Acta Medica Okayama

Volume 18, *Issue* 1 1964 *Article* 2

FEBRUARY 1964

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Abstract

1. In the absorption spectra of crude catalase solution (Stages 2, 3, and 5) of normal blood, three absorption bands characterizing catalase molecules are recognized. 2. The three absorption bands specific for catalase cannot be found in acatalasemic blood extracts (Stages 2 and 3). 3. It is inferred that catalase is not present in the crude catalase extract from acatalasemic red blood cells.

*PMID: 14201063 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 18, 9—14 (1964)

THE CATALASE PROTEIN OF ACATALASEMIC AND HYPOCATALASEMIC RED BLOOD CELLS II. SPECTROPHOTOMETRIC STUDY

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Received for publication, February 10, 1964

With respect to the question of the presence or absence of catalase protein, we have already demonstrated by paper electrophoretic[1] and immunological[2] methods that catalase protein is absent in acatalasemic[3-7] blood while it is one half of the normal in hypocatalasemic[8,9] blood. This seemed to indicate the lack of active catalase molecules in acatalasemic blood. To verify the above assumption, we carried out the spectrometric study on the presence or absence of catalase absorption bands in the acatalasemic blood extracts, Stages 2 and 3 of HERBERT and PINSENT[10]

MATERIALS AND METHODS

Heparinized blood taken from the cubital vein of normal and acatalasemic individuals was used.

The method used for catalase purification was essentially the same as described by HERBERT and PINSENT[10]. The red blood cells were separated from 15 ml of heparinized human blood by centrifugation and washed three times with 0.9 per cent saline solution, and then an equal volume of distilled water was added to the cell suspension (Stage 1). This cell suspension was treated with ethanol-chloroform (3 : 1) in volume ratio of the cell suspension to the ethanol-chloroform mixture, 1 : 0.44, and stirred for 15 minutes.

The precipitated hemoglobin was discarded by centrifugation and the supernatant (Stage 2 solution) thus obtained was divided into two parts. One part to be used for the spectrophotometric experiment was dialyzed against saline solution. The other part was treated with a suspension of $Ca_3(PO_4)_2$ gel prepared according to the method of KEILIN and HARTREE[11]. After centrifugation, the precipitate containing the catalase activity was shaken vigorously with a solution containing 0.5 M potassium oxalate and 0.5 M NaH_2PO_4. The calcium oxalate produced by this procedure was precipitated by centrifugation and discarded.

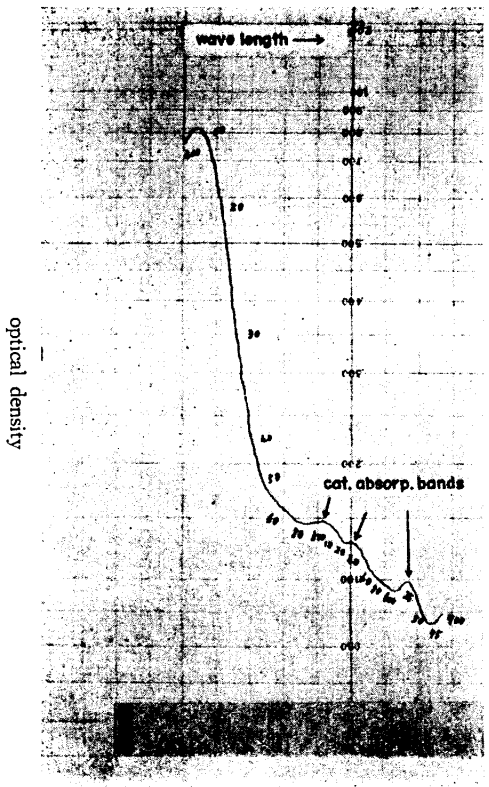

Fig. 1-A Absorption spectrum of crude catalase solution (Stage 5 of Herbert and Pinsent)

