The Functional Significance of Changes in Activity of the Enzymes, Tryptophan Pyrrolase and Tyrosine Transaminase, after Induction in Intact Rats and in the Isolated, Perfused Rat Liver*

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SUMMARY

The possible functional significance of induction of the hepatic enzymes, tryptophan pyrrolase and tyrosine transaminase, has been studied in intact normal and adrenalectomized adult male rats and in the isolated perfused rat liver. In intact, normal rats, several-fold increases in tryptophan pyrrolase activity induced by treatment with hydrocortisone or tryptophan, or both, were associated with at most a 20% increase in the percentage dose of DL-tryptophan-3-14C converted to 14CO2. However, under conditions in which the activity of tryptophan pyrrolase did not increase further, for example, with increasing the tryptophan load from 17.5 to 35 mg, the conversion of labeled tryptophan to 14CO2 was substantially increased.

In the adrenalectomized rat, hydrocortisone elicited a 6-fold increase in tryptophan pyrrolase activity without changing oxidation of a tracer dose of DL-tryptophan-3-14C to 14CO2. Although hydrocortisone added to the labeled tryptophan load led to a further 4-fold increase in tryptophan pyrrolase activity, no change in oxidation of tryptophan to 14CO2 was seen.

In the adrenalectomized rat the percentage dose 1-tyrosine-1-14C converted to 14CO2 increased substantially with a 10-mg or 30-mg load of L-tyrosine in spite of the fact that the level of hepatic tyrosine transaminase remains unchanged; in contrast, treatment with hydrocortisone leads to a 7- to 14-fold increase of enzyme activity, yet the increase in oxidation of l-tyrosine-1-14C to 14CO2 is trivial at several load levels.

In the isolated perfused rat liver induction of tryptophan pyrrolase with hydrocortisone did not alter the rate of clearance of a graded load of L-tryptophan from the perfusate, did not enhance accumulation of kynurenine in the perfusate, and did not increase the rate of conversion of DL-tryptophan-3-14C to 14CO2.

Thus, manifold increases in enzyme activity inducible in the intact or adrenalectomized rat and in the isolated perfused liver are not associated with parallel increases in oxidation of 14C-labeled amino acids to 14CO2. However, increasing the substrate load per se was associated with quantitatively large increases in oxidation to 14CO2 under conditions not associated with altered levels of enzyme activity.

Ever since the early observations of Knox (1) and Lin and Knox (2) the enzymes tryptophan pyrrolase (L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12) and tyrosine transaminase (L-tyrosine:2 oxoglutarate aminotransferase, EC 2.6.1.5) have been studied extensively, particularly to gain some insight into the mechanism of induction by substrate or by adrenal cortical hormones (3-7). Although the quantitative magnitude of the increases in enzyme activity may exceed 2 to 4 times control values, surprisingly little effort has been directed toward elucidating the possible functional significance of the observed changes in enzyme activity. Thus, the loss of body weight, observed by Sankoff and Sourkes (8), and attributed by them to induction of tryptophan pyrrolase, is basically obscure. Similarly, Moran and Sourkes (9), Henderson and Hankes (10), and Hankes, Brown, and Schmaeler (11) have described increased elimination of respiratory 14CO from intact rats given DL-tryptophan-1-14C, or DL-tryptophan-2-14C, incidental to increasingly larger doses of L-tryptophan or DL-α-methyl tryptophan. Without making actual measurements, they suggested that these increments reflected increased activity of tryptophan pyrrolase induced by large doses of substrate or analogue, respectively (8-11).
Altman and Greengard (12) have correlated human hepatic tryptophan pyrrolase and urinary kynurenine; they administered large doses of tryptophan to human subjects previously treated with hydrocortisone and ascribed small increases in urinary excretion of kynurenine to increased tryptophan pyrrolase activity as measured in homogenates of liver biopsies. This report describes studies carried out in normal rats, in adrenalectomized rats, and in the isolated perfused normal rat liver; they were designed to explore the functional significance of changes in activity of the enzymes tryptophan pyrrolase and tyrosine transaminase as measured in vitro after hormonal or substrate induction. Functional activity was estimated in terms of the elimination of $^{14}$CO from L-tryptophan-3-14C, or substrate induction. Functional activity was estimated in adrenalectomized rats, and in the isolated perfused normal rat liver; they were designed to explore the functional significance of such substrates as measured in homogenates of liver biopsies.

