Biphasic Changes of Tryptophan Peroxidase Level in Tumor-bearing Mice and in Mice Subjected to Growth Hormone and Stress*t

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It has been well established that the growth of spontaneous, induced, or transplanted tumors in several species is associated with alterations in the enzymatic activity of nontumorous organs. The decrease in liver catalase during tumor growth has been particularly well documented (7). As yet there is no precise understanding of the mechanisms responsible for these changes, nor of their physiologic and pathologic significance. Therefore, the study of the tryptophan peroxidase-oxidase system (TPO) in liver during the growth of a transplanted neoplasm was undertaken, since its physiological role (16) and some of the factors regulating its level in the tissue are already known.

There are at least two independent ways in which the liver TPO level is regulated. It is increased in vivo by the administration of its substrate, tryptophan, to intact or adrenalectomized animals, but not by the administration of other compounds (11). This change in TPO activity is similar to the substrate-induced enzyme adaptations frequently encountered in micro-organisms. The TPO activity is also increased by the administration of cortisone or hydrocortisone to intact or adrenalectomized rats, or by the administration to intact but not adrenalectomized rats of certain compounds which stimulated the adrenal cortex (11, 24). Adrenocorticotropic hormone, histidine, epinephrine, or histamine increased the TPO activity in this way only in intact rats. Adrenalectomy alone has been shown to lower the liver TPO activity. This new kind of adaptation discovered in the higher animals has been distinguished as hormone-induced adaptation (12).

The observed changes in activity of TPO have all been measured under conditions in which the concentration of the enzyme was the sole known factor limiting the activity. It is, therefore, probable that the activity changes reflect changes in the amount of enzyme in the cells. Supporting evidence that specific protein synthesis must occur for the enzyme activity to increase was provided by the ethionine inhibition of the tryptophan-induced adaptation (14) and by the preferential incorporation of a labeled amino acid into the TPO-containing liver fraction after tryptophan induction (8). Factors affecting the enzyme level during tumor growth would therefore be expected to be relatively specific ones affecting the net synthesis of particular proteins, and possibly the same as the factors known to regulate the enzyme level in normal animals.

MATERIALS AND METHODS

Male and female C57BL/6 mice were maintained on Purina Laboratory Chow and water ad libitum. Mice of two age groups were used, either 2–5 or 5–8 months old. The transplantable tumor, Lewis sarcoma S-241 (28) (hereafter designated as S-241), was implanted by trocar inoculation into the right axillary region. At successive times during tumor growth, the tumor-bearing animals, with their controls maintained under similar conditions, were killed by decapitation. The subcutaneous tumor mass was dissected out and its size measured to the nearest 0.1 ml. by fluid displacement. Liver weights and TPO activities were determined for both tumor-bearing and control mice.

Tryptophan peroxidase assay method.—The activity of tryptophan peroxidase was measured within 45 minutes after death by the spectrophotometric determination of the amount of kynurenine formed during aerobic incubation of the gall bladder-free liver homogenate with L-tryptophan, as previously described for the rat liver enzyme (18). The assay was modified only by reducing the volumes in the reaction mixtures one-half and by adding the glucose oxidase-glucose-catalase system as a source of generated peroxide, which was found to be necessary for optimal activity of the mouse liver enzyme as described below. The homogenate from each animal was tested in two concentrations with corresponding blanks containing no trypto-
phen. The 20-ml reaction beakers contained 0.5 or 1.0 ml of homogenate, 0.5 ml of 0.2 m potassium phosphate buffer (pH 7.0), 0.3 ml of 0.015 m L-tryptophan (replaced by water in the blanks), 0.1 ml of 1.0 m glucose, 1.2 units of glucose oxidase,

1 Glucose oxidase was purified by V. H. Auerbach by an unpublished procedure from "Dee O" obtained from Takamine Laboratories, Clifton, N.J. One unit catalyzed the uptake of 1 μl. O₂ in 10 min. at 37° C in air and in the presence of 0.3 m glucose, 0.07 m phosphate (pH 6.6), 10 μg. catalase, and .005 m Na versenate.

mice assayed in the presence of increasing amounts of glucose oxidase as a generator of peroxide. It is apparent that the activities were highly variable without glucose oxidase but were much more constant and generally higher with a small amount of glucose oxidase. Still larger amounts of glucose oxidase inhibited the reaction. The optimal amount of glucose oxidase which permitted maximal activity was very nearly the same for all the animals studied and was used in the assays reported here (1.2 units for the 2-ml assay system, one-half the optimal amount for the 4-ml assay system in Chart 1). The other conditions of the assay, such as pH and substrate concentration, were optimal. There was no known limitation on activity except the amount of enzyme. No significant removal of kynurenine occurred during the reaction, since L-kynurenine, incubated with the homogenates from the several kinds of animals studied, was completely recovered. The measured activity was linear with time and proportional to the enzyme concentration.

RESULTS

Three groups of mice, differing in sex and age, were used in the present experiments. The mean activities found in the controls for each of these three groups are given in Table 1. The small differences between the different groups of controls were not statistically significant. This indicated the constancy of the enzyme level in this population of animals. The different groups also showed very similar responses to the experimental conditions and are not separately reported.

