SPECIAL FEATURES OF THE REACTION OF SERUM ALBUMIN WITH HEMOGLOBIN IN THE HUMAN EMBRYO

V. I. Yablokova and S. S. Vasileiskii

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In normal physiological conditions very slight hemolysis takes place constantly in the mammalian body and the small quantity of hemoglobin liberated, when it enters the plasma, is bound by a serum protein–haptoglobin [8-12].

The complex of hemoglobin with haptoglobin does not pass through the kidney barrier [5], so that iron is retained in the body. If in some conditions, the amount of hemoglobin entering the plasma exceeds the "haptoglobin capacity," the hemoglobin reacts with the serum albumin, transferring heme to it, and the heme is linked to the albumin in the form of hematin. The complex thus formed has been called hematin-albumin or methemalbulin [4, 6, 7, 11, 13]. The theory of the reaction of transhemation has been studied in detail [1, 3]. This complex can be detected during electrophoresis by means of a benzidine reagent, like hemoglobin. The remainder of the free hemoglobin (in a concentration of approximately 130 mg/100 ml plasma) is excreted through the kidneys, and within a wide range of concentrations the excretion bears a linear relationship to the concentration of hemoglobin in the plasma [14].

In the course of investigations of the blood serum of human fetuses aged between 14 and 40 weeks, it was observed that during electrophoresis of the serum from fetuses of the earlier ages, during treatment with benzidine reagent, the albumin region stained intensively (in the absence of hemolysis), whereas in older fetuses the staining was hardly visible (Fig. 1).

The problem of the competitive fixation of hemoglobin by haptoglobin was not considered, because haptoglobin was absent from both groups (haptoglobin is found very rarely in the serum of full-term fetuses).

An attempt was made to discover whether the increased fixation of hematin by albumin in the embryo is due to a property of the albumin itself or to peculiarities of the embryonic hemoglobin. For this purpose, the ability of embryonic hemoglobin and of adult human hemoglobin to react with pure albumin was compared during countercurrent electrophoresis.

EXPERIMENTAL METHOD AND RESULTS

Wells in agar gel were filled with a mixture of serum with agar as described earlier [2]. A gutter measuring 3 mm was cut out at a distance of 7 mm from the wells on the anode side, measuring 3 mm along the course of the lines of force and 20 mm in a transverse direction. A mixture of hemoglobin solution with agar was poured into the gutter. During electrophoresis, the albumin fraction met the hemoglobin zone, passed through it, and appeared on the other side of the gutter.

Optimal experimental conditions were as follows: dilutions of serum with agar for filling the start wells 1:19; concentration of hemoglobin in the gutter 0.1 mg/ml, duration of electrophoresis 3 h in 2% agar gel, made up in 0.03 M veronal buffer, potential gradient 5 V/cm.

At the end of electrophoresis the surface of the agar was flooded with saturated benzidine solution in 10% acetic acid with the addition of H_2O_2 at the rate of 0.3 ml/10 ml. After 60 sec, the solution was washed off with a stream of water and photographs were taken in standard conditions in reflected light through an interference filter with a transmission maximum at 602 mμ (Isopan F film, sensitivity 17 Din, Agfa). All manipulations connected with photography were completed within one min. Before staining, a row of blocks of agar gel containing different known concentrations of hemoglobin (usually from 0.1 to 0.01 mg/ml) were stuck to the agar. Such a calibration

