Br. J. Pharmac. (1984), **83**, 357–362

The effect of fasting on 5-hydroxytryptamine metabolism in brain regions of the albino rat

Luis D. Fuenmayor[1] & Susana García

Cátedra de Farmacología, José M. Vargas School of Medicine, Central University of Venezuela, Caracas, Venezuela

1 The turnover of 5-hydroxytryptamine (5-HT) in the whole brain and different brain regions was studied in rats fasted for 24 h. These rats showed an increased tissue concentration of the amine in the whole brain and of its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the whole brain, the striatum, the combined pons-medulla and the cerebral cortex.

2 The accumulation of 5-HIAA after probenecid was increased by fasting in the regions mentioned above except for the striatum. The effect of probenecid was also increased by fasting in the midbrain, the hypothalamus and the hippocampus.

3 In the striatum, the administration of probenecid produced a smaller increase in 5-HIAA concentration in fasted than in fed rats.

4 The decay of 5-HT following *p*-chlorophenylalanine (PCPA) was increased in the hypothalamus of fasted rats at 16 h, but not at 4 h, after the intraperitoneal administration of the inhibitor. In the midbrain, the striatum and the combined pons-medulla, food deprivation did not modify the decrease induced by PCPA. However, the inhibitor induced a reduction of food consumption in the fed group, which made this group rather similar to the fasted one and complicated the interpretation of the results in these last three cerebral areas.

5 Our results confirm that food deprivation increases the turnover of brain 5-HT and point out that the increase probably occurs in all brain areas. This increased tunover appears to be accompanied, in the hypothalamus, by an increased neuronal release of the amine. In the striatum, fasting probably blocks the active transport system which removes acid metabolites from the brain.

Introduction

Since 1972 it has been known that after a short fast, rats show an increased synthesis and metabolism of cerebral 5-hydroxytryptamine (5-HT) (Knott & Curzon, 1972; Pérez-Cruet *et al.*, 1972). This has been attributed to an elevation in the cerebral level of tryptophan in response to an increase in the free fraction of the amino acid in plasma (Knott & Curzon, 1972; Curzon *et al.*, 1973). The increase in the free fraction of serum tryptophan seems to be due to the high level of free fatty acids in the serum of fasted animals. The acids compete and displace tryptophan from its binding sites on serum albumin (Curzon *et al.*, 1973; Tagliamonte *et al.*, 1973). A similar effect of food deprivation on the metabolism of brain 5-HT has been observed in mice (Fuenmayor, 1978; 1979).

There are few studies about the effect of fasting on 5-HT metabolism in different brain regions. Knott & Curzon (1974) showed that food deprivation increased the concentration of brain 5-hydroxyindoleacetic acid (5-HIAA) in the pons-medulla pool, cerebral cortex and midbrain-hippocampus pool, but not in the striatum. These authors suggested that differences in the regional effects of fasting were due to the high concentration of tryptophan normally present in some brain areas, which would maintain the enzyme tryptophan hydroxylase more nearly saturated so that effects of a further increase of tryptophan on 5-HT synthesis would not be readily detectable.

It is not known whether the increase in 5-HT metabolism induced by fasting is the only consequence of an increased synthesis of the amine, or whether there is also an increased activity of 5-HT neurones. Therefore, we decided to investigate this by measuring the decay of brain 5-HT in different

[1]Correspondence to L.D.F., Apartado de Correos No 47413, Caracas 1041-A, Venezuela.

regions after blocking its synthesis with *p*-chlorophenylalanine (PCPA) (Koe & Weissman, 1966). If fasting increases the release of 5-HT, the decrease of this amine after blocking its synthesis will be greater in fasted than in fed rats. We also measured the turnover of 5-HT in different cerebral regions by estimating the accumulation of 5-HIAA, the major metabolite of 5-HT, after the administration of probenecid, a drug known to block the active transport which removes acid metabolites from the brain (Neff *et al.*, 1964; Sharman, 1966; Werdinius, 1966).