Changes in enzyme level during tumor growth.—The TPO levels in the mice given inoculations of S-241 differed significantly from those of the controls. During the first phase of tumor growth there was a marked reduction of the liver TPO activity below the level in the controls. During the second phase, the TPO levels were higher than those of the controls. These biphasic changes in the enzyme level in the tumor-bearing animals were equally clear as a function of the duration of tumor growth in days (Chart 2) or as a function of the volume of the tumor found at the time of enzyme assay (Chart 3). With either grouping there was a critical point, after about 18 days of tumor growth or when the tumor volume reached 4.5 ml., at which time the enzyme level changed from the early low level to the late high level. Although no animals were killed earlier than the 5th day after tumor inoculation, the enzyme level was already low at this time. Animals having tumors only 0.2 ml. in volume were found to have a significantly lowered enzyme level.

The results are summarized in Table 1. The activities/gm dry liver found in the 35 animals with tumors of less than a 4.5-ml volume were substantially lower than the levels in the ten animals with larger tumors. Both groups were significantly different from the controls (P < .01). Exactly the same sort of relationship and degree of significance

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activity as a measure of enzyme concentration.—It was desirable that the observed changes in TPO activity with tumor growth be measured under conditions in which the enzyme was the sole limiting factor, so that changes in enzyme activity could be ascribed to changes in enzyme concentration in the tissue. The tryptophan peroxidase activity in fresh homogenates of mouse liver was not maximal in the absence of a source of peroxide, although such an addition had not been required for maximal activity of the fresh rat liver homogenates previously studied. Chart 1 shows the relative activities of the homogenates from a number of control and growth hormone-treated
were found for the animals bearing tumors for less than 18 days and for more than 18 days.

It was possible that the low enzyme levels observed during the early phase of tumor growth represented only changes in enzyme concentration/gram but not in the total amount of enzyme/liver, since the livers of the tumor-bearing animals differed from that of the controls in the same way as the activity/gm dry weight.

Factors changing the enzyme level.—Of the two known ways of increasing the enzyme, by adrenocortical hormone stimulation or by a rise in tryptophan level, the former would appear to be the more probable explanation for the observed terminal in-

<table>
<thead>
<tr>
<th>Age (mo.)</th>
<th>Sex</th>
<th>No. animals</th>
<th>Liver weight (gm. ± S.E.)</th>
<th>TPO activity* ± S.E.</th>
<th>Total TPO activity per liver ± S.E.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3</td>
<td>M</td>
<td>21</td>
<td>1.27 ± 0.05</td>
<td>16.72 ± 0.94</td>
<td>5.80 ± 0.51</td>
</tr>
<tr>
<td>2-3</td>
<td>F</td>
<td>7</td>
<td>1.14 ± 0.06</td>
<td>15.01 ± 1.55</td>
<td>4.54 ± 0.51</td>
</tr>
<tr>
<td>5-8</td>
<td>M</td>
<td>10</td>
<td>1.00 ± 0.05</td>
<td>19.24 ± 1.11</td>
<td>7.78 ± 0.55</td>
</tr>
<tr>
<td>All controls</td>
<td></td>
<td>38</td>
<td>1.33 ± 0.04</td>
<td>16.81 ± 0.63</td>
<td>6.09 ± 0.37</td>
</tr>
</tbody>
</table>

* Expressed as μmoles of kynurenine formed in 1 hour/gm dry weight of liver.

† The differences between the values for all groups of tumor-bearing mice and the pooled controls were highly significant by the "t" test (P<0.01).

may increase in size during the early growth of the tumor (1, 5, 10, 31) and later decrease in size (22). However, the changes in liver size given in Table 1 were not of sufficient magnitude to account for the observed changes in enzyme activity/gm of liver by simple dilution or concentration. The total activity/liver (activity/gm of wet liver times the total wet weight of liver) in the tumor-bearing ani-
renine/gram dry weight), which was comparable to the increase seen in the terminal phase of tumor growth.

In the search for other means by which the enzyme level could be lowered, it was found that growth hormone injections had this effect in normal mice (Table 2). In growth hormone-treated mice, the liver TPO level was reduced about half, compared to the reduction seen during the early phase of tumor growth. This reduction was seen only 6-12 hours after hormone administration, and the control level was regained within 24 hours. A single dose of 3 mg appeared to be equally effective as a series of three such doses, although no real effort was made to determine the minimal effective dose. A single injection of 0.5 mg was, however, not effective in one animal.

**Table 2**

<table>
<thead>
<tr>
<th>HOURS AFTER LAST DOSE</th>
<th>GROWTH HORMONE* (mg/dose)</th>
<th>HORMONE-TREATED ANIMALS</th>
<th>TPO ACTIVITY (u/ml/dry wt.)</th>
<th>DIFF. FROM CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>3</td>
<td>4</td>
<td>13.4±2.12</td>
<td>7</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>7</td>
<td>8.5±1.37</td>
<td>0.1</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>6</td>
<td>10.9±1.38</td>
<td>0.75</td>
</tr>
<tr>
<td>12</td>
<td>0.5</td>
<td>1</td>
<td>8.9</td>
<td>0.001</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>1</td>
<td>9.6</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* Growth hormone (Armour's) was administered intraperitoneally, dissolved in saline, at 24-hr. intervals to the mice receiving multiple doses. The controls were injected with saline at the same time.

