

ELECTROPHORETIC MOBILITY OF HEMOGLOBIN OF THE NORMAL AND THYROIDECTOMIZED CAT

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UDC 612.111.11-06.612.445

It was shown by electrophoresis in agar that by the 5th day after removal of the thyroid gland the mobility of the second fraction of cat hemoglobin changes, whereas the first (leading) fraction moves at the same rate as before. The suggestion is made that hemoglobin circulating in the erythrocytes changes its physicochemical properties.

In consideration of existing views of the probable influence of hormones on protein biosynthesis, the physicochemical characteristics of hemoglobin during hypo- and hyperfunction of the endocrine glands are being studied in this laboratory.

EXPERIMENTAL METHOD AND RESULTS

Thyroidectomy was performed under ether anesthesia on cats of different ages (2 months or older), of both sexes, and weighing from 1 to 3 kg. The gland, which lies on both sides of the trachea at the level of the 3rd-5th tracheal rings, was shelled out of its fascia, thereby facilitating preservation of the parathyroid glands. Blood samples were taken from the heart or femoral vein before the operation, and then daily, starting on the 5th-9th day after the operation. Solutions of hemoglobin were prepared by washing the cells three times with 0.85% sodium chloride solution, followed by hemolysis with distilled water for 15 min and centrifugation. Electrophoresis was carried

out in Difco agar, using potassium-phosphate buffer (pH 7.0). The ionic strength of the buffer in the electrode vessels was 0.05, and in the gel it was 0.0125. Strekalov's cell was used [3]. The thickness of the gel was 2 mm and its concentration 0.8-2%. Distillation was carried out in the freezing compartment of a refrigerator at -13°C , when the temperature in the gel was 8°C . The conditions of distillation were: voltage 16 V/cm, current 1.4 mA/cm², time 1-2 h. The samples were applied to strips of filter paper measuring 1 x 5 mm. Staining was carried out without preliminary fixation, with a mixture of benzidine and hydrogen peroxide prepared before use. The specimens were photographed as the color developed.

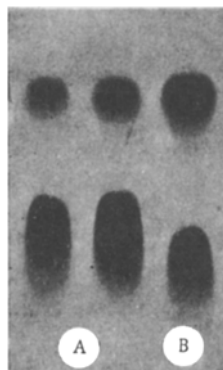


Fig. 1. Electrophoresis of hemoglobin of a normal cat (A) and of a cat 5 days after thyroidectomy (B).

Normal adult cat hemoglobin consists of two fractions separable by electrophoresis on starch [4] and agar [2]. The ratio between the two hemoglobin fractions varies considerably [2, 4]. The first fraction is more compact and the second more spread out (Fig. 1). Analyses on the 5th-9th days after thyroidectomy showed that the position of the second fraction of hemoglobin was substantially altered: it was shortened on account of its leading part (Fig. 1B) and, in some cases, the whole electrochromatogram was retarded a little. This picture was maintained throughout the period of observation, until 60-90 days after the operation. In two animals, however, the normal electrophoretic mobility of the hemoglobin was restored by the 18th-20th day. Since no morphological study of these animals was undertaken, it is difficult to decide what was the cause of the early restoration of the electrophoretic mobility of the hemoglobin.

Laboratory of Age Physiology and Genetics, State Pedagogic Institute, Komi ASSR. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 75, No. 3, pp. 59-61, March, 1973. Original article submitted February 29, 1972.

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An electrophoretic picture similar to that just described was observed earlier in cat fetuses weighing 40-60 g [2] and also in two recently. Similar changes take place as a result of feeding with methylthiouracil. In all cases the electrophoretic mobility of the hemoglobin is an earlier indicator of hormonal changes taking place in the cat than the ordinary clinical indices: a decrease in the red cell count and hemoglobin concentration in the blood and a decrease in the hematocrit index.

Two features must be specially mentioned. First, the change in electrophoretic mobility of cat hemoglobin takes place so quickly that it is evidently not a question of a change in the synthesis but a change in the physicochemical properties of hemoglobin already present in the blood stream. In fact, assuming that the life span of the cat's red blood cells is about 70 days [1], $\frac{1}{70}$ of the total mass of red cells must be replaced every day. In those 4 or 5 days during which the change takes place in the electrophoretic mobility of the hemoglobin after removal of the thyroid gland or after administration of methylthiouracil to cats, the added amount of the new type of hemoglobin resulting from a change in synthesis, if such took place, would be so small that it could not be detected by electrophoresis in agar.

Second, the degree of mobility of hemoglobin which becomes apparent for the first time a few days after the beginning of treatment subsequently remains unchanged, so that the delay of the second hemoglobin fraction is maintained throughout the period of observation.

Both these features of the behavior of hemoglobin draw attention to a class of phenomena, connected with the properties of this protein, which includes physicochemical transformations based on the same primary structure of the compound. It remains to be shown to what extent these transformations influence the physiological properties of hemoglobin.

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