Methods

Male albino rats (Sprague-Dawley derived strain), from the breeding colony of our Department, weighing 200–300 g, were housed in groups of 8–10 with free access to food and tap water. Food was removed from one group (fasted rats) 24 h before killing, whereas the other group (fed rats) had food *ad libitum*. Fasted and fed rats were killed alternately by decapitation between 09 h 00 min and 13 h 00 min.

Some of the fed and fasted rats were used to investigate the effect of fasting on body temperature. A small glass thermometer was introduced into the animal's rectum for 1 min immediately before withdrawal of food from one of the groups. A new reading was taken 24 h later.

Analytical methods

The brains were rapidly removed and placed on an ice-cooled Petri dish. Fluorimetric estimations of 5-HT and 5-HIAA were carried out on the whole brain (without pineal gland and cerebellum), or midbrain, pons-medulla, hypothalamus, striatum, hippocampus and cerebral cortex from one rat by the method described by Haubrich & Denzer (1973). The midbrain was separated from the forebrain by a section extending from the rostral border of the superior colliculi to the posterior edge of the hypothalamus. The combined pons-medulla was separated from the midbrain by a coronal section from the posterior edge of the inferior colliculi to the rostral border of the pons. The striatum and the hippocampus were dissected out after dividing the forebrain along the midline and opening the lateral ventricle. Both striata were detached from deeper tissues by a pair of sharp pointed forceps; one of the hippocampi was dissected in the same way. Cerebral cortex from one hemisphere was obtained after dissecting the striatum, the hippocampus and discarding the diencephalon. The whole procedure for any region was performed within 4 min of killing.

The biochemical data were subjected to Student's *t* test. A probability level of $P<0.05$ was considered as indicative that the samples were not from the same population.

Drug treatment

Probenecid (Merck Sharp & Dohme, Ltd.), 180 mg kg^{-1}, was dissolved in distilled water by the drop-wise addition of 2 N NaOH until the pH was alkaline. The pH of the solution (36 mg ml^{-1}) was then adjusted to 8.5 with HCl immediately before the intraperitoneal injection, which was made 2 h and 15 min before killing. D,L-*p*-Chlorophenylalanine methylester hydrochloride (Sigma Chem. Co.), 310 mg kg^{-1}, was dissolved in 0.85% saline (155 mg ml^{-1}), and given by the intraperitoneal route 4 or 16 h before killing. Some of the vehicle- and PCPA-treated animals were used to investigate food ingestion in individual animals. Food pellets were weighed at the beginning of the experiment, between 09 h 00 min and 13 h 00 min, rats were treated 8 h later, and food was again weighed next day at the same time: 24 h after the first measurement, 16 h after treatment. Doses and concentrations are expressed in terms of the base. Each experiment included animals treated in different ways as well as vehicle-treated animals.

Results

Effect of fasting on the brain concentration of 5-HT and 5-HIAA

After 24 h fasting, rats showed a significant increase over fed rats in the concentration of 5-HIAA in the whole brain (+22%), the combined pons-medulla (+19%), the striatum (+18%) and the cerebral cortex (+20%), whereas the content of 5-HIAA was not altered in the midbrain (+4%), the hypothalamus (+7%) and the hippocampus (+7%) (Table 1). The same Table shows that the concentration of 5-HT was significantly increased only in the whole brain (+18%); the increases observed in the regions were not statistically significant.

Effect of fasting on body weight and body temperature

Fasted rats lost 9.3% of their body weight, whereas fed rats increased it by 2.2%, both changes being significant ($P<0.001$, Student's *t* test for paired samples). Body temperature (°C) of fed and fasted animals did not change during the experiment (fed rats: 0 h = 38.2 ± 0.12; 24 h = 38.4 ± 0.13; fasted rats: 0 h = 38.0 ± 0.26; 24 h = 37.8 ± 0.15; mean ± s.e.mean; $n = 8$).