**DISCUSSION**

The enzyme assay conditions used made it most probable that the observed activity changes were the result of changes in the amount of enzyme per total liver, per gram of dry liver, and presumably per cell. Similar changes are known to occur in the amounts of this and other enzyme proteins under a variety of physiological stimuli (18). These consist of the metabolic adaptations induced by the substrates of the enzymes, by particular hormones, and by more complex stimuli in which the primary agent causing the change in the amount of a protein is still undetermined. A relevant example of the latter type is the decrease in liver catalase in tumor-bearing animals. The early decrease in TPO levels observed here has some parallels with the changes in catalase, since both are liver enzymes, both probably have iron-porphyrin prosthetic groups, and both decrease in tumor-bearing animals. The possibility that these changes result from a tumor-induced limitation of iron-porphyrin synthesis is unlikely in view of the later increase in the amount of liver TPO. The changes in amounts of both enzymes would appear to result from tumor-induced alterations in the rates of synthesis or degradation of these particular proteins.

Changes in the amount of liver TPO analogous to the early decrease and late rise in the tumor-bearing animals might show more precisely whether the terminal elevation is a substrate- or hormone-induced change. At the time these experiments were done, the only procedure known to decrease the enzyme level was adrenalectomy. Subsequently, decreases to less than half the normal level of TPO have also been observed in the livers of partially hepatectomized rats (20). The discovery of the effectiveness of growth hormone in lowering the enzyme level now provides three situations in which this same phenomenon of a 50 per cent decrease in enzyme level occurs, viz., early tumor growth, liver regeneration.

eration, and treatment with growth hormone. The lowered enzyme level could be considered in all three instances to result from the direct action of injected or secreted growth hormone, or to be the result of decreased tissue tryptophan concentrations, secondary to the increased growth and protein synthesis occurring in these animals.

Some of the inter-relationships between early tumor growth, liver regeneration, and growth hormone treatment may be further illustrated. (a) Certain transplanted rat neoplasms (Walker carcinoma 256 and a hepatoma) grew more rapidly in partially hepatectomized than in intact control animals (20). (b) Growth hormone treatment of tumor-bearing mice produced an acceleration in early tumor growth.* (18, 25). (c) Partial hepatectomy of one member of a parabiotic pair causes increased weight and mitotic activity of the liver of the other intact parabiont (4, 27). (d) Growth of experimental tumors is associated with an increase in liver weight (1, 3, 10, 31). (e) Growth hormone treatment increases liver weight (5, 15). Each of these conditions is characterized by an anabolic state. This state may be produced in each instance by a growth-promoting factor like growth hormone.

The nature of the responsible agent in the purified growth hormone preparation has not been identified. In the absence of information as to the minimal effective dose acting on the TPO level and the effect of inactivated growth hormone, growth hormone itself cannot be finally identified with the agent producing this effect on the TPO level.

The expected physiological significance in the whole animal of a low and later of a high TPO activity from whatever causes are operative would be (a) an early anabolic phase with little tryptophan wastage by degradation and (b) a later catabolic phase with degradation of excess tryptophan. Studies on the body and tumor nitrogen metabolism of pair-fed rats bearing Walker carcinoma 256 have shown that such distinct phases occurred, with an initial increase in body weight followed by body wastage, while the tumor continued to grow during both phases (17). Further experiments to determine the liver TPO levels during the growth of other tumors, and in different species, as well as following total extirpation of the primary neoplasm, will be necessary to establish the general validity of this correlation between enzyme level and nitrogen metabolism.

Insofar as liver TPO levels are concerned, growth hormone and hydrocortisone treatment may duplicate, in normal animals, the metabolic stages of the early and late phases of S-241 growth. The possible pathologic significance of these alterations of metabolism has been further investigated in the same animals used here by determining the effect of growth hormone or hydrocortisone administration on the growth of S-241. These studies showed an acceleration by growth hormone of tumor growth rate during the early phase (first 2 weeks), with later refractoriness (29) and prolonged retardation of tumor growth by hydrocortisone. On the other hand, both hormones favored successful lodgement and growth of embolic tumor cells in the lung (29).

**SUMMARY**

Quantitative assays were made of the liver tryptophan peroxidase activity in 38 controls, and 46 C57BL/6 mice bearing subcutaneous implants of the Lewis sarcoma 241. A depression of the enzymatic activity to one-half the control level was found in mice bearing tumors less than 4.5 ml. in volume, and during the initial 18-day period of tumor growth. Mice with tumors of a size larger than 4.5 ml. or after the first 18 days of tumor growth showed significant increases in the enzyme activity over the controls. Changes analogous to this biphasic depression and elevation of the enzyme level in tumor-bearing animals could be produced in control mice by growth hormone and by adrenal-stimulating stress, respectively.

**ACKNOWLEDGMENTS**

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**REFERENCES**


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