In perfusate samples from liver perfusions kynurenine was determined by methods already mentioned (18), tryptophan was estimated spectrophotometrically (20), and free α-amino acid nitrogen was measured by the ninhydrin method of Moore and Stein (21).

**Experimental Results**

*Induction of Tryptophan Pyrrolase in Intact Rats Given Graded Doses of Tryptophan or Hydrocortisone (or Both)*

Experiments were first carried out in intact rats to ascertain whether induction of tryptophan pyrrolase and oxidation of ni-tryptophan-3-14C to $^{14}$CO$_2$ occurred to varying degrees with graded doses of tryptophan (Fig. 1) with or without preliminary injection of hydrocortisone. Fig. 1 reveals that doses of 8 and 17.5 mg of L-tryptophan, given intraperitoneally, are associated with substantial increase in the level of tryptophan pyrrolase activity, and that doses of 35 and 70 mg are without significant further effect. Similarly, in homogenates from livers of rats previously treated with hydrocortisone, there is clearly substantially greater tryptophan pyrrolase activity than that seen in homogenates from rats treated only with a load of tryptophan. With the largest tryptophan load, one sees an approximately 4-fold increase in enzyme activity whether one compares the activity of the rats treated or untreated with hydrocortisone, respectively.

*Rate of Oxidation of L-Tryptophan-3-14C to $^{14}$CO$_2$ in Intact Rats*  
*Effects of Tryptophan Load or Hydrocortisone Treatment (or Both)*—Fig. 2 presents data on the cumulative per cent dose of parenterally administered L-tryptophan-3-14C converted to $^{14}$CO$_2$ and reveals, as has been observed by others (11), that the per cent dose conversion to $^{14}$CO$_2$ is increased with increasing load of tryptophan. Thus, an average of 4.8% of a tracer dose is converted to $^{14}$CO$_2$ in 6 hours. With a load of 17.5 mg of L-tryptophan, the conversion to $^{14}$CO$_2$ is increased to 16% of the dose and with a further increase in load to 35 mg of L-tryptophan to 20.6% of the dose. It is of interest that, in going from a load of 17.5 mg to one of 35 mg, there is no significant increase in tryptophan pyrrolase activity, yet there is a significant increase in per cent dose oxidized to $^{14}$CO$_2$.

Although, as seen in Fig. 1, preliminary treatment of the intact rat with hydrocortisone without a tryptophan load is associated with a 3-fold increase in tryptophan pyrrolase activity, Fig. 2 shows that there is only approximately a 20% increase in conversion of $^{14}$C tryptophan to $^{14}$CO$_2$. It is also of interest that, with increasing load of tryptophan, the additional influence of hydrocortisone leads in each case to a substantial increase in total tryptophan pyrrolase activity. In these experiments carried out with intact animals, it is clear that the preliminary treatment with hydrocortisone is, at every load level, associated with a small but significant increase in the per cent dose of $^{14}$C-tryptophan oxidation to $^{14}$CO$_2$. Neither at the tracer level nor in the rats with tryptophan loads is the increased conversion to $^{14}$CO$_2$ proportional to the increase in tryptophan pyrrolase activity. The results of these experiments in intact animals cannot be unequivocally interpreted as indicating that induction of tryptophan pyrrolase by hydrocortisone causes the observed increase.
in oxidation of substrate to $^{14}$CO$_2$. This difference may be related to nonhepatic effects of hydrocortisone (22) since such differences are not seen in experiments carried out with the isolated perfused liver presented below.

