Metabolic Adaptations in Rat Hepatomas

VI. Substrate-Hormone Relationships in Tryptophan Pyrrolase Induction*

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SUMMARY

The failure of induction of tryptophan pyrrolase (TP) in the majority of hepatomas after the administration of substrate or hormone was shown probably to be the result of a basic defect in the tumor cell itself, and not of the lowered blood flow or the lack of portal blood supply to the tumor.

Two exceptions, Hepatomas H-35 and 7793, were shown to have a significant substrate induction in the intact but not in the adrenalectomized host.

The absence of "pure" substrate induction in these hepatomas was interpreted in the light of modern concepts of protein synthesis and the control of enzyme synthesis. The interpretation suggested that hepatomas may have lost the capacity to maintain a stable RNA template for tryptophan pyrrolase synthesis, so that the substrate induction in the hepatomas no longer occurs unless the RNA template is renewed by corticosteroid stimulation.

In a preceding paper in this series (24) we presented evidence that the Morris hepatoma 5123, although having most of the enzymic and morphologic features of normal rat liver, would not significantly alter its level of tryptophan pyrrolase (TP) in response to the parenteral administration of substrate or cortisone to the (tumor-bearing) host. This failure of enzyme "adaptation" was shown to result not from any intrinsic variables in the assay procedure itself or from the lack of availability of the inducer to the neoplasm. The conclusion arrived at both from these and previous data was that the failure of tryptophan pyrrolase induction in Hepatoma 5123 was the result of some intrinsic defect within the neoplastic cell itself.

Previous work with primary hepatomas and with the transplanted Dunning L-C18 hepatoma (22) gave results similar to those found with the 5123. Furthermore, slices of these tumors showed no induction of tryptophan pyrrolase when incubated in Eagle's medium in the presence of tryptophan. Other workers have reported poor (4) or absent (1) induction of this enzyme in primary hepatomas.

The results of this work show that a few hepatomas are capable of a slight absolute degree of tryptophan pyrrolase induction and even of a "normal" relative degree of induction, but only in the intact host. Furthermore, evidence will be presented to show that the low degree of enzyme induction in the tumor is itself probably a basic abnormality in the neoplastic liver cell and not a result of the relatively low blood flow in the hepatomas as compared with liver.

MATERIALS AND METHODS

The tumor-bearing animals used in these experiments were the Morris hepatoma 5123, sublines A, B, and D, generations 25–35; 7316; 7793; 7800; and the Reuber hepatoma H-35. The Reuber hepatoma H-35 was carried in ACI/N animals, and all other hepatomas carried in Buffalo strain rats at the National Cancer Institute and after 1–2 months shipped to McArdle Laboratory, where they were used in the experiments reported here. Both male and female animals bearing the hepatomas and sublines A, B, and D of Hepatoma 5123 were used in these experiments, no significant difference being noted with respect to sex or sublines of tumor in our hands.

All animals were killed by cervical dislocation, and homogenates were prepared and tissues examined as described in the previous publication (25). The assay of TP was that described earlier (25). It was a modification of that described by de Castro et al. (8) with the coupling procedure of Brown and Price (3). In addition, the assay included the addition of catalase, 50 μg/ml ascorbic acid, 10−4 M (H2O2 generating system [33]) and Mn++, 10−4 M, to the reaction mixture, since in cortisone induction TP

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activity was enhanced 2–3 times more when the H₂O₂ generating system and Mn⁺⁺ were added to the incubation mixture, compared with the activity found in the flask lacking these components. No apparent effect of these components was shown in the liver and tumors of noninduced or tryptophan-treated animals.

**Enzyme induction.** For the substrate induction animals were given injections intraperitoneally at zero time and at 3 hours of 4.0 ml of an 0.25 M suspension of L-tryptophan in 0.9 per cent NaCl and sacrificed 6 hours after the initial injection. For the attemptted induction by phenylalanine and methionine, amounts of these compounds equimolar with the amount of L-tryptophan used for induction were given to animals in the same manner as was the L-tryptophan acetate. Animals intraperitoneally at zero time and 3 hours later. The substrate and hormonal induction of TP of host liver and hepatomas in intact and adrenalectomized rats after administration of tryptophan or cortisone in vivo.