Table 1 Effect of a 24 h fast on the concentration ($ng\,g^{-1}$) of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in different brain regions of the rat

	5-Hydroxytryptamine		*5-Hydroxyindoleacetic acid*	
	Fed rats	*Fasted rats*	*Fed rats*	*Fasted rats*
Whole brain	459.6 ± 27.8 (10)	538.4 ± 23.0* (10)	364.0 ± 14.1 (10)	444.9 ± 22.2** (10)
Midbrain	568.2 ± 53.4 (9)	573.6 ± 34.3 (8)	654.9 ± 34.0 (8)	681.9 ± 31.8 (8)
Pons and medulla	478.3 ± 49.8 (8)	492.2 ± 47.7 (11)	412.6 ± 18.0 (9)	489.6 ± 19.2** (13)
Hypothalamus	704.9 ± 25.3 (16)	730.0 ± 45.0 (16)	659.3 ± 29.7 (17)	707.4 ± 35.1 (14)
Striatum	407.9 ± 16.1 (11)	436.2 ± 20.3 (13)	530.6 ± 23.5 (17)	624.7 ± 24.8** (18)
Hippocampus	381.6 ± 66.2 (9)	446.1 ± 82.6 (9)	458.2 ± 15.0 (9)	491.6 ± 27.1 (9)
Cerebral cortex	363.8 ± 14.0 (9)	396.3 ± 18.1 (8)	300.4 ± 16.4 (9)	361.8 ± 29.7* (8)

Values are expressed as mean ± s.e.mean. Number of estimations indicated in parentheses. $*P<0.05$ and $**P<0.01$ *vs.* fed rats: Student's *t* test for non-paired samples.

Effect of fasting on the increase of 5-HIAA induced by probenecid

The administration of probenecid induced a significant rise in the concentration of 5-HIAA in all regions studied and in both groups (Table 2). However, the increases observed were significantly higher in fasted than in fed rats with the exception of the striatum, in which the accumulation of 5-HIAA induced by probenecid was not modified by fasting (Table 2). The treatment with this drug increased the 5-HT content in the cerebral cortex of both experimental groups (fed rats: $\Delta = +225.0 \pm 59.7\ ng\,g^{-1}$; fasted rats: $\Delta = +171.2 \pm 31.9\ ng\,g^{-1}$; mean ± s.e.mean; $P<0.02$; $n=11$) and in the combined pons-medulla of fed rats ($\Delta = +193.0 \pm 29.4\ ng\,g^{-1}$; mean ± s.e.mean; $P<0.02$; $n=5$).

Effect of fasting on the reduction of 5-HT induced by PCPA

The effect of fasting on the decay of cerebral 5-HT after inhibition of its synthesis with PCPA was studied in only four regions as shown in Tables 3 and

Table 2 Effect of a 24 h fast on the accumulation of 5-hydroxyindoleacetic acid ($ng\,g^{-1}$) in different brain regions of the rat after the administration of probenecid

	Fed rats		*Fasted rats*	
	*Probenecid**	*Increase by probenecid*	*Probenecid**	*Increase by probenecid*
Midbrain	1078.4 ± 70.5 (13)	423.4 ± 70.5 +65%	1327.0 ± 72.6 (13)	645.1 ± 72.6** +95%
Pons and medulla	1275.8 ± 41.7 (7)	863.3 ± 41.7 +209%	1535.1 ± 54.3 (6)	1045.5 ± 54.3** +214%
Hypothalamus	979.1 ± 57.3 (13)	319.8 ± 57.3 +49%	1167.6 ± 52.0 (13)	460.3 ± 52.0** +65%
Striatum	952.3 ± 89.3 (10)	421.7 ± 89.3 +80%	1020.7 ± 72.0 (9)	396.0 ± 72.0 +63%
Hippocampus	679.6 ± 47.4 (13)	221.4 ± 47.4 +109%	913.5 ± 56.4 (13)	422.0 ± 56.4† +86%
Cerebral cortex	628.7 ± 43.1 (11)	328.4 ± 43.1 +109%	808.4 ± 37.8 (11)	446.7 ± 37.8** +123%

