STUDIES ON CATALASE INHIBITION AS RELATED TO TUMORS*

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The inhibition of catalase in vitro by boiled extracts of tumors has been recently demonstrated (1). It was also found that fresh liver and spleen contained small amounts of a factor having similar action. In further work it was found that autolyzed liver homogenates contained large amounts of such inhibitory material. Studies on the rate of formation in liver homogenates, attempts at purification, and studies directed toward the elucidation of the possible nature of the responsible factor have been carried out.

EXPERIMENTAL

Homogenates of perfused beef and rat livers and the Jensen rat sarcoma served as the tissues for the preparation of boiled aqueous extracts (BAE) containing catalase inhibitor. Beef liver catalase (2) crystallized five times was utilized in studies with pure enzyme. The so-called rapid (3), classical (4), and inverse (1, 5) methods of catalase assay were employed. In the last procedure the substrate is added at zero time after a suitable incubation period of the diluted enzyme with buffer. Catalase inhibition by BAE or other substances was determined by incubation of test material with a suitable amount of purified catalase for 2 hours at 20°C prior to enzymatic assay. A control sample of catalase was always studied to correct for the activity losses of catalase in dilute solutions. The catalase activity of liver is expressed as the units of enzyme per gm. of dry weight of perfused tissue, as used previously (1).

Results

Effect of Aging Liver Homogenates on Catalase Activity—Fresh perfused liver was homogenized in a Waring blender, and the gross tissue portions were removed by filtration through gauze. A sample was then assayed for catalase activity by the rapid and inverse methods over an extended period

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of time. The results for rat liver, Fig. 1, A, reveal a marked decrease in enzyme activity with time. As seen from the data of Fig. 1, B, a good deal of this apparent activity decrease is reversible with dilution. The characteristic rise in assay value with time during titrations by the rapid and classical methods has been observed previously in studies on BAE-catalase mixtures (1) and with lysates of sea-urchin embryo (5). The time required for the maximal reversal of enzyme activity as measured by the inverse method, i.e. the period during which the diluted homogenate is allowed to stand in buffer prior to addition of the hydrogen peroxide substrate, increases with the age of the homogenate. At the 24 hour period 4 minutes of incubation of the homogenate in buffer were sufficient to give maximal activity, 60 minutes being required at the 10th day. Previous studies of

![Fig. 1. Catalase activity of 0.07 ml. of rat liver homogenate at 22° measured by (A) the rapid method, and (B) the inverse method.](image)

the effect of contact time of tumor BAE and purified catalase have demonstrated a similar relation (1).

The difference in the catalase activities of the homogenate found by the direct and inverse assays should be a function of the catalase inhibitor formed. A plot of this data is presented in Fig. 2, A. BAE homogenates were prepared at intervals and assayed for their ability to inhibit catalase. The results, presented in Fig. 2, B, reveal qualitative agreement with the inhibitor curve of Fig. 2, A.

The same effect that was noted for rat liver was also found to obtain for the bovine system. From Fig. 3 it can be seen that the catalase activity of this system was quite constant for the first 5 days and then suddenly decreased. The reversal effect was particularly striking at this time.

Purification Studies—The boiled aqueous extracts of Jensen rat sarcomas have higher levels of catalase inhibitor than those of aged liver homogenates and were consequently used in this work. The 80 per cent ethanol-soluble portion of these extracts, prepared as previously described (1), was utilized. The ethanol solution was passed through a Dowex 50 column without loss of inhibitor activity. The filtrate from a Dowex 2 column was inactive,
but attempts to elute catalase inhibitor were unsuccessful. Paper chromatography of the Dowex 50 filtrate was attempted. Difficulties of identification of active material in the eluates and of utilizing sufficient material on the paper to demonstrate clearly catalase inhibition made these experiments inconclusive. A disconcerting feature in attempts at purification of

![Figure 2](image.png)

**Fig. 2.** Catalase inhibitor activity of (A) 0.07 ml. of homogenate, as illustrated by the difference in results of the direct (rapid) and inverse assay methods, and (B) 0.3 ml. of liver homogenate of BAE on an amount of crystalline beef liver catalase (1.5 \times 10^{-9} M) sufficient to give a \( k_i \) of 2 \times 10^{-2} by the rapid method.

