Mechanism of Liver Catalase Depression in Tumor-bearing Animals: A Review

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CONTENTS

INTRODUCTION

Studies of liver catalase have been important in attempts to better understand the malignant process. This enzyme has been extensively investigated because its depression seemed to indicate some unique property of tumor tissue. A review of this vast literature is intended to help clarify the catalase problem and provide useful information for other tumor-host studies.

Emphasis in this review is on the mechanisms possibly involved in the depression of liver catalase activity in tumor-bearing animals. Further information on liver catalase can be found in earlier reviews (27, 38, 48, 69, 70).

Liver catalase provided a useful system for studying the effects of a tumor upon the host because: (a) it seemed to be depressed in almost all animals with growing tumors, and returned to normal when the tumor was removed (71, 74); (b) the amount of depression was related to the size of the tumor (70); and (c) the activity was not affected by rapidly growing nonmalignant tissue such as an embryo (72).

Many different theories have been offered as possible explanations for lowered liver catalase activity in tumor-bearing animals. The reason for so many theories is that a number of factors, such as nutrition, hormones, and others, influence liver catalase activity. The simultaneous control of these variables has been difficult, if not impossible, to achieve. Still another difficulty, which has been emphasized by Busch (38), is the lack of a good standardized method for determining catalase activity and expressing the results.

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This review has been limited to those factors which influence liver catalase in a tumor-bearing host and those which must be controlled in order to obtain useful results. Wherever possible, reference has been made only to reviews or papers having the most direct bearing upon the topic under discussion.

NUTRITION

Starvation caused significant depression of catalase activity in the rat (137, 173), guinea pig (114), and mouse (46). A rapid depression of total liver catalase activity was observed during starvation in the rat (174) owing to a decrease in liver weight, but the rates of synthesis and destruction per gram of liver remained normal for the 1st 5 days of starvation.

Cachexia and diminution of food consumption usually occur during tumor growth, particularly during the latter stages (48). A loss in body weight is therefore a frequent occurrence. Mason et al. (129) looked for catalase depression in groups of patients with cancer or other maladies where an abdominal operation was necessary, and compared the catalase activities in those who were maintaining their weight with those who were losing weight. Their results indicated that human liver catalase depression was related to weight loss. A similar relationship between weight loss and liver catalase depression in human cancer patients was observed by Maldia and Holland (119). Although loss in carcass-weight of tumor-bearing animals has long been recognized as a factor in the lowering of catalase activity, its importance in studying the mechanism of lowering was diminished when Begg and Dickinson...
(28) found that low catalase activity was also present in force-fed tumor-bearing animals who maintained their carcass-weight. However, Begg and Dickinson (28) cautioned that it was still possible that carcass nitrogen was being lost to the tumor in their force-feeding experiments. Some evidence that this was occurring was shown by the increased liver nitrogen, raising the question of whether it was still possible that tumor growth places unusual demands upon the liver which would affect catalase activity. Evidence for such unusual demands might be found in the alterations of plasma proteins and the possibility of selective uptake of plasma proteins by the tumor (38). That tumor growth imposes unusual metabolic demands upon the host, and this in turn affects the liver catalase in much the same way as starvation, still seems to be a good possibility for further investigation. It appears quite unlikely, however, that decreased total intake of food alone is the principal factor responsible for the drop in liver catalase activity in the tumor-bearing animal (27, 177).

In addition to a general diminution of nutrients, tumor growth may result in the lack of specific nutrients. The most obvious need would be for protein and perhaps even specific amino acids. It has been shown that a low or nonprotein diet depressed liver catalase activity (21, 138). Liver catalase also appeared to be sensitive to the elimination of certain specific amino acids from the diet (198). The studies of Weil-Malherbe and Schade (199), in which high- or low-protein diets failed to affect the liver catalase activity of rats bearing Jensen tumor, indicated that dietary protein was not the major factor responsible for liver catalase depression in tumor-bearing animals. Applemann et al. (22) found a further decrease in catalase when tumors were injected into animals on a protein-free diet. It was also shown by Rechcigl et al. (175) that a protein-free diet, while decreasing the liver catalase, did not affect the catalase of a hepatoma in the same animal.

