Clinical Studies on Liver Catalase Activity in Gastric Carcinoma Cases

II. Experimental Supplement

By

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Following the clinical observations reported in the first paper, the aqueous extract of gastric carcinoma tissue was tested for the inhibitory effect on the catalase activity of the mouse liver, being studied the relation of this effect to the nature of the tumor as well as to the liver catalase activity of the patient.

EXPERIMENTAL

Method

1) Objects
Tumor tissues of 39 gastric carcinoma cases, in which subtotal resection of the stomach was performed, were used. They included some of the 58 cases examined in the first report.

2) Method
The aqueous extract prepared from the tissue was injected intraperitoneally into the mouse and the liver catalase activity was measured after 24 hours.

Injection material. A portion of the carcinoma tissue was dissected from the resected stomach, washed to remove the blood, mucus and necrotic matter, sliced, and then dried in a desiccator and powdered finely. The tissue powder so prepared was extracted with ten times as much distilled water and the extract separated by centrifugation was condensed under reduced pressure at a low temperature, and then dried.

Before using, the dried extract was dissolved into sterile physiological saline at 10 mg/cc.

Preparation of enzyme solution. The animals used were male mice of DD strain, each of which weighed about 15 g. They were fed with a definite feed composed of wheat, barley, bean cake and fish-meal.

Injection was performed intraperitoneally with a dosage of 10 mg. (1.0 cc. as a solution) per 10 g. body weight, referring to the previous
communication\(^1\). Twenty four hours after the injection, the mouse was killed by de-
capitation and laparotomized immediately. A piece of the liver weighing
about 0.1 g. was taken from the ventral margin of this organ, washed with
saline to remove the blood completely, then wiped and weighed. This
was then masticated in a mortar with a small amount of quartz sand, to
which was added pH 7.0 phosphate buffer solution to make a 1:1000
suspension, supernatant of the latter being ready for measurement.

*Method for measurement.* See the first paper. Three mice were used
for one material and the data obtained were averaged.

Results

1) Liver Catalase Activities in Mice Injected with Aqueous Extract
   of Gastric Carcinoma Tissue

In Fig. 1 is shown the frequency distribution of the liver catalase
activities in mice injected with 39 aqueous extracts of gastric carcinoma
tissue, and of 22 control mice which were injected with plain physiological
saline at a dose of 1.0 cc. per 10 g. body weight for comparison. The
activities were 23.3 to 60.7, 44.1 ± 2.5 on an average\(^2\), in the injected mice,
being apparently lower than those in the control mice (41.7 to 83.2, 64.4 ±
4.7).

![Graph showing frequency distribution of liver catalase activities](image)

Fig. 1. Frequency distribution of liver catalase activities of mice when
the tumor extracts were injected (heavy lines). Light lines show that of the
control mice.

2) Relation of Catalase Lowering Activity to Morphological Feature
   of Tumor

See Table I. No significant difference was found in the liver catalase
activity between the mice injected with the extracts prepared from the
tumors of Borrmann’s types I or II (three, and 24 cases, respectively) and
those with the extracts of type III (19 cases), nor between the mice injected
with the extracts of carcinoma adenomatosum (17 cases) and those with
the extracts of carcinoma solidum (22 cases).

3) Relation between Liver Catalase Activity of Gastric Carcinoma
   Cases and Catalase Lowering Activity of Tumor Extracts
TABLE I

Liver Catalase Activity of Injected Mice According to Types of Tumor from which the Injection Material was Extracted

<table>
<thead>
<tr>
<th>Types of tumor</th>
<th>Cases</th>
<th>Liver catalase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td>Gross appearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Types I &amp; II</td>
<td>27</td>
<td>23.3</td>
</tr>
<tr>
<td>Type III</td>
<td>12</td>
<td>35.0</td>
</tr>
<tr>
<td>Histological feature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenomatous</td>
<td>17</td>
<td>36.3</td>
</tr>
<tr>
<td>Solidum</td>
<td>22</td>
<td>23.3</td>
</tr>
</tbody>
</table>

In Fig. 2, the ordinate shows the liver catalase activity of the patient with gastric carcinoma and the abscissa the liver catalase activity of the mouse injected with an aqueous extract prepared from the tumor of this patient. No significant correlation was found between the two values.

**DISCUSSION**

Greenstein et al. observed that the lowered catalase activity of the rat liver induced by subcutaneous implantation of hepatoma 31 was restored when the tumor was extirpated, and were led to the conclusion...
that the lowering of the liver catalase activity in tumor-bearing animals is a far-reaching effect elicited from the existence of the tumor. And, failing to produce the lowering of the catalase activity by intraperitoneal injection of the tumor homogenate, they were of the opinion, at that time, that the living tumor is needed for such a phenomenon.4)

Later, Nakahara and Fukuoka5) found that a substance separated from the tissue of the human carcinoma lowers the catalase activity of the mouse liver when injected intraperitoneally, and this was confirmed by Greenfield et al.6) in various tumors and in different animals.