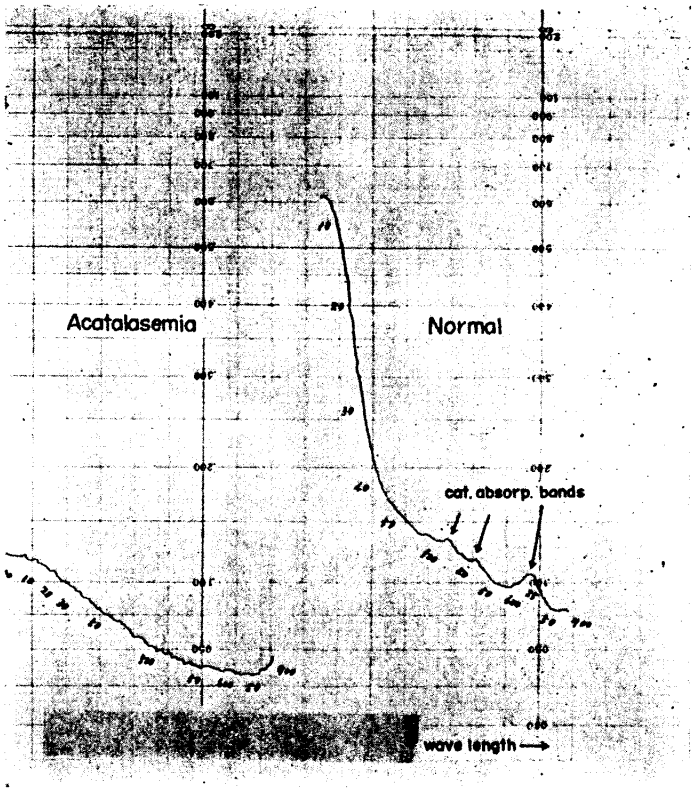

Fig. 1-B Absorption spectrum of crude catalase solution (Stage 2)

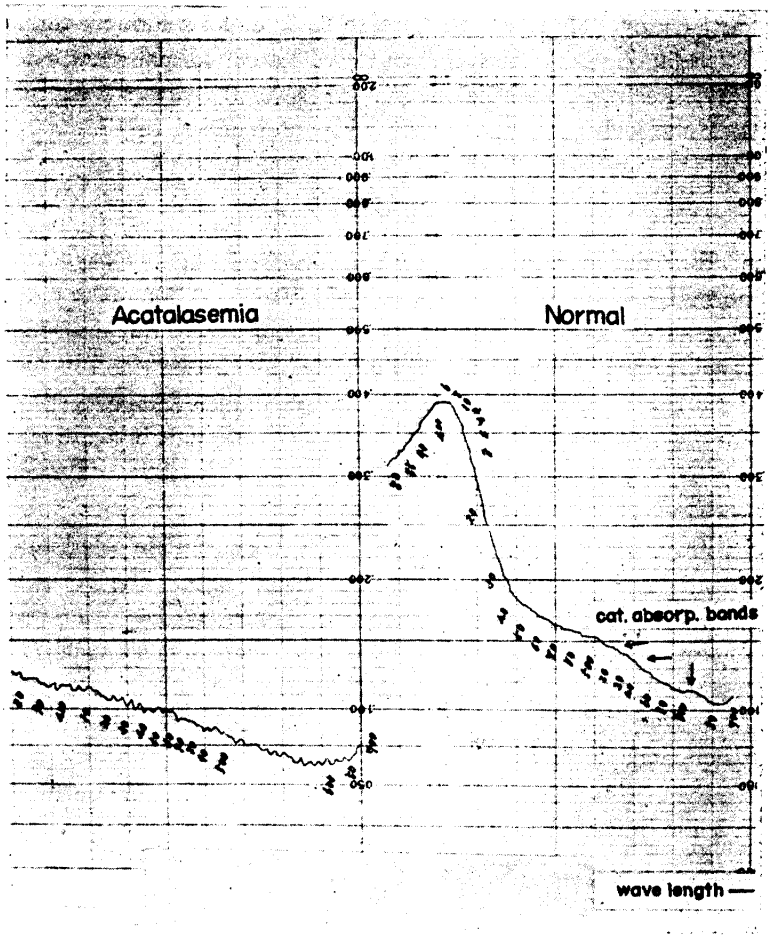

Fig. 2 Absorption spectrum of crude catalase solution (Stage 3)