Thus, although the lack of protein or certain amino acids did not affect the catalase of a hepatoma in the same animal, it will still not provide an adequate explanation for the maintenance of liver catalase is iron. In addition to a general diminution of nutrients, tumor growth imposes unusual metabolic demands upon the host, and this in turn affects the liver catalase in much the same way as starvation, still seems to be a good possibility for further investigation. It appears quite unlikely, however, that decreased total intake of food alone is the principal factor responsible for the drop in liver catalase activity in the tumor-bearing animal (27, 177).

ALTERATIONS IN IRON METABOLISM

Still another specific nutrient that might be required for the maintenance of liver catalase is iron. In addition to the effect that tumor growth has on the iron-containing enzyme, catalase, it has also been implicated and extensively studied in anemia (27, 167), plasma iron concentration (192, 135), iron storage (180, 191), and various iron-containing enzymes such as cytochrome c (99). This has led to speculation that changes in liver catalase in the tumor-bearing animal might be just a small part of a general alteration in iron metabolism.

Anemia and the depression of catalase activity are both frequently present in the same tumor-bearing animal (27, 65). It has also been shown that the production of anemia in an otherwise normal animal lowered liver catalase activity (29); but Begg et al. (29) did not believe that the decrease in catalase activity accompanying anemia explained the marked decrease in enzyme activity which occurred in tumor-bearing animals with mild anemia. Later, it was shown that the anemia of some tumor-bearing animals was corrected by injections of cobalt without increasing the low liver catalase activities (103).

Although Fukuoka and Nakahara (60) reported that additional iron (either fed or injected) prevented the decrease of liver catalase activity in tumor-bearing animals, this could not be confirmed in tumor-host systems used by several other investigators (54, 103, 159, 179). It has also been indicated that no direct relationship existed between the concentration or turnover of transport iron and liver catalase activity (109, 158). It must, therefore, be concluded that the total iron available is not the limiting factor in catalase synthesis in tumor-bearing animals. This still leaves the possibility that the tumor is affecting some specific phase of iron metabolism. One such possibility was suggested by the observation that free porphyrin accumulates in the erythrocytes (193) and liver (162) of tumor-bearing animals. These findings logically led Sugimura et al. (192) to speculate that tumor growth might be inhibiting the insertion of iron into protoporphyrin. When methods became available for measuring the rate of incorporation of iron into heme in the liver (117), no differences could be demonstrated between normal and tumor-bearing animals (92, 164).

There is not sufficient evidence at the present time, to support a conclusion that iron is a limiting factor in catalase synthesis in the tumor-bearing animal. However, the rather general alterations in iron metabolism which frequently accompany tumor growth would suggest that further investigations in this area might be rewarding.

HORMONES

A possible relation between loss of liver catalase activity and altered hormone function in the tumor-bearing animal seems quite likely, especially, since a number of studies have shown that altering the hormone balance of an otherwise normal animal affects liver catalase activity. These studies, prior to 1958, were summarized by Begg (27). Although injection of hormones or removal of endocrine glands changes liver catalase activity, it is still not clear what influence hormones have upon liver catalase activity in tumor-bearing animals.

Hypophysectomy caused an increased liver catalase activity in normal rats (16, 62, 196), but not in mice (197). Gaebler and Mathies (62) found that growth hormone was not involved, or at least was not the only factor involved. Utsugi (196, 197) reported that hypophysectomy prevented the reduction of liver catalase activity in tumor-bearing rats and mice. However, a reduction was again observed when growth hormone was administered. Rechcigl and Wollman (177) found that a transplantable, nonfunctional thyroid tumor caused a depression of catalase concentrations in both the liver and kidney of hypophysectomized and intact rats. They concluded that under their conditions the pituitary gland was not a significant factor in the depression of catalase activity of the tumor-bearing host.

Enlargement of adrenal glands (26, 185), decrease of adrenal ascorbic acid (185), and alterations of adrenal and plasma corticosterone (85) were observed in tumor-
bears catalase activity in both rats (30, 195) and mice (2, 3).

Male rats and mice generally have a higher liver and kidney catalase activity than females (1, 2, 6). Castration reduces the males' liver catalase activity and the activity in both the male castrate and female can be increased by administering testosterone (6). Administration of thyroxin produced a decrease in the liver catalase activity of normal animals (63, 202) and thyroidectomy or thiouracil caused an increase in catalase activity (206).

