dose caused a 4-fold increase in liver tyrosine aminotransferase, and an even lower dose (0·2 mg/kg) significantly elevated the enzyme. These results show that the increase of liver tyrosine aminotransferase by epinephrine is not necessarily mediated by glucocorticoid release, i.e. that the first possibility is unlikely.

Direct evidence for the second suggested possiblity is in Table 2. Epinephrine given to adrenalectomized rats did not increase tyrosine aminotransferase, whereas epinephrine given to such rats after hydrocortisone treatment significantly elevated the enzyme.

Both epinephrine and theophylline induced tyrosine aminotransferase in intact rats. Adrenal corticoids may have exerted at least a permissive effect on the action of both drugs, for theophylline had a smaller effect in adrenalectomized rats, and epinephrine had no effect unless hydrocortisone was administered. Presumably there was an increased amount of enzyme protein after both drugs, although the experiments would not distinguish between an increased rate of synthesis of enzyme versus a decreased rate of degradation. Although there is no evidence that changes in cyclic AMP levels in liver mediated the effect of epinephrine or theophylline on tyrosine aminotransferase, recent studies[5, 9] make that possibility seem worth considering. Recently, Holt and Oliver[10] reported the induction of tyrosine aminotransferase by epinephrine in newborn (2-to 6-day-old) rats, and Reshef and Greengard[11] have published data very similar to those in Table 2.

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REFERENCES

1. O. GREENGARD and G. T. BAKER, *Science, N.Y.* **154**, 1461 (1966).
2. M. CIVEN, B. M. TRIMMER and C. B. BROWN, *Life Sci.* **6**, 1331 (1967).
3. D. HOLTEN and F. T. KENNEY, *J. biol. Chem.* **242**, 4372 (1967).
4. C. B. HAGER and F. T. KENNEY, *J. biol. Chem.* **243**, 3296 (1968).
5. W. D. WICKS, *Science, N.Y.* **160**, 997 (1968).
6. E. C. C. LIN, B. M. PITT, M. CIVEN and W. E. KNOX, *J. biol. Chem.* **233**, 668 (1968).
7. J. H. SOLEM and T. BRINCK-JOHNSEN, *Scand. J. clin. Lab. Invest.* **17**, suppl. 80, 1 (1965).
8. R. J. WURTMAN and J. AXELROD, *Proc. natn. Acad. Sci. U.S.A.* **57**, 1594 (1967).
9. O. GREENGARD, *Science, N.Y.* **163**, 891 (1969).
10. P. G. HOLT and I. T. OLIVER, *Biochemistry, N.Y.* **8**, 1429 (1969).
11. L. RESHEF and O. GREENGARD, *Enzymol. Biol. Clin.* **10**, 113 (1969).

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Modification of the toxic actions of *l*-tryptophan by pargyline and *p*-chlorophenylalanine

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IN THEIR extensive studies on the metabolism *in vivo* of numerous amino acids, Gullino *et al.*[1] observed that the *l*-form of tryptophan possessed the greatest toxicity in the rat. They also demonstrated that it was the only amino acid which exhibited a difference in toxicity between the *d*- and *l*-isomers, the latter being the more toxic form. These observations are of interest since a considerable amount of

l-tryptophan *in vivo* is eventually converted to 5-hydroxytryptamine (5HT, serotonin),[2-4] a biogenic amine possessing both central nervous system and peripheral pharmacological effects. Whether 5HT is involved in the toxicological symptoms and eventual death of the animals with large doses of *l*-tryptophan has yet to be determined. Since a decarboxylase inhibitor reduced the toxicity of tryptophan in animals pretreated with a monoamine oxidase inhibitor, Hodge *et al.*[5] concluded that the amine metabolites, rather than the amino acid, were responsible for the toxic actions. The present study was undertaken to clarify further the role of 5HT in the production of toxicity after tryptophan administration. The compounds *p*-chlorophenylalanine, an inhibitor of tryptophan hydroxylase, and the monoamine oxidase inhibitor, pargyline, were employed as agents to regulate the levels of 5HT in the brain.

Male Sprague–Dawley rats weighing 200–300 g were used throughout this study. *l*-Tryptophan in varying doses was injected intraperitoneally in volumes of 8–10 ml. Because of its low solubility the injections were in the form of slurries suspended in 3% acacia. *p*-Chlorophenylalanine injections were also given i.p. in aqueous suspensions 17–20 hr prior to the *l*-tryptophan. For monoamine oxidase inhibition pargyline HCl (25 mg/kg as the base) was given 2 hr prior to tryptophan administration.

