Summary

The s.c. injection of talc, MgSiO₃, into mice results in a decrease of hepatic catalase activity. A transient, but marked rise in catalase activity was observed on the 4th day following talc injection. This rise in activity was manifest primarily in the mitochondrial fraction. A concomitant increased incorporation of radioactive leucine occurred in the microsomal fraction. The relationship between the increased catalase activity and increased incorporation of leucine is discussed with respect to enzyme biosynthesis.

Introduction

The depression of liver catalase during tumor growth was long considered to be specifically a manifestation of malignancy (17). This view has become less tenable as a number of diverse organic compounds have been shown to depress hepatic catalase to varying degrees (discussed in Refs. 8, 19, 20). Recently, Nishimura and co-workers have shown that liver catalase could be markedly and reproducibly depressed when mice were injected with an aqueous suspension of talc, MgSiO₃ (20). Maximum depression of hepatic catalase occurred at 48 hr. No inhibition of the enzyme was obtained when catalase and talc were incubated together in vitro.

The present study describes the changes in catalase activity in subcellular fractions of mouse liver on successive days following talc injection. Concomitantly, in a preliminary attempt to determine the site of catalase biosynthesis, the extent of incorporation of leucine-¹⁴C in the cell fractions was also measured.

Materials and Methods

Animals

Inbred male Strong A mice were used. The animals, 4–6 months of age, were housed in airconditioned quarters and had free access to Rockland mouse diet and water.

Talc Suspension

The preparation and s.c. injection of talc was similar to that described previously (20), except that each animal received 1.0 ml of a 0.67% suspension of talc. The smaller quantity of talc was easier to inject and produced an effect equivalent to the larger dose used previously.

Enzyme Preparations

The following cell fractionation procedure was used except where noted otherwise. A 1% total homogenate of mouse liver was prepared by homogenizing 80-100 mg of tissue with 2 ml of 1% Triton (prepared in 0.1 M phosphate buffer, pH 6.8) in a cold all-glass Potter-Elvejhem homogenizer for 1 min. The homogenate was diluted to volume with 0.1 M phosphate buffer (pH 6.8) and centrifuged at 800 x g for 15 min at 5°C to remove cellular debris not solubilized by Triton. The supernatant was diluted 1:1 with phosphate buffer immediately before catalase was to be assayed.

Pellet and high-speed supernatant fractions were prepared from the liver in a manner similar to the above procedure, except that the tissue was homogenized in 2 ml of phosphate buffer instead of Triton. After low speed centrifugation, a 5-ml aliquot of the supernatant was again centrifuged at 18,400 x g for 20 min at 5°C. The supernatant was decanted, and the pellet was dissolved and made to 5 ml with 0.25% Triton (in phosphate buffer). Both supernatant and pellet were used without further dilution. Enzyme preparations were kept in ice water. This type of simple fractionation was used in the early experiments when the aim was only to observe whether or not modification in enzyme activity occurred in the particulate (presumably mainly mitochondrial and lysosomal rich particles (9, 11)) or in the supernatant fraction (which would include most of the microsomal particles).

Catalase Assay

The permanganate titration method (5, 25) was modified with respect to small reaction volume (3 ml), short reaction time (30 sec), and a single titration. The procedure and kinetic data of reliability are described below.

To a 25-ml Erlenmeyer flask was added 1.9 ml of phosphate buffer (0.1 M, pH 6.8) and 0.1 ml of enzyme preparation. The
mixture was stirred with a magnetic mixer at a slow to moderate speed. The size of stirring bar and speed of mixing were not critical, but excessive agitation was avoided. At zero time, 1 ml of hydrogen peroxide (0.1 M) was blown into the reaction mixture. The tip of a 1-ml volumetric pipet was cut off to facilitate rapid delivery (2). A stop watch was started when the peroxide was in the last 1–2 inches of the pipet. At 30 sec, 1 ml of 2 N sulfuric acid was added to stop the reaction. The hydrogen peroxide not decomposed by catalase was titrated with 0.05 M potassium permanganate using a micro buret. Blank determinations were made on samples in which sulfuric acid was added prior to the hydrogen peroxide.