### RESULTS

The substrate and hormonal induction of TP of host liver and hepatomas in intact and adrenalectomized animals is seen in Table 1. The enzyme levels of the host

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**TABLE 1**

<table>
<thead>
<tr>
<th>Hepatoma</th>
<th>Control</th>
<th>Tryptophan*</th>
<th>Cortisone*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Tumor</td>
<td>Liver</td>
</tr>
<tr>
<td>5123</td>
<td>1.0 ± 0.1 (2)</td>
<td>0.2 ± 0 (2)</td>
<td>2.2 ± 0.8 (2)</td>
</tr>
<tr>
<td>H-35</td>
<td>2.5 ± 1.1 (8)</td>
<td>0.3 ± 0.2 (8)</td>
<td>23.8 ± 4.9 (8)</td>
</tr>
<tr>
<td>7316</td>
<td>2.0 ± 0.5 (6)</td>
<td>0.1 ± 0.01 (6)</td>
<td>21.0 ± 9 (3)</td>
</tr>
<tr>
<td>7793</td>
<td>2.7 ± 1.2 (5)</td>
<td>1.2 ± 0.6 (5)</td>
<td>27.0 ± 9.5 (4)</td>
</tr>
<tr>
<td>7800</td>
<td>2.2 ± 0.7 (6)</td>
<td>0.3 ± 0.2 (6)</td>
<td>19.0 ± 6.6 (4)</td>
</tr>
</tbody>
</table>

* The enzyme units are expressed in μmoles kynurenine/hr/gm tissue. The single numbers are the average of the groups ± standard deviation. The numbers in parentheses denote number of animals used. See "Materials and Methods" for dosage employed.
liver in the intact hosts varied from 0.7 to 3.8 units as seen from the range of individual values. The normal livers of tumor-free Buffalo strain rats possess 2–3 units (25). The lower activities in some host livers may reflect the pathological changes seen in some, such as fatty metamorphosis, etc. After the administration of tryptophan, the enzyme activity of the host liver in intact animals increased about tenfold over the control levels, whereas cortisone produced slight and variable degrees of induction of the enzyme in the host liver.

The great majority of tumors growing in intact hosts possessed enzyme levels between 0.1 and 0.3 units, as seen in Table 1. This range of TP activities was also found previously in the Dunning L-C18 and some primary hepatomas (22). The Morris hepatoma 7793 is the exception to this rule. The enzyme activity of this tumor in the intact host was within the normal range found for host livers. From the table it is obvious that no stimulus gave any significant change in the low enzyme levels seen in the tumors, with two exceptions: the Reuber hepatoma H-35 and the Morris hepatoma 7793 growing in intact hosts. The H-35 hepatoma in the intact host was capable of a significant induction of TP after the administration of tryptophan but showed only a slight response to the administration of cortisone. Hepatoma 7793, which in the intact host possessed an exceptionally high basal level of TP, responded to both the substrate and hormonal stimuli by a significant rise in TP.

When rats bearing Hepatoma 7793 were adrenalectomized, the TP level in the neoplasms fell to the usual low value of 0.1 unit, and the administration of tryptophan no longer had an effect on the enzyme level of the tumor but gave a significant rise in the TP of the host liver. Nevertheless, the response to the administration of cortisone was still retained in Hepatoma 7793 in the adrenalectomized host. Interestingly, Hepatoma 5123 in the adrenalectomized host as well as the host liver showed an increased responsiveness of the TP-forming system to corticosteroid hormones. Adrenalectomy of rats bearing H-35 hepatomas produced the same effect as with 7793 in that tryptophan induction was lost in the hepatoma but not in the liver. Thus, two hepatomas—the H-35 and 7793—retained some response of TP to substrate in the intact but not in the adrenalectomized host. In connection with these data, the induction mechanism of tyrosine transaminase reported by Lin and Knox (19), and recently by Kenney and Flora (15), became of interest. They have reported that the induction of tyrosine transaminase is clearly not the result of a specific action of substrate per se, but appears to be entirely mediated by adrenal hormones, since induction by tyrosine is effective only in the presence of adrenal hormones. Tyrosine induction may be surpassed by other amino acids and also by insoluble inorganic preparations. This observation promptly led to the question whether TP induction in hepatomas H-35 and 7793 is, like the transaminase, exclusively controlled by adrenal hormones with no substrate-inducer specificity. If such were the case, induction of TP of these tumors in the intact host would occur not only by tryptophan but also by other amino acids which would stimulate the release of adrenal hormones. The experiment in Table 2, however, shows no induction of TP by L-methionine or L-phenylalanine in the tumor as well as in the host liver. However, in transaminase induction in the intact animal (15) it was shown that tryptophan was as effective as tyrosine, causing a seven- to tenfold stimulation, and the enzyme activity was also elevated fourfold by phenylalanine and threefold by methionine. Thus, when TP induction occurs in hepatomas it appears to retain the substrate inducer specificity seen in normal liver (16).