Much more information is required about the hormones of the tumor-bearing animal. Most of the alterations which have been shown to affect liver catalase activity do not seem sufficiently specific to describe the situation which occurs in the tumor-bearing animal.

GENETICS

Most small-animal studies on liver catalase activity have been conducted on animals bearing transplantable tumors. There exists, however, evidence which shows that primary tumors will also depress liver catalase activity (67, 69, 175). Nevertheless, it still seems possible that in some of the studies involving transplantable tumors, the heterologous tissue or the site of transplantation may have influenced the catalase activity. The necessity for further investigation in this area is emphasized by differences reported in catalase activity when the same tumor is used in different laboratories (108). Some investigators observe only slight depression in liver catalase activity with the Jensen and Novikoff tumors (105, 108), whereas others have reported marked depression with these tumors (21, 22, 175, 199).

An eventual understanding of the role of genetics in liver catalase depression may be provided by the type of studies initiated by Rechcigl and co-workers (172, 175, 176). They determined the catalase activity in the tumor, liver, and kidney of animals bearing various transplantable, induced, or spontaneous hepatomas (175). Their findings showed that many induced and spontaneous tumors had a high catalase activity; but even when the catalase was high in the tumor, the catalase in the liver and kidney of these animals was lowered (175). They also observed that a protein-free diet decreased the liver catalase without significantly affecting the tumor catalase of that animal.

Transplantation of a primary ethionine-induced hepatoma gave rise to two lines, one with high- and the other with low-tumor catalase (176). These lines have remained stable after transplantation and are similar in most other aspects which have been investigated.

It seems entirely possible that genetic mutations may produce variations in liver catalase activity between substrains of animals (172). Mutations may also be involved in determining the amount of catalase in the tumor (176).

The information available at the present time does not suggest, however, that either of these is a major determinant of the effect the tumor will have upon the host's liver catalase activity. The numerous studies which show a depression of liver catalase activity in animals bearing primary tumors suggest that incompatibility of tumor and host was not a major factor in the lowering of liver catalase activity. The possibility still remains that some of the variability noted in transplantable tumors might be due to genetic changes in either the tumor or the host.

TOXOHORMONE

Greenstein and co-workers (73, 74) in their early studies on liver catalase, postulated the existence of a toxic material from the tumor. However, incubation of normal rat liver with tumor extracts, serum, or other tissues of the tumor-bearer had no effect on liver catalase activity (67). They also were unable to influence the liver catalase activity by injecting extracts of the tumor into normal rats. In later work, Nakahara and Fukuoka prepared concentrated extracts from human gastric or rectal carcinomas (140—143, 145). When these extracts were injected into normal mice a marked decrease in liver catalase occurred. This material was named toxohormone and has been the subject of several reviews (38, 139, 145, 146).

Activity of normal and necrotic tissues.—Nakahara and Fukuoka (142) compared their toxohormone extracts with similarly prepared extracts from human gastric tissues. They found normal tissues to be inactive. The first indication that normal tissues acted in some way to depress liver catalase activity was presented by Greenfield and Meister (64). These investigators observed that fractions obtained from certain normal tissues, by methods similar to those which gave an active toxohormone from tumor tissues, also possessed the ability to lower liver catalase activity, although the activity was considerably less than that obtained from tumor.

Riley (178) obtained a depression of liver catalase activity in mice by injecting homogenates of normal liver, kidney, or spleen. Day et al. (49) found that spleen depressed catalase to an extent comparable to tumor tissue. The injection of normal tissue homogenates according to Adams (7) caused a depression of liver catalase activity comparable to injections of Sarcoma 37 homogenates. Kampschmidt et al. (100) reported that toxohormone material prepared from Walker tumor was about 100 times as active as a normal tissue preparation made from the rat after removal of the intestines, skin, and bones. A further study (105) indicated that the preparations made from Walker carcinoma 256 and Flexner-Jobling carcinoma were markedly different from the normal tissue preparations, while the Jensen sarcoma and Novikoff hepatocarcinoma were similar to normal tissue. The active fractions from the Walker and Flexner-Jobling tumors were then shown to be due to bacterial contamination so that none of the tumors, when freed of bacteria, differed significantly in ability to reduce catalase activity from that of the more active normal tissues (108).