The supernatant (Stage 3 solution) containing the catalase activity was dialyzed against saline solution at 5°C overnight. This dialysate, having Kat. *f.* of 8,000 by Euler's method[12], was used for the spectrophotometric experiments.

The Stage 5 solution was obtained by the use of ammonium sulfate from the stage 4 solution[10], prepared from Stage 3 by the method of ammonium salfate fractionation. These samples, Stage 2, 3, and 5 solution, were adjusted to pH 6.8 by diluting with two volumes of 0.2 M, pH 6.8 phosphate buffer, and used for the spectrophotometric measurements. All analyses were carried out with a Beckman DK spectrophotometer with automatic recorder.

RESULTS

The absorption spectrum of purified catalase solution (Stage 5) obtained from a normal subject shows three absorption bands at 505, 520 and 625 mμ. These results are shown in Fig. 1-A. This coincides with the absorption bands of the crystalline red blood cell catalase reported by HERBERT and PINSENT[10].

The absorption spectrum of crude catalase solution (Stage 2) from normal red blood cells, is observed to have three absorption bands which are the same as in Stage 5. These results are shown in Fig. 1-B. In the acatalasemic blood absorption bands of catalase cannot be detected.

Fig. 2 illustrates the absorption spectra of crude catalase solution (Stage 3) obtained from normal and acatalasemic red blood cells. The normal extract (Stage 3) has three maximum absorption bands, but their peaks are lower than those of Stage 2. This seems to be due to the lower catalase concentration in Stage 3, probably because a portion of the catalase is lost in the purification process from Stage 2 to Stage 3. In the case of acatalasemic extract no absorption bands of catalase are visible.

From these results it is concluded that there are no catalase molecules in the stage 2 and 3 extracts of acatalasemic red blood cells.

DISCUSSION

To determine whether catalase is present or absent in acatalasemic blood we have conducted an assay for catalase by a number of different methods. In the previous experiment we found that the extracts of acatalasemic blood did not react with catalase antibody. From this immunological study we concluded that the catalase protein was lacking in these extracts. Also in the paper electrophoretic studies of the Stage 3 extract of acatalasemic blood, no catalase peak could be recognized at all. This finding further confirms the absence of catalase protein in agreement with the immunological studies.

In the paper electrophoretic experiment using normal blood, we observed brown coloration at the catalase position in the Stage 3 extract before staining with brom-phenol-blue. In Stage 3 extract of acatalasemic blood we could not detect the brown color. Since the brown coloration itself is characteristic of catalase molecules, these results suggested that catalase molecules are absent in acatalasemic blood.

In this spectrometric study, our previous findings have been further confirmed by the fact that acatalasemic blood extracts (Stages 2 and 3) did not show any absorption bands of catalase.

CONCLUSION

1. In the absorption spectra of crude catalase solution (Stages 2, 3, and 5) of normal blood, three absorption bands characterizing catalase molecules are recognized.

2. The three absorption bands specific for catalase cannot be found in acatalasemic blood extracts (Stages 2 and 3).

3. It is inferred that catalase is not present in the crude catalase extract from acatalasemic red blood cells.

ACKNOWLEDGEMENT

We want to thank the late Prof. K. Ohtahara, Department of Public Health, for his valuable suggestions during this work.

This investigation was supported in part by Public Health Service Research Grant AM 06793 —01 GEN, from the National Institute of Arthritis and Metabolic Diseases.

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