The rats were placed under observation and all observable symptoms were recorded. No attempt was made to quantify the responses except for the number of deaths which occurred. All deaths occurring within the first 24 hr after the administration of tryptophan were included in the final analysis.

Brains from surviving animals (24 hr after tryptophan), as well as from those dying from drug administration, were removed, frozen and subsequently assayed for 5HT levels according to the method of Bogdanski *et al.*[6] Only brains which were removed from animals shortly after death or from survivors were used for 5HT determinations. 5HT levels are expressed as the μg per g wet weight of tissue.

The administration of a dose of 12 m-mole/kg of *l*-tryptophan to Sprague–Dawley rats caused death in seven of fourteen animals, with correspondingly fewer animals dying at lower doses (Table 1). The symptoms exhibited by rats receiving the larger amounts include those described by Gullino *et al.*,[1] such as dyspnea, hypothermia, prostration and muscle incoordination. Considerable central nervous system depression also occurred. Death usually resulted between 8 and 12 hr after tryptophan

TABLE 1. EFFECT OF *p*-CHLOROPHENYLALANINE (PCPA) AND PARGYLINE ON THE LETHALITY OF *l*-TRYPTOPHAN IN RATS

Treatment (m-moles/kg)	No. dying/total No.	P compared to:
l-Tryptophan		
(a) 12	7/14	
11	0/6	
10	1/5	
9	0/3	
8	0/4	
PCPA (200 mg/kg) + *l*-tryptophan		
12	7/14	(a) N.S.
PCPA (300 mg/kg) + *l*-tryptophan		
12	4/9	(a) N. S.
Pargyline (25 mg/kg) + *l*-tryptophan		
(b) 4	5/6	
(c) 2	5/9	
1	0/5	
PCPA (200 mg/kg) + pargyline + *l*-tryptophan		
12	4/7	
8	4/12	
4	0/12	(b) $< 0{\cdot}001$
2	0/8	(c) $< 0{\cdot}05$
PCPA (300 mg/kg) + pargyline + *l*-tryptophan		
12	10/17	
8	9/18	
4	0/9	(b) $< 0{\cdot}05$

administration at the 12 m-mole/kg dose. Pretreatment with *p*-chlorophenylalanine did not alter significantly the toxicity or the symptoms of tryptophan administration, since five of seven animals died with the 12 m-mole/kg dose.

In rats pretreated with pargyline, the dose of *l*-tryptophan necessary to produce death in approximately half of the rats was reduced to about 2 m-moles/kg. The symptoms in this instance were quite different from the control tryptophan animals in that a considerable amount of excitation, piloerection, increased respiration, hyperthermia, and other signs of sympathetic stimulation were evident. In the more severe cases animals would lose motor coordination, and symptoms of tremors and forepaw clonus were observed. Increasing the dosage produced more violent reactions with death ensuing more rapidly and with greater frequency.

Pretreatment of rats with 200–300 mg/kg of *p*-chlorophenylalanine 18–24 hr prior to pargyline resulted in a marked protection upon challenge with tryptophan (Table 1). Whereas 4 m-moles/kg was lethal to five out of six rats in MAO-inhibited animals, after *p*-chlorophenylalanine at 200 mg/kg the same dose of tryptophan produced no deaths in twelve animals ($P < 0{\cdot}001$). Even at 8 m-moles/kg of tryptophan only four of twelve animals died, and at 12 m-moles/kg death occurred at the same frequency as in control animals receiving tryptophan (12 m-moles/kg) alone. The symptoms at this dose level, however, were different from control animals receiving the high dose of tryptophan. These animals initially exhibited symptoms similar to animals receiving tryptophan alone, such as hypothermia, muscle weakness and depression, but within an hour after the amino acid administration the symptoms were modified to resemble more of the MAO inhibitor plus tryptophan-treated animals; namely, signs of hyperthermia, hyperactivity and excessive salivation followed by a comatosed state and death within 3–5 hr in those that succumbed to this dose of tryptophan.

Of the rats receiving tryptophan alone, only those with the 12 m-moles/kg were assayed for brain 5HT. As indicated in Table 2 this dose of tryptophan caused an elevation of some 60 per cent above normal levels. This increase was abolished in animals treated with 200–300 mg/kg of *p*-chlorophenylalanine. 5HT levels in brains from rats pretreated with pargyline and tryptophan were elevated far above control levels. Even with 2 m-moles/kg there was a 3-fold increase in 5HT after MAO inhibition. Increasing the dose of tryptophan from 2 to 4 m-moles/kg did not increase appreciably the 5HT levels, although the lethality rose considerably (Table 1). As in the case of the rats given tryptophan alone, the pretreatment of rats with 200–300 mg/kg of *p*-chlorophenylalanine prevented the