Other effects produced by toxohormone.—Fukuoka and Nakahara (61) observed that in addition to its effect on liver catalase activity, injections of toxohormone produced thymus involution. This observation was confirmed (100, 106) and the effects of toxohormone extended to include a variety of changes. A slight anemia developed 24—48 hr. after a single injection of toxohormone and became more severe after multiple injections (61, 100, 128, 181, 203). Plasma iron was very sensitive to change with small doses.
of toxohormone; the decrease reached a minimum 12 hr.
after injection, and returned to normal after 1 to 2 days
(59, 100, 105, 106, 156, 158). An increase in the weight of
liver (96, 100, 105, 106, 120, 121, 130), spleen (96, 100,
105, 106, 121, 183), and adrenals (61, 100) followed toxo-
 hormone injection. Toxohormone also lowered ferritin
storage in the liver (90, 91); depressed liver ascorbic acid
and riboflavin content (91); inhibited tumor growth (50);
damaged the reticulum network of the intraganglion (32);
and decreased liver arginase (34), coenzyme A (116),
tryptophan pyrrolase (113), and the synthesis of DPN
(161). In addition, toxohormone increased liver por-
phyrin (162) and plasma copper concentration (94, 203),
and altered the serum proteins (94). Most of these
changes have been shown to be similar to those which occur
in the tumor-bearing animal.

Purification of toxohormone.—Many different methods
have been used to yield a variety of active toxohormone
preparations. This must indicate that there are several
active materials or that a highly active material was
present as an impurity in many of the preparations. The
most active preparations have been proteins which usually
contain bound lipid (51).

In order to isolate the active fraction, several investi-
gators have employed methods involving the extraction
of the tumor tissue with methanol and acetic acid (155,
163, 183). This has usually been an initial step before
further purification by column chromatography on DEAE-
cellulose (155) or Celite (183). These methods generally
yielded a material which was active in doses of 100–500
µg/mouse. The most active preparations of toxohormone
have been reported by Yunoki and Griffin (204). The
active fractions were separated by column chromato-
graphy on Amberlite XE-64, and 1–5 µg/mouse was found
to depress liver catalase activity. However, there may
be considerable variation in activity depending upon the
starting material or other factors, since Sato and Yunoki
(182) using similar methods found an effective dose to be
500 µg. The analysis of these preparations indicated 50 %
protein and 20 % lipid (162, 205). The lipids were pre-
dominately phospholipids (205). Arginine was shown to
dominate phospholipids (205). Arginine was shown to
protein and 20 % lipid (182, 205). The lipids were pre-

starting material or other factors, since Sato and Yunoki
active fractions were separated by column chromatog
raphy on Amberlite XE-64, and 1—5 gig/mouse was found
further purification by column chromatography on DEAE

Further work appeared to show that homogenates or extracts (145) of
many different tumor tissues will, upon injection into normal rats or mice, depress liver catalase activity. There
remain, however, two questions which have not been satisfactorily answered. First, is the toxic material a
product of the tumor tissue? Most of the investigations
have ignored the possibility that tumor tissue may be a
better medium for the growth of bacteria and viruses than
normal tissues. This question will be discussed in greater
detail in the section on bacterial contamination. Second,
does the toxic material reach the general circulation? The
parabiotic experiments of Lucké et al. (118) indicate that
the effect on liver catalase can be effective across the
parabiotic union, but this would not necessarily prove
that the same toxic factor which can be extracted from
the tumor was being carried by the bloodstream. Simi-
larly, the many experiments showing extracts of urine
(36, 124, 125, 139, 181, 184), ascitic fluid (96, 126, 128),
gastric juice (95), or sputum (115) to be active in lowering
liver catalase are generally unconvincing: The activity
was usually low. There was no assurance that these ma-
terials were the same as those found in the tumor, and
the bloodstream has usually given negative results. This
problem will be discussed further in the section on the
reticuloendothelial system.

The name toxohormone as defined by Nakahara and
Fukuoka implies that the toxin is produced by all cancer
tissues and is then transported to its site of action in the
liver. This gives the erroneous impression that the two
questions raised here on site of production and transport
have already been solved, whereas actually the production
of toxin by all tumor tissues has not been established and
some evidence (108) indicates that the tumor itself may
not be the source of the toxin. Finally, there is no evi-
dence that the toxin as it exists in the tumor is being trans-
ported.