Values are expressed as mean ± s.e.mean. Number of estimations indicated in parentheses. Animals were killed 2 h and 15 min after probenecid ($180\ mg\,kg^{-1}$). $*P<0.001$ vs. controls (see control values in Table 1). Increase by probenecid is significantly higher ($\dagger P<0.01$ and $**P<0.005$) in fasted than in fed rats: Student's *t* test for non-paired samples.

Table 3 Effect of a 24 h fast on the loss of 5-hydroxytryptamine in different brain regions of the rat 4 h after the injection of *p*-chlorophenylalanine (PCPA) 310 mg kg^{-1}

	Fed rats		*Fasted rats*	
	PCPA	*Reduction by PCPA*	*PCPA*	*Reduction by PCPA*
Midbrain	476.6 ± 32.8	93.5 ± 32.8	499.3 ± 11.8*	74.3 ± 11.8
	(10)	−17%	(10)	−13%
Pons and Medulla	285.9 ± 11.5†	192.4 ± 11.5	254.2 ± 19.4†	238.0 ± 19.4
	(4)	−40%	(4)	−48%
Hypothalamus	675.8 ± 44.1	29.1 ± 44.1	661.1 ± 45.4	68.9 ± 45.4
	(9)	−4%	(9)	−9%
Striatum	278.2 ± 18.4**	129.7 ± 18.4	280.6 ± 23.0**	155.6 ± 23.0
	(10)	−32%	(10)	−36%

Values are expressed as mean ± s.e.mean. Number of estimations indicated in parentheses. Concentrations are expressed in ng g^{-1}. *$P<0.05$; †$P<0.01$ and **$P<0.001$ vs. controls (see control values in Table 1). Student's *t* test for unpaired samples.

4. Four h after the injection of PCPA there were marked reductions in the concentration of 5-HT in the combined pons-medulla and the striatum of both fed and fasted rats while in the midbrain and the hypothalamus the reductions observed were slight and non-significant. The magnitude of 5-HT reduction 4 h after PCPA was not altered by fasting in any of the regions studied (Table 3). When PCPA was administered 16 h before killing there was a large decrease in the concentration of 5-HT in the four regions studied and in both the fed and the fasted groups (Table 4). The reduction of the amine was significantly larger in the combined pons-medulla of both groups than in the other brain regions (Table 4). This table also shows that in the hypothalamus the reduction of 5-HT by PCPA was larger in fasted animals than in fed animals, whereas there was no such difference in the other brain regions studied. The treatment with PCPA elicited large significant reductions in the level of 5-HIAA in all regions and at both times of treatment (data not shown).

Fed rats treated with PCPA 16 h before killing showed a significant reduction in their body weight of 12.7 ± 1.8 g (4.7%) when compared with their weight 24 h earlier. The fasted group treated in the same way lost 21.3 ± 2.1 g (7.9%), which was a reduction similar to the one observed in the untreated fasted group (−24.0 ± 2.6 g; −9.2%). The amount of food consumed during 24 h by untreated rats (fed group) was 24.4 g, whereas rats treated with PCPA consumed 13.0 g. Thus, the reduction in body weight after PCPA was the consequence of a reduction in food consumption by these rats.