The absence of substrate induction in the tumors of the adrenalectomized host led to further experimentation on the relationship of the hormonal and substrate induction in the tumor. In the experiment shown in Chart 1 adrenalectomized animals bearing the H-35 hepatoma were maintained on 0.3 mg cortisone/day for 7 days after surgery and were then tested for substrate induction of TP. It is seen that under this treatment the substrate induction was restored in the tumor. This effect of the hormone, which allows a "normal level" (the induced level seen in intact animal) of substrate induction in the adrenalectomized animal, has been termed a "permissive" effect of the hormone by Lee and Baltz (18).

Since normal liver has a dual blood supply consisting of

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>L-Methionine</th>
<th>L-Phenylalanine</th>
<th>L-Tryptophan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.5†</td>
<td>1.4</td>
<td>1.5</td>
<td>23.8†</td>
</tr>
<tr>
<td>Tumor</td>
<td>0.3†</td>
<td>0.3</td>
<td>0.2</td>
<td>2.5†</td>
</tr>
</tbody>
</table>

* See legend of Table 1.
† Values from Table 1.
portal and arterial blood whereas transplanted hepatomas possess only an arterial blood supply, it is possible that the differences in blood supply between the liver and tumor might, at least in part, be responsible for the different levels of TP in these tissues following the administration of inducer. In fact, Gullino (13) has shown the markedly decreased blood flow of transplanted hepatomas as compared with liver. Thus, portal ligation of a single lobe in normal liver was undertaken to determine whether or not a hepatic lobe not supplied with portal blood (i.e., supplied only by arterial blood) is capable of as much TP induction as the hepatic lobe supplied with both portal and arterial blood. The result is shown in Chart 2, where one sees no significant difference in the induction of TP between ligated and nonligated hepatic lobes. Further confirmation of this result is seen in the experiment with porta-caval-shunted animals bearing Hepatoma 5123. The response of the hepatoma and host liver, when devoid of a complete portal blood supply, to the administration of tryptophan is seen in Table 3. The noninduced TP level of the host liver was rather low, but the induction was to normal range if not supernormal (ten- to twentyfold over control). No induction occurred in the 5123. Therefore, a liver devoid of portal blood supply is capable of normal TP induction, and hepatomas growing in such hosts are still not capable of TP induction. In Table 4 the question whether or not the inducer, tryptophan, was readily available to the neoplasm in vivo was answered more directly with the use of tryptophan-C14. Previous studies with intact animals bearing the 5123 and induced with tryptophan-C14 (24) had shown no appreciable difference in tryptophan pools in liver and tumor at 4 and 8 hours. However, since Knox (6) had shown the peak of intracellular tryptophan concentration to occur within 40 minutes of injection, earlier time points were studied (Table 4). One may see from the results that the specific activity of the acid-soluble fraction of the hepatoma at 20 and 40 minutes was slightly lower than that of the host liver, but the difference between host liver and tumor was not significant. Conversely, the specific activity of the protein fraction of the tumor was a little higher than that of liver, but again probably no significance can be attached to this observation. Chromatography of small aliquots of the acid-soluble fraction both from liver and tumor (see "Methods") showed a single radioactive peak having $R_f$ values of 0.80–0.82 which were reasonably close to the $R_f$ value of standard tryptophan-C14, 0.79. These findings confirm the fact that there is no difference in the availability of the inducer to host liver and Hepatoma 5123 in shunted animals when tryptophan is administered intraperitoneally.