The lack of correlation between hepatic tryptophan pyrrolase as measured in vitro and the oxidation of the amino acid to $^{14}$CO$_2$ is amplified and extended by the data from experiments in adrenalectomized rats shown in Table I; here it is clear that 6-

![Graph showing hepatic tryptophan pyrrolase activity 6 hours after variable L-tryptophan load in intact rats.](http://www.jbc.org/)
administration of a tryptophan load of 70 mg is associated with a 3-fold increase in enzyme activity (compared with a tracer dose), but the per cent dose conversion to $^{14}$CO$_2$ is 6 times that seen with the tracer dose. Further enhancement of the enzyme activity secondary to the administration of hydrocortisone with the amino acid load leads to a further 3-fold increase in activity with no change in the per cent dose of substrate converted to $^{14}$CO$_2$. These data are more in keeping with the view that the increased oxidation is secondary to the increased amount of substrate per se rather than to the enhanced enzyme activity.

**Effect of Prior Substrate Induction of Tryptophan Pyrrolase on Oxidation of $l$-Tryptophan-$3^{-14}$C to $^{14}$CO$_2$ in Intact Rats**—Fig. 3 reveals that the preliminary daily injection of a tryptophan load for 7 days is associated with persistence of hepatic tryptophan pyrrolase activity at levels comparable with those seen in Fig. 1 after hydrocortisone treatment with no tryptophan load. It is noteworthy that treatment of these rats with hydrocortisone resulted in no further induction of tryptophan pyrrolase activity. It is significant that Fig. 4 reveals that the per cent dose conversion of labeled tryptophan given in a tracer dose has not increased in any way paralleling the enhanced tryptophan pyrrolase activity recorded in Fig. 3. However, with the load of tryptophan, there is a 4- to 5-fold increase in conversion of labeled tryptophan to $^{14}$CO$_2$. Thus the increased conversion to $^{14}$CO$_2$ is far out of proportion to the relative increase in tryptophan pyrrolase activity associated with the load and may be presumed to be related primarily to the increased amount of substrate available for oxidation rather than to the increased enzyme activity per se.
Hydrocortisone induced a 7- to 14-fold increase in transaminase levels have not changed. It is equally interesting that, although with the load in spite of the fact that the tyrosine transaminase tyrosine load, yet, as revealed in Fig. 6, the per cent n-tyrosine-is further enhanced by the simultaneous administration of the induction of 7- to 1Pfold increase in transaminase activity which Hepatic Tyrosine Transaminase Activity and on Oxidation of L-
with measurable alteration in tyrosine transaminase activity.

Effects of L-Tyrosine Load and Hydrocortisone Treatment on Tyrosine-14C to 14CO2 in Isolated Perfused Rat Liver—It is clear from Fig. 7 that the introduction of an increasing load of tryptophan is associated with induction of tryptophan pyrrolase activity most prominent at the 70- and 150-mg load levels. The introduction of hydrocortisone along with the tryptophan is in most instances associated with a significant further increase in tryptophan pyrrolase activity and confirms, qualitatively at least, observations made by Goldstein, Stella, and Knox (23) and others (24). The observations of Fig. 7 are to be kept in mind in attempting to evaluate the significance of the results in Figs. 8 and 9. The former reveals that, in spite of the significant differences in tryptophan pyrrolase activity associated with the action of tryptophan load and hydrocortisone, the per cent dose converted to 14CO2 is not affected by the hydrocortisone treatment and that whatever changes are seen are probably secondary to the size of load per se rather than to tryptophan pyrrolase activity. This conclusion is supported by the detailed data of Fig. 9 in which the time course of tryptophan 14C oxidation to 14CO2 is plotted. Close correspondence of the curves for the livers perfused with and without hydrocortisone in no way reflects the induction of tryptophan pyrrolase activity which is known to occur between 2 and 4 hours of the perfusion as noted by Goldstein et al. (23).

