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AN AUTOMATED DETERMINATION OF LIVER CATALASE

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Introduction

A number of manual methods and their modifications have been used for the determination of catalase activity (1, 2, 3, 4, 6, 7, 9, 11), but little has been accomplished with automation. The automated procedure reported here is an adaptation of the colorimetric technique (4, 6) which utilizes titanyl sulphate as a chromogenic reagent and Triton X-100 as a surfectant to obtain total catalase activity. As reported preveiously (10), catalase activities determined by the titanyl sulphate method and potassium iodate method correlated well and the coefficients of correlation on rat and mouse liver catalase activity were 0.946 and 0.957, respectively. Even with the former method, which is currently in wide use due to its simplicity and reliability, difficulties were encountered when attempts are made to cope with a large number of enzyme samples. Although minor modifications were necessary to adapt the manual procedure to the automated method, the results obtained by either of the procedures correlated excellently and fluctuation of data seen in manual determination was almost completely eliminated.

Materials and methods

I. Materials

1) Animals. Male albino mice, dd strain, weighing approximately 20g and rats of Wistar strain weighing approximately 130 g were used. Animals were fed for at least two weeks with commercial Oriental solid diet with water, ad libitum, before the beginning of each experiment.

2) Toxohormone preparation. Crude toxohormone was prepared from the non-necrotic portions of Yoshida sarcoma tumors produced in rats as an ethanol precipitate by the method of Nakagawa et al. (8).

Subsequent fractionation on DEAE-Sephadex column was carried out as reported previously (5).

II. Methods

1) Preparation of enzyme solution. The animals were killed by decapitation and exanguination. The liver was immediately removed and weighed. The entire liver was homogenized with 9 volumes of ice cold deionized water in a Potter-Elvejhem type glass homogenizer and the homogenate was further diluted 30 times with ice cold deionized water and transferred into a sample cup. On each homogenate, dry weight was measured.

2) Preparation of reagents.

a) Buffer and substrate solution. Von Euler and Josephson's direction was followed: 7.5 ml of 3% H_2O_2, 15.1 g of $Na_2HPO_4.12H_2O$ and 3.55 g of KH_2PO_4 were dissolved in 1 litter of deionized water and used as the substrate. A similar solution without hydrogen peroxide was used as the buffer solution. Both solutions was kept in an ice water bath

during determination.

b) Titanyl sulphate reagent. Eight per cent solution of titanyl sulphate in 2 N H_2SO_4 was prepared and filtered before use.

c) 10 N H_2SO_4.

d) 0.2% aquanous Triton X-100 solution.

3) Assay procedure. The method employs a proportioning pump, sampler II, large bore time delay coil, phototube colorimeter and recorder. The flow diagram for the catalase determination is illustrated in Fig. 1.

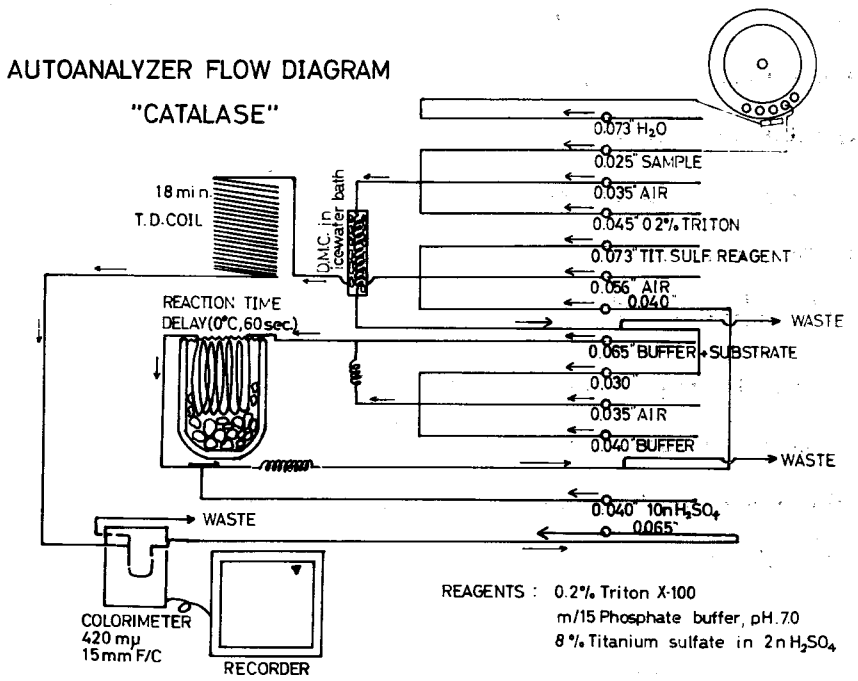

Fig. 1. Schematic of apparatus used in automated catalase determination.