As a part of a series of our studies on toxic substances contained in the cancerous tissue,17) we have also reported previously that the aqueous extract of gastric carcinoma tissue inhibits the liver catalase activity of mice in vivo, and that a nucleoprotein fraction of the tissue constituent is the substance responsible for this effect.1)

In the first report of the present work, it was revealed that the catalase activity in the liver is lowered in gastric carcinoma cases parallel to the extent of spread of the tumor, but with little dependence on the morphological features of the tumor. If so, how does the catalase lowering activity of the tumor extract vary according to the types of the tumor from which it was prepared? Is there any relation between this activity and the liver catalase level of a patient with the tumor? These are the subjects to be discussed here.

As shown in Table I, however, no close relation was found between the catalase lowering activity on one hand, and the macroscopic as well as microscopic types of the tumor on the other hand. This suggests that the catalase depressing substances are contained, at least in human gastric carcinoma, equally in various types of the tumor, and corresponds to the fact proved clinically in the first paper that the liver catalase activity had little relation to such morphological types of the tumor.

Greenstein and Andervont10) reported that the liver catalase activities of tumor-bearing animals had close relation with the kind of tumors implanted to, or originated in, them. Though the results of the present work, clinical as well as experimental, seem to be incompatible with the report mentioned above, it can be understood that such varieties as various types in gastric carcinoma differ essentially from those among the experimental tumors produced from the different matrices by different procedures, and that the variety in the liver catalase activity of gastric carcinoma cases was due little to the difference in morphological types of the tumor, but greatly to the difference in growing or invasive energy of the tumor which is actualized as the peritoneal dissemination and the lymph-nodal involvement.

Moreover, no distinct correlation was obtained between the catalase
lowering activity of the tumor extract and the catalase activity of the liver of the patient with the tumor from which the extract was prepared.

But, it is very important to notice here that the "catalase-lowering activity" in this work is that of a unit weight of the extract, and may be that of a unit weight of the tumor tissue even supposing the existence of an almost equal content of water-extractible substances in tumor tissues. Therefore, when we make a reciprocal of the liver catalase activity of the mouse (in µl. of oxygen) injected with the extract present the catalase depressing activity per unit weight of a tumor and use a square measure of a tumor (a product of the longitudinal by transverse diameters, in cm.²) in place of the weight of the tumor, the product of these two values may be an index showing more precisely the catalase lowering activity of a tumor in vivo, and hereafter in this paper, it will be called the "presumed catalase-depressing activity" of the tumor.

<table>
<thead>
<tr>
<th>Presumed catalase-depressing activity</th>
<th>Cases</th>
<th>Liver catalase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Minimum</td>
</tr>
<tr>
<td>Class I</td>
<td>10</td>
<td>67.9</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>59.4</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>60.6</td>
</tr>
<tr>
<td>IV</td>
<td>9</td>
<td>35.3</td>
</tr>
</tbody>
</table>

Such presumed activities of 39 tumors examined were 0.04 to 2.64, showing a wide range. Of course the numerical values themselves are meaningless. When we group the examined tumors into four groups according to their presumed catalase-depressing activities, each of them containing the same number of cases, the less liver catalase activities were found in the group including cases with tumors of the greater catalase-depressing activity (Table II). An inverse correlation was thus demonstrated between the liver catalase activity of cancer cases and the catalase-depressing activity of the tumor.

As to the patho-physiological mechanism of the catalase inhibition in tumor-bearing animals, there are also opinions that the product of the tumor does not affect the catalase activity, but that the synthesis of catalase is disturbed for lack of its constituents due to the rapid growth of the tumor. But, the catalase lowering activity of the tumor components is a matter approved in general, and that the same process should be carries actually in tumor-bearing animals was demonstrated also by a parabiotic
experiment by Lucké et al.\textsuperscript{12)}

Close correlation of the two values, the liver catalase activity of cancer cases and the catalase-depressing activity of the tumor which was calculated, taking the tumor size into consideration, from the liver catalase activity of the mouse injected with the tumor extract, is thought to be a fact supporting the humoral mechanism in general patho-physiological effects of the malignant tumor.

**SUMMARY**

Aqueous extracts were prepared from 39 gastric carcinoma tissues and injected into mice intraperitoneally, the liver catalase activity of which was measured. The results obtained are summarized as follows.

1. Liver catalase activities in mice injected with the tumor extracts were significantly lower than those in the control mice.

2. Catalase-lowering activity of a tumor extract was not dependent upon the macroscopic or microscopic features of the tumor.

3. Decrease in liver catalase activity of gastric carcinoma cases had little relation to the catalase lowering activity of the tumor extract, but showed close parallelism with the catalase-depressing ability of the tumor itself calculated under some assumptions.

**References and note**

2) In the text, as well as in the tables, arithmetical means were presented with 95 per cent confidence limits.
4) Greenstein, \textit{ibid.}, 1943, 3, 397.
9) Yamaguchi \textit{et al.}, \textit{ibid.}, 1959, 69, 185, 191.