INHIBITION OF LIVER CATALASE IN VITRO

Hargreaves and Deutsch (79) reported that in vitro
inhibition of liver catalase occurred when rat liver homog-
enates were incubated with the supernatant from boiled
Jensen tumor. This factor was also effective in inhibiting
the activity of a number of other heme-containing en-
zymes. Fresh liver and spleen were observed to contain
small amounts of factor with similar inhibitory prop-
erties. In further work it was found that autolyzed liver
homogenates contained large amounts of such inhibitory
material (187).

Many different investigators were able to confirm that
boiled aqueous extracts of tumor, and in some instances
normal tissue, would inhibit catalase in vitro (37, 41, 53,
88, 122). The cysteine content of these extracts appeared
to be largely responsible for this activity (37, 86, 187).
Cysteine and some related compounds were then shown
to be active both in vitro and in vivo (44, 86, 187).

The mechanism of in vitro catalase inhibition by boiled
aqueous extracts containing sulfhydryl groups was attrib-
uted to the production of H2O2 during auto-oxidation
of the extract (18, 122). The in vivo inhibition by cysteine
or boiled aqueous extracts could be reversed with ethanol,
but ethanol had no effect on the reduced liver catalase of
tumor-bearing animals (18). This would suggest a differ-
ent mechanism than the depression of catalase activity
produced by toxohormone or in the tumor-bearing animal.
Further indication that the in vitro inhibition of liver catalase was not associated with the depression which occurs in tumor-bearing animals was provided by studies which showed a reduction of the enzyme concentration in the liver of tumor-bearing animals (150, 166). The in vitro inhibition produced a reversible repression of catalase activity and not a lowering of the actual enzyme concentration (18, 122). It would therefore appear that the in vitro inhibition of liver catalase cannot be used to help explain the lowering of liver catalase which occurs during the growth of malignant tissue.

**BACTERIAL CONTAMINATION OF TUMORS**

Bacteria were believed to have no effect upon liver catalase activity during the period when many of the early studies on liver catalase depression in tumor-bearing animals were conducted (68). Even when it was shown by Dounce and Shanewise (52) that rats with the leprosy bacillus had a lower than normal liver catalase activity, very few investigators mentioned examining the tumor material for bacteria. Kato et al. in 1959 decreased the liver catalase activity of mice by injecting virulent tuberul bacteria (112). They attributed the decreased liver catalase activity to an allergic tissue reaction. Contamination of tumor-bearing rats with Salmonella typhimurium, or injection of this bacteria into normal animals, was shown by Kampschmidt and Upchurch (111) to produce a depression in liver catalase activity. It was further demonstrated that many different bacteria caused a reduction in liver and kidney catalase activity in normal rats by showing that it occurred after injection of lipopolysaccharides from Escherichia coli, Shigella flexneri, Staphylococcus aureus, and Serratia marcescens (108). It was therefore suggested (111) that many mild infections with bacteria may go unobserved in tumor-bearing animals or in the cancer patient, and these infections may be responsible for the marked decrease in liver catalase.

Although these results require confirmation by other laboratories, it would still seem advisable to eliminate the possibility of such contamination from all future studies on liver catalase activity. A depression of liver catalase activity was shown in some tumor-bearing animals in which bacteria could not be demonstrated (111). However, Kampschmidt and Schultz (108) were unable to demonstrate toxohormone in any mouse or rat tumor they studied that was free of contamination.

This would still leave the possibility that the effects noted from human tumors were not due to bacterial contamination. It is worth noting, however, that gastric and rectal carcinomas have been the tumors most frequently used for the preparation of toxohormone from human patients. These tumors might be expected to be contaminated with Gram-negative bacteria from the intestinal tract. Numerous laboratories have indicated that bacterial contamination in the cancer patient is a frequent occurrence (19, 23, 33–35, 47, 76, 78, 131, 132, 148, 171, 201).

Some of early toxohormone experiments by Nakahara and Fukuoka (142, 144, 146) would on first inspection appear to rule out the possibility of bacterial contamination. Upon closer examination, however, it may be observed that a bacterial contaminant within the tumor would probably not be detected. One example of this would be the biosynthesis of toxohormone which was demonstrated to occur in vitro (144). In recent unpublished experiments the author found that he could repeat these experiments with tumors which were contaminated by bacteria, but no biosynthesis could be demonstrated in the absence of bacteria.