Sourkes (32) has reported that the analog α-methyl-tryptophan is a highly efficient inducer of TP in the intact animal over a prolonged period of time. We have substantiated and extended this result to rats hypophysectomized for 6 months. As seen in Chart 3, an excellent response to α-methyltryptophan, but little response to tryptophan (as reported by Rosen [29]), was elicited. The response of TP of several hepatomas to this analog is seen in Chart 4. Again, little or no induction occurred in the hepatomas tested, although a significant induction
DISCUSSION

From the results presented here the substrate induction of tryptophan pyrrolase in the host liver of tumor-bearing animals was within the normal range; however, the response of TP to the administration of cortisone appeared to be somewhat altered (Table 1) with respect to the degree of the response. The enzyme level found in the host liver of the intact animal after the administration of cortisone was variable and generally lower than that found in the substrate-induced animal. In normal, tumor-free rats the levels of the substrate and cortisone induction of tryptophan pyrrolase are equal (16). Furthermore, the responses to cortisone administration were enhanced in the host livers of adrenalectomized rats bearing Hepatomas 5123 and H-35 as compared with the intact animals bearing these hepatomas. This result is in contrast to normal rats as reported by Knox and Auerbach (17), wherein both substrate and hormonal induction was lower in adrenalectomized than in intact rats. Studies (23) of other enzymes, such as threonine dehydrase and tyrosine transaminase, also have shown the variable degree of induction by cortisone in the host liver of rats bearing these hepatomas. These findings may reflect a complicated host-tumor interaction, the mechanisms of which are not understood at present. One possible explanation is that the neoplasm acts as a "corticosteroid" trap having a somewhat greater affinity for the steroid than liver. Experiments are now under way to test this hypothesis.

In the majority of hepatomas (Table 1), TP was shown to be at very low levels and not significantly responsive to any of the controls, hormonal or substrate, that are known to influence the synthesis of this enzyme (10). The data presented in Chart 2 and Table 3 demonstrated that the failure of a response to be effected in these hepatomas was not a function of the decreased blood flow or of the lack of portal blood supply to the hepatomas. Furthermore, by means of a labeled inducer (Table 4), no difference was found in the labeling of the acid-soluble

or-insoluble fractions between hepatoma and liver, substantiating the fact that as much inducer is available to the tumor as to liver. Dyer et al. (9) have also shown that the failure of TP induction in hepatomas is not the result of lowered substrate availability. In addition, the nonmetabolizable analog a-methyl-tryptophan, known to be a more efficient inducer of tryptophan pyrrolase (32), had no effect on the TP induction in these hepatomas.

These observations further substantiate the hypothesis that virtual absence of induction of TP in hepatomas is probably the result of some defective mechanism(s) within the tumor cell itself.

There were, however, two exceptions (Table 1), the H-35 and 7793 hepatomas, which retained some response of TP to substrate administration in the intact host. However, upon adrenalectomy of the hosts the substrate induction in these hepatomas was lost. The failure of substrate induction of these hepatomas in the adrenalectomized animal is in contrast to normal (tumor-free) adrenalectomized liver (18) wherein a "pure" substrate induction occurs in the absence of endogenous corticosteroids. TP is the only enzyme in the mammal known to be under dual—i.e. specific-substrate and hormonal control. The independence of the hormonal and substrate induction of TP was first emphasized by Knox and co-workers (6) who showed that cortisone does not operate by raising the level of tryptophan in the liver and that induction by tryptophan does not require adenocortical secretion. Later, Greengard and Feigelson (12) also presented evidence that the availability of hemin, cofactor for TP, is different in substrate as opposed to hormonal induction of this enzyme. However, it is also known (15) that the administration of tryptophan stimulates corticosteroid secretion, and thus TP induction by tryptophan in intact rats is partially attributable to hormonal induction. Thus, "pure" substrate induction of TP can be demonstrated only in the adrenalectomized animal. Interestingly, the failure of "pure" substrate induction of hepatoma in the adrenalectomized host was restored with the aid of a "permissive" dose (18) of cortisone (Chart 1), suggesting the complete dependence of substrate induction on the presence of corticosteroids in these hepatomas.