The enzyme solution, which is diluted to 1:300 with deionized water as described above, was aspirated under contonuous mixing and diluted with 0.2% Triton X-100 solution and mixed in a double mixing coil immersed in an ice water bath. Then a portion of this suspension was diluted with chilled buffer and mixed. This was followed by the addition of the substrate solution. The mixture was allowed to react exactly for 60 seconds in a reaction time delay coil immersed in an ice water bath and the reaction is terminated by the addition of 10 N H_2SO_4. To one volume of the reaction mixture was added 0.3 volumes of the titanyl sulphate reagent and coloration was completed in a large bore time delay coil. The stream was fed to a colorimeter with a 15 mm flow cell and absorbancy at 420 mμ was recorded automatically on a log scale chart paper. A water washout cup was used between samples at a 20/hour sampling rate.

The enzyme activity is expressed as a reaction constant, k, by calculating from the following equation:

$$k^{*} = \frac{1}{t} \log \frac{a}{a-x} \cdot 0.327^{**}$$

where t, a and x represent time, the initial concentration of substrate, and the amount of substrate decreased per unit of time, t, respectively.

Since the method of von Euler and Josephson (11) is for 50 ml of substrate solution, the specific activity (k/unit dry weight of the enzyme) in the present method should be divided by 2 to compare with the value obtained by their original method.

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* reaction constant of catalase corresponding to 1 mg wet weight of the liver

** coefficient computed from the dilution factors of the manifold

Results and discussions

In general, such conditions as temperature, concentration of reagents, and automation setup were adopted directly from the manual procedure described by Hozumi, Sugimura and Nakahara (6, 9).

The addition of the surfectant Triton X-100, which did not show any interference with color development, in the present procedure facilitated the determination of total liver catalase activity.

In a preliminary experiment, however, it was found that the addition of Triton to the diluted enzyme solution caused a significant reduction in enzyme activity, which may possibly be due to an inactivation of enzyme, if the solution is left standing for more then 30 minutes at room temperature.

The use of one double mixing coil for this step fulfilled the requirement to obtain a total catalase activity without inactivation.

To test the reproducibility of the assays, 10 samples of the same mouse liver homogenate were run simultaneously and the results are shown in Fig. 2. Standard deviation was less than 3% of the mean catalase activity.

The automated method was compared with the manual procedure and is presented in Fig. 3. The coefficient of correlation on 40 mouse liver catalase activities determined by the two procedures was 0.889 ($p < 0.01$). It should be added that the manual procedure was performed in another laboratory by different operators.

By the automated procedure, the mean normal value of liver catalase activities for mice and rats were 398 ± 54.9 ($n = 24$), 333 ± 42.9 ($n = 20$),