Highly reproducible results were obtained when the enzyme concentration was adjusted so that approximately 1/3 of the substrate was decomposed within 30 sec. Catalase activity is expressed in terms of the 1st order velocity constant (k) and is calculated:

\[ k = \frac{2.3}{t} \log \frac{x}{a} \]

where \(x\) is the hydrogen peroxide present at zero time, \(a\) is the hydrogen peroxide remaining at time \(t\), and \(t\) is in sec. Data presented as \(k/N\) and \(k/p\) refer to \(k\) divided by mg of nitrogen or protein, respectively, of the enzyme preparation. Nitrogen was determined by a micro-Kjeldahl method (14) and protein by the Lowry procedure (15).

CATALASE AND SUBSTRATE CONCENTRATION. Establishment of a quantitative relationship between catalase activity (breakdown of hydrogen peroxide) and catalase concentration is based upon 2 criteria (4): catalase concentration must be linearly proportional to the 1st order velocity constant \((k)\), and secondly, the 1st order velocity constant must be independent of substrate concentration and of time. The validity of the 1st order equation with respect to catalase activity has been shown by Chance and Herbert (7) and by others (3, 23) in detailed kinetic studies.

Linear proportionality between the 1st order rate constant and enzyme concentration was obtained, except at very low enzyme concentration (1:30 dilution of a 1% homogenate). This deviation from linearity is probably due to inactivation of enzyme due to excessive dilution (5).

Substrate concentration between 0.008 and 0.033 M did not affect the value of \(k\) under the conditions of this procedure. A slight decrease in \(k\) values was obtained between 0.033 and 0.067 M hydrogen peroxide. The independence of \(k\) through a greater range of substrate concentration has been obtained by others (3) using the spectrophotometric procedure. The results of the present experiments show that \(k\) and, therefore, the \% of hydrogen peroxide, was independent of the hydrogen peroxide originally present; and the amount of hydrogen peroxide destroyed was proportional to the original concentration of substrate.

EFFECT OF TIME. Although the 1st order velocity constant should be independent of the time of the reaction, this condition is difficult to achieve with simple titration procedures. The \(k\) value usually decreases with increasing reaction time. Extrapolating the \(k\) values to zero time may not necessarily improve the accuracy, since a straight line relationship between \(k\) and reaction time is not always attainable (5).

The calculation of \(k\) at the 30-sec reaction time interval was found to be the most expedient and simple measure of catalase activity for routine determinations under the conditions described. The 30-sec time interval was based also on the results obtained when \(\log a/a - x\), from the integrated form of the 1st order equation was plotted against time \(t\). The slope of the resulting straight line should equal \(k/2.3\), and is a test for the 1st order equation (18). The 30-sec \(k/2.3\) values obtained with a total liver homogenate, supernatant, and pellet fraction were 0.0128, 0.0056, and 0.0179 for slopes of 0.0110, 0.0049, and 0.0157, respectively.

EFFECT OF TEMPERATURE. No appreciable change in \(k\) was observed in a temperature range of 2°–45°C. The energy of activation obtained from an Arrhenius plot yielded a value of 1.5 kcal for liver pellet fraction, and 1.2 kcal for a highly purified (19) mouse liver catalase preparation. These results are in general agreement with those obtained with other short reaction time methods: horse blood catalase, 1.7 kcal (5), beef liver catalase, 5.1 kcal (23), and bacterial catalase, 1.4 kcal (7).

Results

Charts 1 and 2 compare and show the similarity between hepatic catalase activity on successive days following implantation of Ehrlich ascites tumor and following s.c. talc injection. The catalase activity remained low throughout the period of tumor growth. Of particular interest is the transient rise of catalase activity of the pellet fraction at the 4th day of growth. No significant change in red cell catalase or kidney catalase occurred; although in some animals a small (10–15%) decrease in kidney catalase was noted after about 12 days of tumor growth. The over-all pattern of hepatic catalase activity following talc injection is similar to the tumor experiments, even to the transient rise of catalase activity on the 4th day. The general pattern

Chart 2. Hepatic catalase activity following talc injection. The results are representative of 2 experiments. Each point is an average of 3 mice.

Chart 3. Kidney catalase activity in total homogenate (T). Each point is an average of 3 mice.

Chart 4. Hepatic catalase activity in mitochondrial (M), microsomal (μ) and supernatant (S) fractions following talc injection. The results are representative of 2 experiments. Each value is an average of 2 mice.

Chart 5. Leucine-$^{14}$C incorporation following talc injection. Same animals as in Chart 4. The labeled leucine, 3.3 μc, was injected i.p. 45 min before livers were removed. The ordinate represents disintegrations/min/mg protein (dpm/mg protein). Trichloroacetic acid-insoluble material of the various fractions was solubilized with Hyamine (Packard Instrument Co.) and counted in a Packard Tri-Carb liquid scintillation spectrometer. Each sample was recounted with an internal standard to obtain disintegrations/min.