It is this reviewer's opinion that most of the effects upon liver catalase activity attributed to a toxin liberated by the tumor have been due to bacterial contamination (108). It is further suggested that bacteria may be involved in the association between the patient's clinical condition and the fall in liver catalase activity (119).

**INVolvEMENT OF THE RETICULOENDOTHELIAL SYSTEM**

The diversity of injected materials which have been shown to depress hepatic catalase activity of the normal animal suggests that the reticuloendothelial system (RES) may be involved. Some of the materials which have been shown to depress liver catalase activity when injected into normal animals are shown in Table 1. From the variety of substances listed it is fairly obvious that the depression of catalase is not a very specific measurement for a particular type of chemical structure. There is even some suggestion that physical properties, such as the size of the colloidal particles injected, may be just as important as chemical properties in determining the ac-

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th><strong>SUBSTANCES WHICH HAVE DECREASED LIVER CATALASE ACTIVITY WHEN INJECTED INTO NORMAL ANIMALS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Reference</td>
</tr>
<tr>
<td>Talcum</td>
<td>151, 152</td>
</tr>
<tr>
<td>Colloidal carbon</td>
<td>108</td>
</tr>
<tr>
<td>Colloidal sulfur</td>
<td>108, 189</td>
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<tr>
<td>Methocel</td>
<td>108</td>
</tr>
<tr>
<td>Zymosan</td>
<td>102, 153</td>
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<tr>
<td>Endotoxins</td>
<td>102, 103, 153</td>
</tr>
<tr>
<td>Turpentine</td>
<td>104</td>
</tr>
<tr>
<td>Bacteria</td>
<td>108, 109</td>
</tr>
<tr>
<td>Certain viruses</td>
<td>4, 154</td>
</tr>
<tr>
<td>Secondary antigen</td>
<td>81</td>
</tr>
<tr>
<td>Cysteine</td>
<td>86, 187, 189</td>
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<tr>
<td>Tyrosine</td>
<td>17</td>
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<tr>
<td>n-Glutamic acid</td>
<td>20</td>
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<tr>
<td>Ferritin</td>
<td>179</td>
</tr>
<tr>
<td>Denatured albumin</td>
<td>17</td>
</tr>
<tr>
<td>Sodium caseinate</td>
<td>178</td>
</tr>
<tr>
<td>Egg yolk</td>
<td>178</td>
</tr>
<tr>
<td>Various protein fractions</td>
<td>40, 127</td>
</tr>
<tr>
<td>3-Amino-1,2,4-triazole</td>
<td>25, 57, 83, 84, 123, 147, 149, 190</td>
</tr>
<tr>
<td>Cobalt</td>
<td>21</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>58, 165</td>
</tr>
<tr>
<td>Mutant strains of yeast</td>
<td>39, 134</td>
</tr>
<tr>
<td>Several anticancer drugs</td>
<td>77, 87</td>
</tr>
<tr>
<td>Several antibiotics</td>
<td>170</td>
</tr>
<tr>
<td>Various purines</td>
<td>80</td>
</tr>
<tr>
<td>Various carcinogens</td>
<td>15</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>5, 15, 45, 82, 186</td>
</tr>
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</table>
activity. Several different investigators have suggested that the RES may be involved in the reduction of liver catalase activity (51, 102, 104, 109, 153). Evidence that toxohormone effects may also involve the RES appeared when a tolerance to toxohormone was developed, so that it had less effect upon liver catalase after repeated injections (93, 100, 102, 104, 153, 203).

Effects of toxins and the reticuloendothelial system have been carefully studied for their effects upon the production of fever (24), and on the lowering of plasma iron (110). Under these conditions an endogenous factor was shown to be formed (24, 110). It seems quite likely that a similar situation might also exist for liver catalase. The formation of endogenous factors would provide a reasonable explanation for the effects upon liver catalase activity of such materials as turpentine and talcum (104, 132).

The studies on plasma iron concentration during tumor growth provide a very clear demonstration of the rapid removal by the reticuloendothelial system of toxic substances from the bloodstream (101, 107, 109, 110, 111). A marked decrease in plasma iron occurred while the tumor was still very small (105, 111). It also occurred in normal rats after injection of very minute amounts of tumor tissue or bacteria (109, 110). After a single injection, the toxic material was demonstrated in the bloodstream (110), yet during tumor growth, or after the development of tolerance by repeated injections, the toxins cannot be shown when plasma from a tumor-bearer is injected into a normal animal. The presence of toxins has been clearly demonstrated (118). The inability to demonstrate them during tumor growth was probably due to their more rapid removal from circulation and the formation of opsonins (98). The injection of opsonins along with the toxic material promotes the rapid removal of the toxin in the normal animal (75). It seems entirely possible that a similar situation may exist for liver catalase and tumor growth.