Lack of Effect of Hydrocortisone on Clearance of Tryptophan from Blood by Isolated Perfused Rat Liver—If the activity of tryptophan pyrrolase bears any relationship to the capacity of the liver to clear L-tryptophan from the blood then measurement of tryptophan levels in the course of the perfusions detailed in Figs. 8 and 9 should be revealing. However, the data of Fig. 10 show that L-tryptophan is rapidly cleared from the perfusate at a rate which keeps pace with the infusion and which decreases only with the cessation of the infusion after the 5th hour. The lack of effect of hydrocortisone on the hepatic clearance or oxidation to 14CO2 of L-tryptophan during perfusion is reflected in the absence of significant disparity in the final plasma concentrations of kynurenine, α-amino acids, and tryptophan presented in Table II.

**Table II**

<table>
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<tr>
<th>Infusion for 6 hr</th>
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<tr>
<td>L-Tryptophan</td>
<td>Hydrocortisone</td>
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DISCUSSION

These studies have sought to examine quantitative functional changes associated with the induction of the hepatic enzymes, tryptophan pyrrolase and tyrosine transaminase, which occurred in response to a tryptophan load or a tyrosine load or treatment with exogenous adrenocortical hormones (hydrocortisone), or both. The observations made in intact and adrenalectomized rats as well as in the isolated perfused rat liver lead to the conclusion that the quantitatively impressive, often manifold increases in enzyme activity as measured in vivo are not associated with demonstrable quantitatively parallel changes in the oxidation of L-tryptophan-3-14C or L-tyrosine-1-14C to 14CO2 or in the clearance of L-tryptophan from the blood by isolated perfused rat livers.

The use of the rate of 14CO2 evolution from the oxidation of tryptophan-3-14C or tyrosine-1-14C as a quantitative measure of functional activity in vivo of tryptophan pyrrolase or tyrosine transaminase, respectively, must necessarily depend on two conditions. First, the enzyme must catalyze the rate-limiting reaction in the sequence of reactions ultimately producing 14CO2. In these studies, direct evidence for such a rate-limiting behavior is not available and, for this reason, measurements of tryptophan disappearance and kynurenine formation were carried out in the liver perfusion experiments. However, for tyrosine transaminase, Lui and Greenberg (25) have presented evidence that the transaminase step is rate-limiting in the oxidative release of CO2 from tyrosine carboxyl by rat liver acetonemia. Second, even if the enzyme in question were that of the rate-limiting reaction, an increase in the amount of enzyme could be anticipated to mediate a parallel increase in function to the extent that the enzymes would be acting in vivo in a manner predictable from and dependent on the tacit assumptions of the Michaelis-Menten relationship.

In the light of published values of 0.5 × 10−3 m and 3.4 × 10−4 m for Km of tryptophan pyrrolase (9) and tyrosine transaminase (26), respectively, we must conclude that normal physiological blood concentrations of substrate and maximum estimated concentrations associated with the largest load used in our studies are less than or at most equal to the Km concentration. However, it is not clear to what extent one might disregard the known ability of the liver to concentrate amino acids from 2 to 4 times the blood level; disregarding the latter, one might anticipate that in these loading studies a major effect would be related to increasing saturation of the enzyme by substrate.

The enhanced per cent dose conversion of tryptophan-3-14C to 14CO2 associated with the increased amount of substrate involved in our loading experiments is analogous to that observed by Moran (9), Hanks et al. (11), and Yamaguchi, Shimoyama, and Ghoshal (27). These authors have speculated that the increased output of 14CO2 was secondary to induction of tryptophan pyrrolase. However, they did not evaluate the possible effect of a quantitative increase in the load of substrate per se. It is reasonable to expect that, if a constant dose of 14C is given to a rat in the form of a graded increase in the load of an oxidizable amino acid, the per cent of the dose of 14C converted to 14CO2 will increase with the size of the load. This should occur up to the saturation limit of the oxidation system in question.