Several interpretations of these results are possible. First, it may be proposed that the enzyme in the hepatoma is different in its structure from that of the liver and that such a lesion results in a defective control of enzyme synthesis. This proposal was tested (5) with a purified (1000-fold) TP of liver and Hepatoma 7793. No significant difference was noted either in certain of the kinetic constants or in the electrophoretic mobilities between liver and hepatoma. In addition, partially purified TP from Hepatomas 5123 and H-35 gave similar Km values to that of the liver enzyme. Thus it appears that the molecular structure of TP of hepatoma is probably similar to that of the liver.

Second, it is conceivable that TP in these hepatomas may be under exclusive hormonal control, as has been reported for tyrosine transaminase, instead of having a dual (substrate and hormonal) control as in normal liver. This possibility was excluded by the experiment
shown in Table 2 wherein the amino acids methionine or phenylalanine, which induce tyrosine transaminase in the intact rat probably indirectly by adrenal stimulation (15), showed no induction of TP in the H-35 hepatoma, whereas tryptophan elicited a significant rise in the enzyme level.

Finally, a more hypothetical but at present most plausible interpretation of these data can be proposed according to modern concepts of protein synthesis and the control of enzyme synthesis. Such a hypothesis is tenable, since the induction of tryptophan pyrrolase has been shown (10) to be a reflection of the actual synthesis of this enzyme.

The mediation of protein synthesis in both bacteria and mammalian liver by an unstable, rapidly turning over RNA fraction designated messenger RNA (mRNA) has been well supported by much experimental evidence (21). However, the knowledge of the fate of the mRNA molecule during the formation of protein has been somewhat meager. The antibiotic, actinomycin D (an inhibitor of DNA-directed RNA synthesis [27], has been recently used in the determination of the time that a given mRNA functions in protein synthesis—i.e., the lifetime of the template for protein synthesis.

Reich et al. (28) observed a considerable if not absolute, stability of some of the protein-synthesizing templates in mammalian tissue culture cells through experiments showing that a substantial amount of protein synthesis continued for prolonged periods under conditions wherein the concentration of actinomycin D inhibited 99 per cent of RNA synthesis. More recently, Davidson et al. (7) have also reported, in a study of mammalian tissue culture cells, that the specific activity of succinic dehydrogenase was not diminished in the cells suffering with near-total blockage of nuclear RNA synthesis by actinomycin D for many hours. They concluded that the gene action determining this enzyme system appears to have a low degree of "immediacy," (i.e., immediate genetic control dependent on constant RNA synthesis) despite the fact that there is available experimental evidence (20, 26) demonstrating the genetic control of mitochondrial systems. Hemoglobin synthesis in mammalian reticuloocytes has also been shown to require a low degree of immediate genetic control by the experimental evidence that hemoglobin synthesis was active for days after the loss of the cell nucleus (31) and that actinomycin D does not inhibit this synthesis (28), although it is well known that hemoglobin synthesis is under genetic control. These observations suggest the presence of ribosomal protein synthesis which does not require an immediate gene control—i.e., the presence of stable mRNA templates capable of protracted independent function in mammalian cells. Recently, Greengard and Acs (11) made an interesting observation on TP induction in normal adrenalectomized rat liver in connection with the stability of an RNA template for TP synthesis. They have shown that actinomycin D inhibits cortisone induction of TP in the adrenalectomized animal but has no effect on the "pure" substrate induction. With a knowledge of the mechanism of actinomycin D they suggested that the hormone-induced elevation of enzyme levels but not the substrate induction requires the renewal of some fraction of cellular RNA—probably mRNA. The fact that the "pure" substrate induction of TP occurs in liver in the absence of mRNA synthesis suggests the existence of a stable mRNA template for TP synthesis in normal liver. Through this type of reasoning the absence of pure substrate induction in the hepatomas can be explained in that the hepatomas have lost the capacity to maintain a stable RNA template for TP synthesis. Consequently, the specific substrate induction of TP in the hepatoma in the adrenalectomized host could be restored only if there is a renewal of RNA template by corticosteroid stimulation. This was in fact the case with H-35 hepatoma in the adrenalectomized host (Chart 1). Of course, experiments testing more directly the stability of RNA template in TP synthesis would be required for the further confirmation of the thesis. Furthermore the test of the stability of an RNA template in other enzyme synthesis in hepatomas would be of importance for a more precise understanding of the essential characteristic of neoplasia responsible for the conversion of one normal cell type to its malignant counterpart. Such is currently under investigation in this laboratory.

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