Table 4 Effect of a 24 h fast on the loss of 5-hydroxytryptamine in different brain regions of the rat after the injection of *P*-chlorophenylalanine (PCPA) 310 mg kg^{-1}

	Fed rats		*Fasted rats*	
	*PCPA**	*Reduction by PCPA*	*PCPA**	*Reduction by PCPA*
Midbrain	345.8 ± 21.5	222.3 ± 21.5	311.9 ± 23.1	261.7 ± 23.1
	(6)	−39%	(8)	−46%
Pons and medulla	159.8 ± 14.5	318.5 ± 14.5**	162.9 ± 18.7	392.3 ± 18.7**
	(7)	−67%	(7)	−67%
Hypothalamus	513.9 ± 23.5	191.0 ± 23.5	453.4 ± 34.6	276.6 ± 34.6***
	(10)	−27%	(10)	−38%
Striatum	215.6 ± 19.8	192.3 ± 19.8	223.0 ± 8.4	213.2 ± 8.4
	(10)	−47%	(10)	−49%

Values are expressed as mean ± s.e.mean. Number of estimations indicated in parentheses. Concentrations are expressed in ng g^{-1}. *$P<0.01$ vs. controls (see control values in Table 1). **$P<0.01$ vs. the reduction that occurred in the other regions. Decrease by PCPA is significantly larger (***$P<0.01$) in fasted than in fed rats: Student's *t* test for unpaired samples.

Discussion

There is now considerable experimental evidence indicating that whole brain 5-HT synthesis and metabolism is increased by fasting in the rat (Knott & Curzon, 1972; Pérez-Cruet *et al.*, 1972; Curzon *et al.*, 1973; Tagliamonte *et al.*, 1973; Gessa & Tagliamonte, 1974) and the mouse (Fuenmayor, 1978; 1979). In the present study fasting increased the brain concentration of 5-HT and 5-HIAA suggesting that the amine is being synthesized and metabolized more rapidly than under normal conditions. This extra 5-HT is either stored, elevating its brain concentration, or may be metabolized when the storage capacity of the neurones is exceeded. The results of the regional study suggest that food deprivation increases 5-HT turnover in the combined pons-medulla, the striatum and the cerebral cortex, but not in the midbrain, the hypothalamus and the hippocampus. This interpretation partially agrees with the findings of Knott & Curzon in 1974. They found that food deprivation induced an elevation in the concentration of 5-HIAA in the combined pons-medulla, but not in the hypothalamus and the striatum. They thought that the apparent lack of effect of fasting on hypothalamic 5-HT turnover was a consequence of the high concentration of tryptophan normally present in this area, which produced saturation of the enzyme tryptophan hydroxylase and so prevented an elevation in the synthesis of 5-HT (Knott & Curzon, 1974). Nevertheless, this lack of effect of fasting on the concentration of 5-HIAA and the one observed here in the midbrain, the hypothalamus and the hippocampus (see Table 1), could be the result of the existence in these brain regions of a very efficient active transport removing 5-HIAA from the brain. In order to investigate this possibility, we studied the effect of fasting on the accumulation of 5-HIAA in various cerebral areas after the administration of probenecid, a drug that blocks the active transport which removes 5-HIAA from the brain (Neff *et al.*, 1964; Sharman, 1966) and, therefore, increases the concentration of the metabolite according to the rate of its formation. The regional differences in the effect of fasting seen in Table 1 were not present when probenecid was given. With the exception of the striatum, the accumulation of 5-HIAA by the drug was potentiated by food deprivation in all regions studied (Table 2). This result does not support the conclusion of Knott & Curzon (1974) pointed out above. Our results probably mean that the active transport, which removes 5-HIAA from the brain to the blood, is more effective in the midbrain, the hypothalamus and the hippocampus than in the pons-medulla pool and the cerebral cortex. Therefore, even when fasting increases the turnover of 5-HT in these regions, the removal mechanism prevents an elevation of 5-HIAA in some of them, which was unmasked by probenecid. In the striatum, the elevation in the concentration of 5-HIAA observed in fasted animals is suggestive of the existence of an increased synthesis and metabolism of 5-HT. Another possibility is that the increase in 5-HIAA concentration after fasting in this brain area reflects its decreased removal from brain. When this possibility was tested by giving probenecid, fasted rats accumulated less 5-HIAA than fed rats. A similar phenomenon has been reported for homovanillic acid, a major metabolite of dopamine, in mice (Fuenmayor, 1978; 1979). Mice fasted for 20 h showed a higher concentration of homovanillic acid in the striatum than fed mice, but they accumulated less homovanillic acid after probenecid. Thus, the possibility that fasting is interfering with the transport mechanism which removes acid metabolites from the striatum should be taken into consideration.