TABLE 2. CHANGES IN BRAIN 5HT LEVELS IN RATS TREATED WITH TRYPTOPHAN, PARGYLINE AND/OR *p*-CHLOROPHENYLALANINE (PCPA)

PCPA (mg/kg)	Pargyline (mg/kg)	Tryptophan (m-moles/kg)	No.	Brain 5HT ± S. E. (μg/g)
Control			14	0·48 ± 0·03
		12	6	0·78 ± 0·25
200		12	6	0·19 ± 0·09
300		12	12	0·28 ± 0·05
	25	2	10	1·15 ± 0·10
	25	4	5	1·40 ± 0·12
200	25	8	4	0·75 ± 0·20
200	25	12	4	1·00 ± 0·16
300	25	8	7	1·22 ± 0·19
300	25	12	5	1·05 ± 0·19

excessive rise in brain 5HT even in the presence of MAO inhibition plus 12 m-moles/kg of tryptophan. Under this condition the 5HT levels in the brain were one-third to one-half as compared to animals not treated with *p*-chlorophenylalanine but with pargyline and 2–5 m-moles/kg of tryptophan.

The following conclusions may be drawn from these results: (1) The toxicity of large doses of tryptophan in the rat is not related to an increased level of brain 5HT; (2) after pretreatment with pargyline the lethality of tryptophan increases markedly, and this is associated with a 4- to 5-fold increase in brain 5HT levels; (3) *p*-chlorophenylalanine reduces the toxicity of tryptophan in pargyline-pretreated animals, indicating that 5HT is involved in the toxicity of tryptophan in such animals and (4) although 5HT is apparently involved in the toxicity of tryptophan in pargyline treated animals, the total brain levels of 5HT do not correlate with this toxicity.

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REFERENCES

1. P. GULLINO, M. WINITA, S. M. BIRNBAUM, J. CORNFIELD, M. C. OTEY and J. P. GREENSTEIN, *Archs Biochem. Biophys.* **64**, 319 (1956).
2. S. M. HESS and W. DOEPFNER, *Archs int. Pharmacodyn. Thér.* **134**, 89 (1961).
3. H. GREEN, S. M. GREENBERG, R. W. ERICKSON, J. L. SAWYER and T. ELLISON, *J. Pharmac. exp. Ther.* **136**, 174 (1962).
4. L. J. WEBER and A. HORITA, *Biochem. Pharmac.* **14**, 1141 (1965).
5. J. V. HODGE, J. A. OATES and A. SJOERDSMA, *Clin. Pharmac. Ther.* **5**, 149 (1964).
6. D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmac. exp. Ther.* **117**, 82 (1956).

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Specific inhibition by ethidium bromide of the incorporation of ^{3}H thymidine into the kinetoplastic DNA of *Trypanosoma cruzi*

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WE NOW know that the kinetoplast of Trypanosomatids is a part of the mitochondrial apparatus in which a great deal of DNA is accumulated. In *Trypanosoma cruzi* the kinetoplastic DNA represents more than 20 per cent of the total DNA. The kinetoplastic DNA has a higher AT content than the nuclear DNA; this property is used for its fractionation. Ethidium bromide (EB) is a trypanocidal drug which binds specifically to the kinetoplastic DNA when used at low concentration (0·5–5 μg/ml of culture medium). Electron microscope studies of the ultrastructure of trypanosomes growing in a medium supplemented with 0·5 μg EB/ml reveal alterations of the kinetoplastic DNA with a progressive loss of this DNA after a few divisions of the trypanosomes.[1] A high percentage of dyskinetoplastic trypanosomes were obtained when cells were treated with ethidium bromide. Nuclear DNA was not affected by EB at this concentration. Dyskinetoplastic trypanosomes are not viable and they can only survive for 4 weeks after weekly transplantation in a new medium without EB.[2] In this paper we report studies on the incorporation of ^{3}H thymidine into the kinetoplastic and nuclear DNA. We noted a specific inhibition of the incorporation of the radioactive compound by EB, in the kinetoplastic DNA of trypanosomes.

The methods used were autoradiography of trypanosomes and liquid scintillation counting of the DNA. Trypanosomes (*Trypanosoma cruzi* strain Institut Pasteur) were cultured as described in previous works.[3, 4] After 4 days culture, during the exponential phase of trypanosomes' growth, EB in sterile water solution was added to the culture medium to obtain a final concentration of 0·5 μg/ml. After 2 hr culture, Me^{3}H thymidine (specific activity 9·7 c/mM) was added to the medium to a concentration of 0·5 μc/ml. After 2 hr the pulsing medium was removed, the trypanosomes washed twice