Differs from the tumor experiments in that there is a gradual return to normal levels after the 12th day. No significant changes were noted in red cell catalase. However, kidney catalase showed a marked but transient depression of activity on the 2nd day (Chart 3).

A previous report from our laboratories indicated, by immunochimical evidence, that the decrease in liver catalase was due to repressed catalase synthesis (19). Thus, the rise in catalase activity on the 4th day following talc injection may be related, in part, to increased protein synthesis. Accordingly, experiments were done in which the livers were fractionated by the method of Hogeboom (13) so that catalase activity in the mitochondrial and microsomal fractions could be delineated. Also, the mice were injected with leucine-$^{14}$C and the cell fractions assayed for incorporation into acid-insoluble material. Chart 4 shows the catalase activity in the mitochondrial, microsomal,
increasing in the mitochondrial fraction (Chart 4) there was a concomitant increase of leucine-\(^{14}\)C incorporation in the microsomal fraction.

**Discussion**

The results of this investigation show the similarity in pattern between hepatic catalase depression in tumor-bearing and talc-injected mice. The pattern does differ in the talc-injected animals in that there is a gradual return to normal values, whereas tumor-bearing animals continue to maintain low levels of catalase. Greenstein et al. (10) were the 1st to experimentally demonstrate that rat liver catalase is depressed as long as tumor growth is maintained. This difference could be expected if one considers the tumor to be a chronic progressive process and the talc granuloma a self-limited reparative process.

The unusual observation in these experiments was the fact that on the 3rd day following tumor growth or talc injection there was a transient increase of hepatic catalase activity, exceeding normal values, and this rise was manifested primarily in the pellet fraction (Charts 1, 2). The total homogenate did not reflect this increased activity on the 4th day. This was apparently due to the formation of the inhibitory catalase-H\(_2\)O\(_2\) complex (7). Increased catalase activity in unpurified tissue preparations has been obtained by incubating with ethanol before assay (2, 11). Catalase activity of mitochondrial or microsomal preparations does not seem to be effected in this manner (11). Recent tests on tissue preparations similar to those described under "Enzyme Preparations" concur with these observations.

The results obtained in the pellet fraction suggested the series of experiments in which the liver was fractionated into mitochondrial, microsomal, and high-speed supernatant fractions. The fractionation studies showed that most of the catalase activity as well as the transient rise of catalase activity was localized in the mitochondrial fraction (Chart 4). Very little of the particulate enzyme activity was present in the microsomal fraction, as has been shown previously for normal liver by others (9, 11, 16). The high initial proportion of intracellular hepatic catalase activity in the mitochondrial fraction and the rapid rise in activity under the conditions described is evidence for the possibility that the mitochondrion is the site of catalase formation (1). Moreover, results obtained previously, using immunochromical technics, indicated that the lowering of hepatic catalase activity represented a repression of catalase biosynthesis (19). That the depression of hepatic catalase is not due merely to an inhibitor of catalase has been indicated by others using different technics (6, 21, 22, 24). Higashi and Peters (12) have indicated that mitochondrial catalase may be formed by the ribosomes of the rough-surfaced reticulum and then transported to the mitochondria. The rapid uptake of labeled leucine into the microsomal fraction (Chart 5) at the same time that mitochondrial catalase activity was increasing is consistent with this hypothesis. The small peak of radioactivity in the mitochondrial fraction may represent mitochondrial incorporation of labeled leucine into protein or may be a result of rapid transfer (12) of labeled protein synthesized in microsomes. Contamination of the mitochondria fraction could also explain this peak but is partially ruled out by the difference in the shapes of the curve.

Additional work is necessary to determine whether the sudden rise in mitochondrial catalase represents synthesis or assembly of preformed parts of the molecule.

The immunoelectrophoretic technic was in an exploratory phase at the time these experiments were done, and no conclusive data were obtained at the time. The application of this technic to this type of study may indicate qualitative differences in the various components of the cell fractions. Immunochromical procedures will be required to quantitate the changes of the catalase molecule during the course of injury. Such technics may also aid in explaining whether or not the rapid changes in catalase activity could be due to the rapid breakdown of particles containing catalase or due to an altered rate of enzyme destruction.

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**References**

15. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall,
Hepatic Catalase Changes and Leucine Incorporation in Talc-injected Mice


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