Food deprivation did not significantly increase the concentration of 5-HT in any of the regions studied (Table 1). Nevertheless, there were small increases that did not achieve statistical significance. This may suggest that the regions studied did not have an appreciable storage capacity for the newly synthesized molecules of the amine. The small increments observed are probably responsible for the increase seen in the whole brain. Another possible explanation for the absence of regional increases in the concentration of 5-HT is that fasting increases not only the synthesis of brain 5-HT, but also the release of the amine from tryptaminergic nerve endings; thus, fasting would be increasing the activity of 5-HT neurones. This hypothesis was investigated by measuring the utilization of 5-HT in fed and fasted animals. Food deprivation did not modify the decay of cerebral 5-HT seen 4 h after the injection of PCPA. However, the diminution of 5-HT induced 16 h after PCPA in the hypothalamus was potentiated by fasting (Table 4). This result suggests that hypothalamic tryptaminergic nerve endings are activated by fasting and therefore they release more 5-HT in this condition than tryptaminergic neurones in the other three regions. However, treatment with PCPA reduced food consumption by the animals, in agreement with other reports in which PCPA has been administered by the intraperitoneal route (Borbely *et al.*, 1973; Marsden & Curzon, 1976). Fed animals treated with the drug consumed about half of the amount of food consumed by fed animals not given PCPA. This finding creates a difficulty in the interpretation of the results with PCPA because the appetite depressant effect of the drug made both groups, fed and fasted, rather similar. Therefore, while we suggest that fasting is increasing the activity of hypothalamic tryptaminergic endings, we cannot

conclude that fasting does not have a similar effect in the other three regions. In support of our results are the findings of Kantak *et al.*, (1977, 1978). They reported the existence of an increased 5-HT turnover rate in the lateral hypothalamus of food deprived rats; tryptophan uptake and 5-HT synthesis were enhanced as a function of hours of food deprivation. The values shown in Tables 3 and 4 also demonstrate that the reduction of 5-HT by PCPA was larger in the combined pons-medulla than in the other regions indicating a higher turnover of 5-HT in this region than in the others. The treatment with PCPA also induced a large reduction in the concentration of 5-HIAA, as a consequence of the blockade of the synthesis of 5-HT (Koe & Weissman, 1966; Marsden & Curzon, 1976). The reduction of 5-HIAA occurred even before any reduction in the concentration of 5-HT. This finding suggests that 5-HIAA is normally formed from the newly synthesized 5-HT and that this recently formed amine is preferentially released by tryptaminergic nerve endings.

The data presented here confirm that fasting increases the turnover of brain 5-HT and suggest that this condition may also increase the activity of some tryptaminergic pathways. Many other behavioural disturbances can induce changes in the activity of cerebral 5-HT and dopamine neuronal systems (Bliss, 1973; Fukui & Vogt, 1976). It follows that, under normal conditions, the activity of these and other neuronal populations in the central nervous system might be at least partially controlled by changes in environmental conditions, such as food intake, acting on the animal.

This work was supported by a grant to L.D.F. (Project No M.02-8/80) of the Consejo de Desarrollo Científico y Humanístico, Universidad Central de Venezuela. The authors would like to thank Mr José Acosta for his technical assistance.

References

BLISS, E.L. (1973). Effects of behavioural manipulations upon brain serotonin and dopamine. In *Serotonin and Behaviour.* ed. Barchas, J. & Usdin, E. pp. 315–324. London: Academic Press.

BORBELY, A.A., HUSTON, J.P. & WASER, P.G. (1973). Physiological and behavioral effects of parachlorophenylalanine in the rat. *Psychopharmacology*, **31,** 131–142.

