placed in Ringer's solution.

Results of assay. The results listed in Table 1 were obtained from assay of MSH preparations from 10 placentas. Average concentration was about 3.05×10^2 units/mg. This activity is much less than the reported value for pure MSH from hog pituitary of 5×10^7 units/mg(5). Our material had a potency 1/200-1/300 of MSH preparations from hog pituitary which we tested.

Discussion. Because the placenta is an organ with large blood volume (estimated 10% plasma)(12), this contribution is a matter of concern. The highest reported value for MSH in serum during pregnancy is 7.9 U/ml (or

* This work was partially supported by grant from Am. Cancer Soc.

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TABLE I. Assay of MSH Preparations from 10 Placentas from 10 Patients.

U*/mg	U*/mg
3.60×10^2	2.65×10^2
2.75 "	4.80 "
1.30 "	2.70 "
4.00 "	3.20 "
3.10 "	2.40 "

* Unit of activity is defined as degree of darkening, measured with photoelectric reflection meter, produced by .04 μg of lyophilized water extract of beef pituitary powder on isolated frog skin(10).

about 7.9 U/g)(6). This makes a hypothetical placenta: whole blood gradient of more than 30,000:1. This material seems present in concentrations greater than can be accounted for by its being derived from placental blood.

This study does not differentiate between placenta as site of storage or site of production of an MSH-like substance. Experiments attempting to answer this question are projected.

It is known that melanocyte stimulating activity is intrinsic to corticotropins. In this study it was noted that after alkalinization and heating, which destroys much of the corticotrophic activity(9), the melanocyte stimulating activity was enhanced 20 to 30 times. In addition, the darkening was qualitatively somewhat different, with a prolonged and slower darkening caused by the alkali treated material.

Conjecture regarding the biological significance of placental MSH may be summarized as follows: The rapid decline of MSH values in blood and in urine soon after delivery (6) might be due to loss of the placenta containing large quantities of this substance. Whether the formation and rapid post partum loss of much of the increased dermal and mucosal pigmentation commonly noted during pregnancy (chloasma) is also related to placental MSH requires further study.

Conclusion. An MSH-like substance was present in considerable concentration in human placenta.

I am grateful to Dr. R. W. Wissler for encouragement and support, to Dr. R. Stoughton for helping initiate this work. Research Laboratories of Armour & Co. were extremely generous in providing material. The following have kindly given me criticism and suggestions: Dr. Z. Hruban, Dr. E. J. Plotz, Dr. J. Fisher and Mr. L. Frazier.

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Received January 8, 1959. P.S.E.B.M., 1959, v100.

Prevention of Viral Lymphoid Leukemia of Mice by Thymectomy.*† (24717)

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Most animals of the high leukemia mouse strains Ak and C58 die of spontaneous leukemia, and all transmit the genetic liability for leukemia to their offspring, whether or not the parents die of leukemia. These genetically determined leukemias originate in the thymus

* Supported by grants of Nat. Inst. of Health and Am. Cancer Soc.

† We acknowledge the skillful technical assistance of Mrs. Sah Sook Cho.