Single Radial Diffusion Using Catalase Antibody as Screening Method of Hypocatalasemia

MASANA OGATA and JUNKO MIZUGAKI
Department of Public Health, Okayama University Medical School, Okayama


Hypocatalasemia blood showing one half the catalase activity and catalase protein (Ogata and Takahara 1963) of normal blood is heterozygous or genetic carrier state of acatalasemic gene. If it is possible to examine closely the incidence of hypocatalasemia over wide geographical areas, it will enable us to determine the gene flow or the flow of races. A screening method of hypocatalasemia was reported by Takahara et al. (1960). However, the method involves a rather complicated procedure to measure the catalase activity and has disadvantage such as that the activity itself decreases during preservation. The authors devised a new screening method using the single radial diffusion (Fahey and Mckelvey 1965; Mancini et al. 1965) between anti-crystalline beef liver catalase rabbit serum and human blood catalase based on immunological cross reaction.

MATERIALS AND METHODS

Hypocatalasemia blood samples were obtained from individuals whose genotypic and phenotypic characterization of the biochemical variation have been studied thoroughly. Catalase activity was determined by the perborate method (Feinstein 1949).

Specific antiserum for the catalase antibody was prepared by weekly twice injection of 9.2 mg of crystalline beef liver catalase (Sigma 30,000 unit/mg) with Freund’s complete adjuvant to a rabbit.

The erythrocytes from normal and hypocatalasemia subjects were removed from heparinized or EDTA-added human blood by centrifugation, and washed three times with saline. The packed erythrocytes were hemolyzed with 2 volumes of distilled water and incubated at 4°C overnight. The supernatant of hemolysate thus obtained was served as antigen for the single radial diffusion plate.

Three grams of special agar-noble (Difco) were added to 100 ml of barbiturate buffer, pH 8.4 and 0.05 in ionic strength, and melted. Antiserum was diluted with the same barbiturate buffer in proportion of 1:10. Equal volumes of antiserum and 3% agar-gel were mixed at 56°C and poured onto a horizontally placed clean glass plate with a dimension of 55 x 75 mm. Circular wells were punched out in the gel, using a needle of 5 mm bore.

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361
Each of the wells received 10 μl of antigen solution. The plate was kept in a strictly horizontal position in a moist box at 20-25°C. Antigen-antibody precipitate was formed in the agar in a concentric ring around the antigen well. The precipitate ring diameters were measured at 24 hr after pouring antigen.

RESULTS AND DISCUSSION

A graph (Fig. 1) was prepared for comparing the common logarithms of the beef liver catalase concentration to the precipitate ring diameters, indicating that there is a straight line relationship between them.

Typical precipitate patterns obtained with normal and hypocatalasemia hemolysates from heparinized blood are shown in Fig. 2. Bubble formation was observed on the agar plate after washing with saline and pouring H₂O₂ solution.

Hemolysates from 4 volunteers of normal subjects and from 2 hypocatalasemias were allowed to diffuse in a plate containing 1:20 dilution of antiserum. The diameters were plotted as a function of catalase activity expressed as common logarithms of PU/gHb. Data indicated that there was a linear relation between

![Graph](attachment:graph.png)

Fig. 1. Effect of increasing amount of crystalline beef liver catalase upon the diameter of the precipitate.

![Image](attachment:image.png)

Fig. 2. Diffusion rings obtained in the antibody-agar plate with normal and hypocatalasemia hemolysates. Photographed after washing off hemoglobin with saline. N: Normal hemolysate; H: hypocatalasemia hemolysate.
Fig. 3. Relationship between common logarithm of the human blood catalase activity and the diameter of the precipitate.
- *: Normal blood with heparin; 
- #: Hypocatalasemia blood with heparin;
- o: Normal blood with EDTA; 
- #: Hypocatalasemia blood with EDTA.

Fig. 4. Frequency distribution of the diameters of the precipitate with catalase in normal blood by the single radial diffusion method.

The common logarithm of the blood catalase activity and the diameter of the precipitate within the range of antigen activity between 1,000 and 6,500 PU/gHb (Fig. 3). Frequency distribution of the diameters of hemolysate from EDTA-added blood of 46 normal subjects is shown in Fig. 4. Common logarithms of the blood
catalase activity per hemoglobin of normal subjects fit to the normal distribution (Ogata et al. 1972). Therefore the frequency distribution of diameters from normal blood catalase determined by the single radial diffusion (Fig. 4) appears to be similar to the normal distribution, though it is not fit perfectly. From this result, it is considered that the standard deviation calculated from the diameter will be available for an approximate value of standard deviation of normal distribution. The value of \( m-\sigma \) was calculated as 9.89—0.44=9.45 mm, and that of \( m-3\sigma \) was 8.57 mm. The diameters obtained from two cases of hypocatalasemia are 8.0 mm and 7.1 mm, respectively, and they are lower than the value of \( m-3\sigma \) from the normal blood. Therefore the single radial diffusion method can be used as the screening method of hypocatalasemia.

This method especially fits for the screening method of hypocatalasemia owing to the simplicity. In addition to this, this method is also useful for the confirmation of hypocatalasemia which was checked only by the catalase activity, since the parallel relationship between the catalase activity and the catalase protein can be examined at the same time.

References