CURZON, G., FRIEDEL, J. & KNOTT, P.J. (1973). The effect of fatty acids on the binding of tryptophan to plasma protein. *Nature*, **242,** 198–200.

FUENMAYOR, L. (1978). The effect of fasting on the formation of 4-hydroxy-3-methoxy-phenylacetic acid (homovanillic acid) and 5-hydroxyindolylacetic acid in the brain of the mouse. *Br. J. Pharmac.*, **62,** 407P.

FUENMAYOR, L.D. (1979). The effect of fasting on the metabolism of 5-hydroxytryptamine and dopamine in the brain of the mouse. *J. Neurochem.*, **33,** 481–485.

FUKUI, K, & VOGT, M. (1976). Increase by visual stimuli in turnover of 5-hydroxytryptamine in the superior colliculi of the rabbit. *J. Physiol.*, **254,** 519–529.

GESSA, G.L. & TAGLIAMONTE, A. (1974). Possible role of free serum tryptophan in the control of brain tryptophan level and serotonin synthesis. *Adv. Biochem. Psychopharmac.*, **11,** 119–131.

HAUBRICH, A.R. & DENZER, J.S. (1973). Simultaneous extraction and fluorometric measurement of brain serotonin, catecholamines, 5-hydroxyindolacetic acid and homovanillic acid. *Analyt. Biochem.*, **55,** 306–312.

KANTAK, K.M., WAYNER, M.J. & STEIN, J.M. (1978). Effects of various periods of food deprivation on serotonin synthesis in the lateral hypothalamus. *Pharmac. Biochem. Behav.*, **9,** 535–541.

KANTAK, K.M., WAYNER, M.J., TILSOM, H.A. & SVED, A. (1977). Turnover of ^{3}H-5-hydroxytryptamine to ^{3}H-5-hydroxyindoleacetic acid and the ^{3}H-5-methoxyindoles in nondeprived and 24 hr food deprived rats. *Pharmac. Biochem. Behav.*, **6,** 221–225.

KNOTT, P.J. & CURZON, G. (1972). Free tryptophan in plasma and brain tryptophan metabolism. *Nature*, **239,** 452–453.

KNOTT, P.J. & CURZON, G. (1974). Effect of increased rat brain tryptophan on 5-hydroxytryptamine and 5-hydroxyindolylacetic acid in the hypothalamus and other brain regions. *J. Neurochem.*, **22,** 1065–1071

KOE, B.K. & WEISSMAN, A. (1966). p-Chlorophenylalanine: a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.*, **154,** 499–516.

MARSDEN, C.A. & CURZON, G. (1976). Studies on on the behavioural effects of tryptophan and p-chlorophenylalanine. *Neuropharmacology*, **15,** 165–171.

NEFF, N.H., TOZER, T.N. & BRODIE, B.B. (1964). Indole metabolism. Part II. A specialised transport system to transfer 5-HIAA directly from brain to blood. *Pharmacologist*, **6,** 194.

PÉREZ-CRUET, J., TAGLIAMONTE, A., TAGLIAMONTE, P. & GESSA, G.L. (1972). Changes in brain serotonin metabolism associated with fasting and satiation in rats. *Life Sci.*, **11,** Part II, 31–39.

SHARMAN, D.F. (1966). Changes in the metabolism of 3, 4-dihydroxyphenylethylamine (dopamine) in the striatum of the mouse induced by drugs. *Br. J. Pharmac. Chemother.*, **28,** 153–163.

TAGLIAMONTE, A., BIGGIO, G., VARGIU, L. & GESSA, G.L. (1973). Free tryptophan in serum controls brain tryptophan level and serotonin synthesis. *Life Sci.* 12, Part II, 277–287.

WERDINIUS, B. (1966). Effect of probenecid on the level of homovanillic acid in the corpus striatum. *J. Pharm. Pharmac.*, **18,** 546–547.

(Received November 11, 1983. Revised April 30, 1984.)