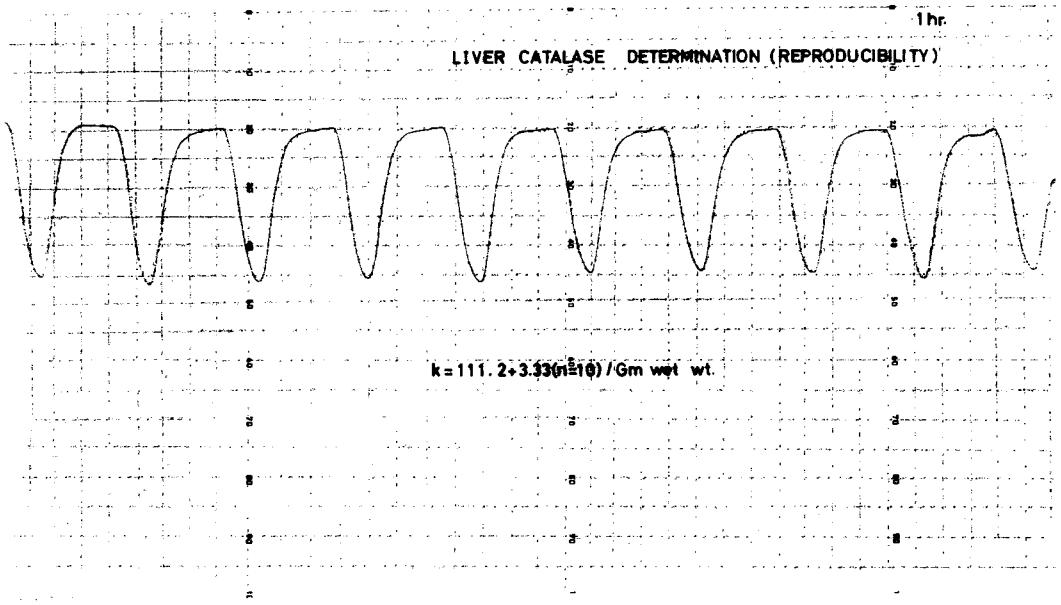

Fig. 2. Recording of mouse liver catalase activity on a single specimen to test reproducibility of the procedure.

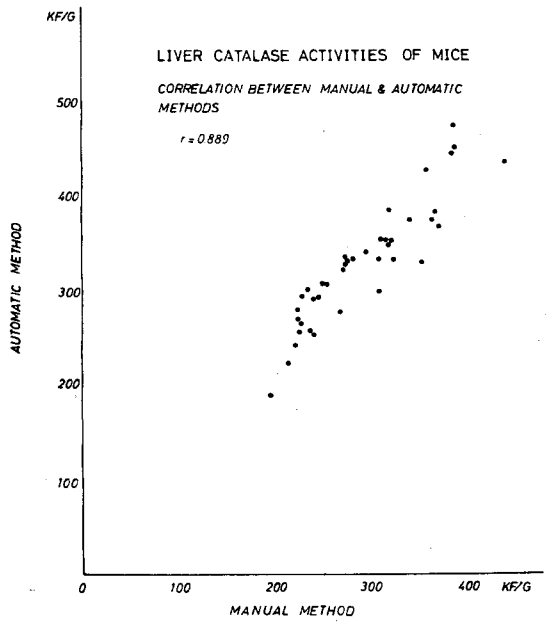

Fig. 3 Correlation of liver catalase activities of mice determined by the automated and manual procedures.

respectively.

Fig. 4 shows an example of bioassay experiment of toxohormone fractions prepared from subcutaneously inoculated Yoshida sarcoma.

The liver catalase activities of mice were determined 24 hours after an intraperitoneal injection of 2 mg of each toxohormone fraction dissolved in 1 ml of distilled water. A moderate inhibition of mouse liver catalase was apparent in the groups of mice injected with Fractions 1, 2 and 3.

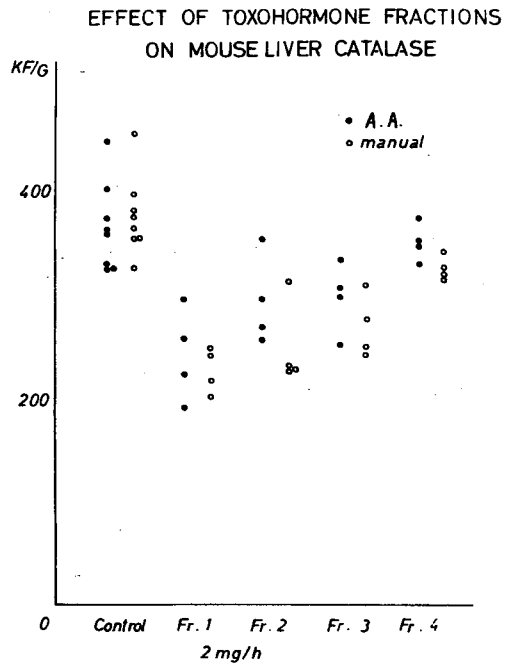

Fig. 4. Bioassay experiment of toxohormone fractions prepared from Yoshida sarcoma tumors by the automated determination of mouse liver catalase.

Summary

An automated procedure for measurement of total liver catalase activity by the titanyl sulphate method is presented. The method based on the

reaction of $TiOSO_4$ and H_2O_2 to produce yellow pigment $TiO(SO_4H)_2$, which is stable in sulfuric acid solution.

Procedure details, precision and accuracy are described. The method is especially suited for the determination of enzyme activity on a large number of samples such as in the bioassay of the toxohormone fractions obtained by column chromatography, and also catalase isozyme study by the use of isoelectric fractionation.

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