![Figure 3](image.png)

**Fig. 3.** Catalase activity of 0.03 ml. of beef liver homogenate at 22° measured by the direct (rapid) and the inverse methods.

the inhibitor is the rapid loss of activity. This appears to be oxidative in nature. Evaporation of inhibitor preparations with small amounts of \( \text{H}_2\text{O}_2 \) completely destroy the activity. This cannot be restored by treating with hydrogen in the presence of a palladized charcoal catalyst. Because of the extreme lability of the inhibitor following preliminary concentration to approximately 10 times the activity of starting BAE, this work was temporarily abandoned.

**Effect of Sulfur-Containing Amino Acids on Liver Catalase Activity in Vivo**—Sulfide and sulfhydryl compounds such as glutathione and cysteine
are known to be strong inhibitors of catalase (6-9). Several reports suggestive of a difference in sulfur-containing compounds of normal and tumor tissue are of interest in this respect. While the total sulfur content of tumors does not differ significantly from that of normal tissues, there is a marked increase in the alkali-labile sulfur (10). In liver tumors this is reflected in an increase in cystine and a decrease in the methionine level compared to the normal tissue (11). It was found that in rats given small amounts of cystine intraperitoneally there were substantial decreases in liver catalase activity compared with saline-treated controls. Typical results are summarized in Table I. Similar experiments with alanine, methionine, cysteic acid, glutathione, and cysteine showed no effects.

**Table I**

*Effect of Intraperitoneal Administration of Cystine on Level of Rat Liver Catalase*

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Sex</th>
<th>Cystine injection</th>
<th>Catalase activity*</th>
<th>Per cent lowered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg.</td>
<td>days</td>
<td>Experimental</td>
</tr>
<tr>
<td>5</td>
<td>♀</td>
<td>25</td>
<td>3</td>
<td>138</td>
</tr>
<tr>
<td>4</td>
<td>♂</td>
<td>25</td>
<td>5</td>
<td>281</td>
</tr>
<tr>
<td>5</td>
<td>♀</td>
<td>5</td>
<td>5</td>
<td>282</td>
</tr>
</tbody>
</table>

*Mean average in units per gm. of dry liver at 12 to 20 hours after the final cystine injection.

The depression of liver catalase activity by cystine is not as marked as that usually seen in the case of rats bearing large and rapidly growing tumors. This could possibly be due to various factors, including the cachexia associated with rapid tumor growth. Fasting has been reported to lower the liver catalase activity of rats (12) and mice (13). A similar effect is observed in rats on protein-free diets (14). In an experiment designed to determine whether fasting would augment the effect of cystine, a group of male rats weighing 72 to 80 gm. was placed on a restricted diet for 31 days prior to the daily intraperitoneal administration of 25 mg. of cystine for 5 days. The rats on the restricted diets gained an average of 40 gm. compared to 157 gm. for the controls. In the cystine-treated animals there was a 26 per cent depression of liver catalase activity compared with the restricted controls. This is the range found for rats fed *ad libitum* (see Table I) and indicates that growth restriction does not augment the effects of cystine. Dietary cystine supplementation sufficient to provide an additional 40 mg. daily over the controls caused no depression in liver catalase. Experiments of the latter type must be carefully controlled, for,
as shown in Fig. 4, the catalase activity of normal rats varies considerably with the age and weight of the animal.

The above experiments clearly demonstrated that cystine \textit{in vivo} was a good inhibitor of liver catalase activity. Microbiological assays of the blood serum of normal rats and rats bearing large Jensen sarcomas for cystine and methionine, however, reveal no differences. The methionine level of both groups ranged from 0.8 to 1.0 and the cystine from 1.4 to 1.5 mg. per cent.\(^1\)

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fig4.png}
\caption{Catalase activity of the livers of normal rats of different weights}
\end{figure}

\begin{table}
\centering
\caption{Effect of Cystine on Catalase Activity* of Homogenate of Perfused Rat Liver}
\begin{tabular}{l|ccccc}
\hline
 & Incubation time  \\
 & 0 hr. & 1 hr. & 2 hrs. & 3 hrs. & 8 hrs. \\
\hline
Homogenate only & 1.0 & 0.93 & 0.98 & \\
" + cystine & 0.95 & 0.90 & 0.84 & 0.75 & 0.73 \\
\hline
\end{tabular}
\end{table}

\* Activities expressed as first order reaction constants \(\times 10^2\) given by 0.04 ml. of homogenate at 22\(^\circ\) assayed by the rapid method.