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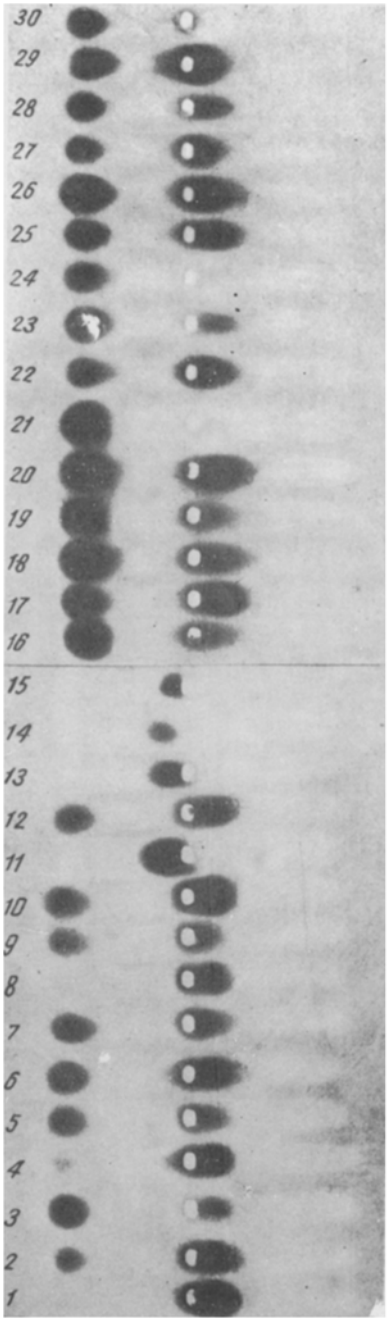

Fig. 1.

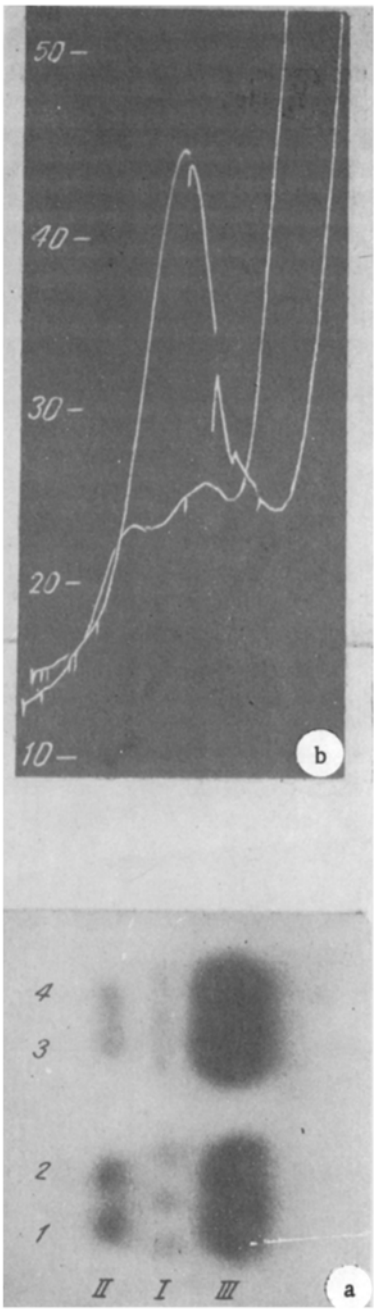

Fig. 2.

Fig. 1. Electrophoresis of blood serum of human fetuses of various ages, after staining with benzidine reagent. Electrophoresis in 1% agar gel with 0.05 M veronal buffer (pH 8.6). 1-10, 12) Serum of full-term fetuses; 11, 13-15) adult human sera; 16-30) serum of fetuses from 36 to 9 weeks.

Fig. 2. Comparison of reaction power of embryonic hemoglobin and adult human hemoglobin during counter-current electrophoresis. a: Electrophoresis in 2% agar gel with 0.03 M veronal buffer (pH 8.6); 1, 2) embryonic hemoglobin; 3, 4) adult human hemoglobin; I—start cells; II—albumin region; III—excess of hemoglobin driven off; b—densitograms recorded from the negative of the photograph a; along the axis of ordinates —transmission in %; I—densitogram of electrophoretic record one; II— densitograms of electrophoretic recording four.

series was present in each experiment, and the results of the photographic photometry, obtained by means of the MF-4 microphotometer, were verified in relation to each series.

In these experimental conditions the albumin was saturated intensively with hematin. The reaction power of the hemoglobin in relation to saturation of the albumin with hematin was always 50-100% higher in the case of the embryo than in the case of the adult human (Fig. 2).

Hemoglobin also differed in the experiments in which it reacted with adult human serum and in experiments with fetal serum. Consequently, the greater saturation of the fetal albumin with hematin was evidently due to peculiarities of the embryonic hemoglobin. The more intensive transhemation reaction in the fetus may be provisionally explained as a compensatory mechanism associated with the absence of a haptoglobin system.

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