It would therefore seem advisable to give the reticuloendothelial system serious consideration in studies investigating what mechanism(s) lowers liver catalase activity in tumor-bearing animals. This would be particularly true of studies designed to demonstrate a toxic material such as toxohormone from the tumor. The physical as well as the chemical characteristics of the materials injected may be important in determining their effect upon the liver catalase activity. It would also seem advisable, when looking for possible blood-borne toxic materials from the tumor, to consider the formation of endogenous factors, formed by the host instead of the tumor. Studies of such materials after the injection of turpentine have been conducted by Menkin (133).

**CATALASE SYNTHESIS AND INTRACELLULAR DISTRIBUTION**

In many of the early studies on liver catalase, the liver homogenate was centrifuged and the catalase assay determined on the supernatant (49). In 1949, Euler and Heller (55) found that the catalase activity of liver was about equally divided between the mitochondria and supernatant, but in tumor-bearing animals the supernatant liver catalase activity decreased first. In most subsequent studies the whole liver homogenate was used for assay, but this also resulted in difficulties if inadequate homogenization was employed (11). Several investigators have shown that this problem can be greatly reduced by adding various nonionic detergents to the homogenate (8, 56, 109, 137).

In a series of papers by Adams and co-workers (8—14) the distribution of catalase assumed importance as a mechanism that possibly lowers liver catalase in tumor-bearing animals. They observed that the leakage of liver catalase from the large granules occurred rapidly during in vitro incubation (12, 13) and indicated several factors which affect permeability of the large-granule membrane (14). The effect that androgenic hormones have upon granule permeability was suggested as a possible explanation for the differences observed in liver catalase activity between the sexes (8).

Adams (7) found that the intracellular distribution of liver catalase was changed during tumor growth, which suggested that tumor growth was altering membrane permeability. He observed further (8) that a variety of injected materials would not result in changes in intracellular distribution even though the liver catalase was decreased. It must be stated, however, that the depression of liver catalase activity after injection of various materials was less and of shorter duration than that observed in the tumor-bearing mice. Additional studies will, therefore, be required before this can be established as a valid difference in the mechanism of liver catalase depression between tumor-bearing animals and animals given injections of various other materials. With the present understanding of the manner in which injected materials affect the RES (see "Involvement of Reticuloendothelial System") it should be possible to duplicate by injection the extent and duration of liver catalase depression that occurs in a tumor-bearing animal. Only after overcoming tolerance, which has been shown to occur for injected materials, can it be clearly established that the mechanism of lowering is different in the tumor-bearing animal.

It is also apparent that altered permeability alone cannot explain the decrease in liver catalase activity. Since a decrease in enzyme content as well as activity has been shown (150, 166), there would seem to be two possible explanations: (a) either the release of more enzyme through the membrane causes increased destruction, or (b) there must be less synthesis of catalase in the large granules of tumor-bearing animals. One of these two must occur in addition to the effect upon membrane permeability.

Ceriotti et al. (42) investigated the synthesis of catalase in mice bearing Sarcoma 180. They observed increased incorporation of Fe59 into liver catalase 5 days after tumor implantation. After 10 and 15 days of tumor growth the incorporation of Fe59 was depressed. They concluded that there was an early destruction of catalase followed by a block in synthesis. These experiments need confirmation under conditions where the amount of labeled iron available for synthesis is known to be the same in the normal and tumor-bearing animal and with an improved method for the isolation of catalase. Price et al. (168)
developed another method for determining the rates of catalase synthesis and destruction, and these kinetics have been shown in the normal rat (169), but information about the tumor-bearing animal is still unavailable by this method. A quantitative immunochemical procedure for determining hepatic catalase has been developed by Nishimura et al. (150). This method indicated that catalase synthesis was markedly inhibited in the liver of mice during the latter stages of growth of Ehrlich ascites tumor.