\textbf{Catalase Inhibition in Vitro}—Cystine does not inhibit the activity of crystalline catalase. However, when it is added to liver homogenate, a definite inhibition results (Table II). A pronounced odor of \(\text{H}_2\text{S}\) can be detected within a few minutes after the cystine addition, owing to the well known desulferase activity of liver. The level of inhibition (25 per cent) is near that found \textit{in vivo} in studies with cystine. Our result with cystine is in contrast to the work of Balls and Hale (15) who found that cystine activated the catalase of liver extracts.

In further preliminary experiments it was found that cysteine, glutathione, and sulfide markedly inhibited purified catalase, whereas cysteic

\(^1\) The authors wish to thank B. E. Kline for these assays.
acid and alanine were without effect. Since both cysteine and sulfide are readily formed by liver tissue from cystine more detailed experiments utilizing the latter two compounds were carried out in an attempt to deter-

Fig. 5. Effect of iodoacetate (0.053 M) on cysteine (0.026 M) inhibition of catalase \(7.8 \times 10^{-10}\) M).

Fig. 6. Effect of iodoacetate (0.1 M) on sulfide (0.02 M) inhibition of catalase \(7.9 \times 10^{-10}\) M).

Fig. 7. Effect of iodoacetate (0.1 M) on inhibition of catalase \(7.8 \times 10^{-10}\) M) by tumor BAE.

mine whether these substances might be responsible for or related to the active material of BAE of tumors and liver autolysates.

Reversal of Cysteine and Sulfide Inhibition of Catalase by Iodoacetate—Both sulfide and cysteine strongly inhibit catalase. Iodoacetate has a slight inhibitory effect on catalase, but, as shown in Figs. 5 and 6, strongly
counteracts the inhibitory effects of cysteine and sulfide. In contrast, iodoacetate is ineffective in reversing the inhibition of catalase by BAE. Fig. 7 clearly illustrates that in this case the inhibitory effect of iodoacetate is additive to that of the BAE. Thus the action of the latter material does not appear to be due to sulfide or to a sulfhydryl compound. Further proof of this is seen in the failure of iodoacetate to elevate the catalase activity of homogenates prepared from the livers of rats bearing tumors.

**DISCUSSION**

The presence of a substance in BAE of aged liver homogenates capable of inhibiting catalase *in vitro* in a manner similar to that of BAE of tumors (1) and sea-urchin lysates (5) provides a ready source of material for study. The depressing action of cystine on liver catalase activity *in vivo* is interesting in view of the apparent rise of this amino acid in tumor tissue (10, 11). The daily intraperitoneal administration of as little as 2 to 3 mg. per 100 gm. of rat over a 3 to 5 day period leads to definite depression of liver catalase activity. It appears that cystine is converted by the liver into an at present unknown active compound. Whether the substance in the BAE discussed here is the same as that formed by tumor tissue is not known. The work of other investigators on tumors has only provided material capable of inhibiting catalase *in vivo* (16–19).

The question as to whether a tumor depressed the concentration of catalase or merely its activity is still a matter of speculation. It would appear that a means of measuring catalase accurately independent of activity would be of great value in the study of this problem.

**SUMMARY**

1. Aging liver homogenates in the cold results in the formation of a heat-stable substance capable of inhibiting catalase.
2. The inhibitory material is relatively labile, which has to the present time made its concentration difficult.
3. The intraperitoneal administration of cystine to rats markedly lowers liver catalase activity.
4. Iodoacetate can partially reverse the inhibition of catalase activity by sulfide and cysteine *in vitro*, but is ineffective against boiled aqueous extracts of tumors.

**BIBLIOGRAPHY**

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