However, in the case of tryptophan pyrrolase, in which increasing the load (with a fixed amount of 14C) leads to both increased apparent enzyme activity and increased oxidation of the amino acid to 14CO2 it is impossible to ascribe the increased oxidation clearly to one or the other factor. This difficulty has been partially circumvented by examining the effects of hydrocortisone induction on the time course of oxidation of tryptophan-3-14C at several load levels. The liver perfusion experiments of Fig. 9 reveal that there is a load-dependent increase in the per cent dose of substrate oxidized to 14CO2 with no apparent change in the curves of oxidation over the interval (3 to 5 hours) during which enzyme induction is occurring. Furthermore, the livers perfused with hydrocortisone yielded oxidation curves which are essentially indistinguishable from the untreated controls, in spite of the fact that the apparent tryptophan pyrrolase activity (Fig. 7) is very substantially greater in the hydrocortisone-treated livers.

Since a tyrosine load does not induce tyrosine transaminase in the adrenalectomized rat, the effects of increased substrate load and enzyme induction on oxidation of L-tyrosine-1-14C to 14CO2 can be dissociated. In this regard the observations made with tryptophan in the adrenalectomized rat (Figs. 5 and 6) reveals clearly that the oxidation of L-tyrosine-1-14C is greatly enhanced merely by increasing the tyrosine load under conditions in which no enzyme induction occurs. In the same figures, it is equally clear that the 7- to 14-fold increase of transaminase activity induced by hydrocortisone is associated with relatively minimal increase in conversion of L-tyrosine-1-14C to 14CO2. These results resemble, however, the small but significant enhancement of oxidation of labeled tryptophan to 14CO2 in intact normal rats (Fig. 2). Both are most likely referable to the recognized protein catabolic effect of hydrocortisone on nonhepatic tissues (22). The resulting release of amino acids could substantially increase the pools of available tyrosine and tryptophan and secondarily affect the apparent degree of oxidation of labeled substrate to 14CO2.

The phenomenon of enzyme induction as studied by the biochemist is documented by measurements in vivo of the activities of the particular enzymes under conditions which do not necessarily correspond to those prevailing in the intact organ or animal. Thus, it has become customary to measure tryptophan pyrrolase in the presence of saturating levels of hemin (28); similarly, the measurement of tyrosine transaminase activity is carried out in the presence of excess quantities of pyridoxal phosphate (26), and both enzymes are measured in the presence of saturating levels of substrate. Although such measurements are obviously reproducible, they leave unanswered the important question of whether the measurements of enzyme activity in vivo correspond to or characterize functional enhancement of the enzyme in the intact organ or animal. Perhaps the possible functional significance of changes in tissue enzyme activities could be better evaluated if such activities are measured in vivo in the presence of naturally occurring levels of substrate and of various cofactors.

In seeking to explain the increased urinary excretion of kynurenine observed in a variety of human disease states, Altman and Greengard (12) suggested that it was secondary to enhanced hepatic tryptophan pyrrolase activity, particularly since an increase of 90 to 200 μmoles of urinary kynurenine followed an oral load of 2 g (9800 μmoles) of L-tryptophan in three humans previously treated with 250 mg of hydrocortisone. This conclusion is not supported by the observed failure of hydrocortisone to alter the clearance and oxidation of tryptophan or the accumulation of kynurenine in perfused livers under conditions leading to marked enhancement of tryptophan pyrrolase activity.

Although the induction of tryptophan pyrrolase and tyrosine transaminase has afforded valuable model systems for exploring factors controlling the levels of tissue enzyme proteins, the func-
tional importance of these changes in the living organism remains to be shown.

REFERENCES
The Functional Significance of Changes in Activity of the Enzymes, Tryptophan Pyrrolase and Tyrosine Transaminase, after Induction in Intact Rats and in the Isolated, Perfused Rat Liver
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