Further studies on catalase synthesis and intracellular distribution are needed to determine whether the mechanism of lowering liver catalase activity is different in the tumor-bearing animal from that produced by injecting materials. If they are similar, the mechanism of decreased catalase activity may be more readily studied after injections of turpentine, endotoxin, and other agents. Recent observations suggested that liver catalase was associated with the uricase particle which differs from both the large mitochondria and the lysosomes (136). The permeability of some of these particles was affected by cortisone, thiorzot, and endotoxin-tolerance (97). This would suggest a relationship between the RES and the release of enzymes from these particles. Studies on the rates of catalase synthesis and leakage in normal, tumorbearing and endotoxin-treated animals should yield valuable information about mechanisms and possible differences among these conditions.

**DISCUSSION**

Liver catalase activity can be altered by a variety of experimental conditions. Many of these conditions have assumed lesser importance in tumor-host systems because the depression was smaller than that which occurred in the tumor-bearing animal. Thus, in most cases, it has not been shown that anemia, nutritional factors, or hormones were nonoperative, but only that the effects of the tumor on liver catalase were great in proportion to these effects. In all studies, however, the possibility of bacterial contamination exists. Thus it may be necessary to reinvestigate many of these conditions in tumor-bearing animals which are known to be free of bacteria.

Greenstein stated that lowered liver catalase must be due either to the extraction of essential materials from the blood stream by the tumor or to the elaboration of toxic materials by the tumor (69). Most of the studies in recent years have emphasized the production of toxic materials by the tumor tissue. Toxic materials still seem to provide the best possible explanation for the marked lowering of liver catalase which occurs in many tumor-bearing animals. It appears unlikely, however, that the tumor tissue itself is the source of this toxin even though the tumor must be present in order for the depression to occur. Other possible sources would be bacterial or viral contaminants or reactions of the host animal to certain abnormal conditions.

The possible elaboration of material by the host, which could affect liver catalase activity, is shown by the relationship of catalase activity to the functional state of the RES (102, 104, 109, 153), and by the many diverse materials which when injected into a normal animal will depress liver catalase activity. These changes in liver catalase activity are similar to those which have been characterized as the “autonomic shift” (89) or “general adaptation syndrome” (188). Therefore, information in these areas should not be overlooked as a possible aid in tumor-host studies. The autonomic shift, for example, can result from the injection of hemoglobin into an otherwise normal animal (89). It has also been shown that subcutaneous injections of blood (66), as well as numerous other normal body constituents, will depress liver catalase activity. This information offers a possible explanation for the lowering of liver catalase in many tumor-bearing animals. Bleeding in and around tumor tissue has been shown to occur frequently in animals with transplantable tumors (167); and the animals which bleed around the tumor frequently have a lowered liver catalase (66). The previous discussions on nutrition and iron metabolism indicate that the loss of protein or iron probably was not responsible for the decrease in liver catalase.

The tumor may therefore depress liver catalase activity by causing the release of a normal body constituent in an unusual location. These materials may be placed in an unusual environment by the rupture of capillaries during tumor growth. A reaction similar to that following a subcutaneous or intramuscular injection of these materials might therefore be expected, and under these circumstances many materials are known which will depress liver catalase activity.

Much additional information is required on how these toxic materials, either from the host, tumor, or bacteria, can result in lowered liver catalase activity. Some of the evidence about toxins, such as endotoxin, indicates that they will cause the in vivo release of enzymes from large subcellular particles of the liver (97, 200). Since the distribution of catalase in the liver cell has been shown to be altered during tumor growth (7), a more detailed study of the release of catalase from the uricase particle during tumor growth should give valuable information. These studies must be supplemented with more information on the rates of synthesis and destruction of catalase in the tumor-bearing host.

The studies on the mechanism of liver catalase depression in tumor-bearing animals have emphasized the many variables which must be controlled in studies on the tumor-host relationship. Several possible additional variables still deserve further investigation. Two are the effect of site of tumor growth and, in transplantable tumors, the genetic relationship to the host. The investigations in this area are still such that lowered liver catalase activity in the tumor-bearing animal seemingly can occur through many different mechanisms. There is, however, the strong possibility that all of the stresses imposed upon the host by the growth of the tumor have many things in common. This is suggested by the similarity of the changes which occur during tumor growth to those which can be produced by toxins. A better understanding of how all of these occur and what they have in common will be useful in all studies of tumor-host relationships, and perhaps in other diseases.
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