HEME TRANSFER FROM FETAL HEMOGLOBIN IN THE PRESENCE OF SODIUM HYDROSULFITE

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In physiological conditions destruction of the erythrocytes is constantly taking place and the hemoglobin entering the plasma is attached to a special serum protein—haptoglobin. United in this complex, the hemoglobin does not pass through the kidney barrier, so that the iron is retained in the body.

In certain conditions the amount of hemoglobin entering the plasma exceeds a certain limit and the reaction of transhemation takes place, i.e., heme is transferred from hemoglobin to serum albumin [1, 2].

The reaction of transhemation has been found to take place more intensively in the fetus than in the adult human. However, it was not known whether this phenomenon is due to oxygenation of the hemoglobin or is independent of it. This problem has arisen because the velocity of the transhemation reaction is dependent on the reversible fixation of oxygen: oxyhemoglobin is less reactive than hemoglobin [2]. Meanwhile, fetal hemoglobin (HbF) in solution, if other conditions are equal, is known to be less oxygenated than adult hemoglobin (HbA); the opposite relationships are found inside the erythrocytes [4, 5].

In the present investigation the reaction of transhemation of HbF was studied in conditions excluding oxyhemoglobin formation.

EXPERIMENTAL METHOD AND RESULTS

The experimental method was as follows. Adult human serum was mixed in one tube with adult human hemoglobin (HbA), and in another tube with HbF. The concentration of the hemoglobin solution was strictly equalized before the experiment. For this purpose samples of both solutions were taken and their hemoglobin concentration determined after preliminary detachment of the oxygen by the addition of sodium hydrosulfite (2 ml of 0.01% hemoglobin + 20 mg $Na_2S_2O_4$), followed by conversion of both the HbA and HbF into hematin by the addition of 0.05 ml of a 0.1 N NaOH solution. In this way the hemoglobin concentration was determined from the heme concentration; in these strict conditions the readings of the photometer were independent on differences in the character of formation of complexes between hematin and the denatured globins of HbA and HbF, as is observed, for example, when the hemoglobin concentration is determined by Sahli's method.

After the determination of the concentrations of the initial solutions of HbA and HbF, solutions of equal concentrations were prepared. Next, the reducing agent $Na_2S_2O_4$ was added simultaneously to both tubes, to convert both hemoglobins into the same form of "reduced" hemoglobin. Saturation of the albumin with hematin took place at room temperature for 30 min. Wells cut out of an agar gel were then filled with a mixture of serum and hemoglobin with agar.

The optimal experimental conditions were: dilution of serum with agar for filling starting wells 1:24, concentration of hemoglobin in well 0.12 mg/ml; duration of electrophoresis 1 h 30 min 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 covered with a saturated solution of benzidine 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 the result photographed immediately in standard conditions.

Before staining, a calibration series was prepared in each experiment: the wells in the agar plate were filled with a mixture of agar and different concentrations of hemoglobin (usually from 0.015 to 0.0004

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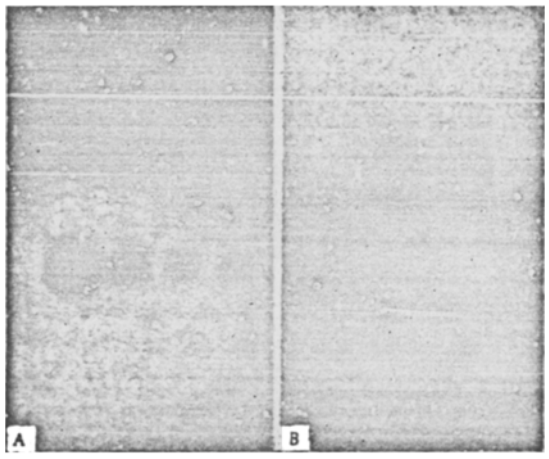

Comparison of reactivity of embryonic hemoglobin and adult human hemoglobin. A) Calibration series, hemoglobin concentration from 0.015 to 0.0004 mg/ml; B: 1) adult hemoglobin 2) fetal hemoglobin; I) region of albumins; II) starting wells; III) zone of hemoglobin (excess migrating during electrophoresis).

mg/ml). This scale was treated with benzidine reagent at the same time as the main preparation was stained (see figure).

The results of photometry, obtained with the MF-4 microphotometer, were checked against this series.

Despite the change in the experimental conditions in the presence of the reducing agent, the results were found to be similar to those obtained earlier, i.e., the greater degree of saturation of the fetal albumin with hematin was due to differences in the HbF, and not simply to the phenomena of oxygenation.

What is the biological significance of the phenomena discovered? Possibly the more intensive transhemation reaction in the fetus is a compensatory factor directed toward the retention of iron in the body because of the absence of a haptoglobin system, which performed this important function, from the fetus.

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