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RAPID EFFECTS OF ENVIRONMENTAL DISTURBANCE ON RAT PLASMA UNESTERIFIED FATTY ACID AND TRYPTOPHAN CONCENTRATIONS AND THEIR PREVENTION BY ANTILIPOLYTIC DRUGS

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1 Changes of plasma unesterified fatty acid (UFA) and tryptophan concentration in group-housed rats following removal of their cage-mates and the effects of antilipolytic drugs on these changes were investigated.

2 Removal of group-housed 24 h fasted rats but not fed rats from cages resulted in increased plasma UFA concentration in the remaining rats which was associated with significant increases of the proportion of free tryptophan but significant falls of total tryptophan concentration. These rapid changes were not associated with brain tryptophan changes. Plasma tyrosine concentration was unaffected.

3 The fall of plasma tryptophan did not appear to be due to passage into red cells as erythrocyte tryptophan concentration remained unchanged.

4 Plasma UFA concentrations correlated positively and significantly with corticosterone concentrations which were also increased following removal of cage-mates.

5 Plasma UFA increases and tryptophan changes in the fasting rats were both prevented by nicotinic acid or propranolol. Corticosterone concentration was increased by nicotinic acid but unaffected by propranolol.

6 The possible importance of these rapid changes of plasma tryptophan and of their prevention by antilipolytic drugs is discussed.

Introduction

Tryptophan is normally bound largely to albumin in the plasma of man (McMenamy, Lund & Oncley, 1957) and other warm blooded vertebrates (Fuller & Roush, 1973). Only the unbound form is directly available for transport to tissues. When plasma unesterified fatty acid (UFA) concentration increases, the free fraction of tryptophan also increases as UFA binds to albumin which results in weakened binding of tryptophan (McMenamy & Oncley, 1958; Curzon, Friedel & Knott, 1973).

Plasma UFA derives from lipolysis of body fat, a mechanism initiated by sympathetic activity and mediated by cyclic adenosine-3′,5′-monophosphate (AMP). This process has been described as a 'unique confluence of biochemistry with psychology' (Brodie & Maickel, 1963). Its importance is perhaps emphasized by the demonstration that it can alter the availability of tryptophan to the brain and thus lead to altered synthesis therein of the neurotransmitter 5-hydroxytryptamine (5-HT). Various drugs can alter brain 5-HT metabolism apparently because they alter the binding of plasma tryptophan to albumin by interacting with the sympathetic – cyclic AMP system. Also, in stress situations increased plasma UFA may be associated with increase of brain tryptophan and 5-HT turnover, e.g. in the rat when deprived of food or immobilized (Knott & Curzon, 1972; Curzon & Knott, 1974). These are prolonged or extreme changes in the physical state of the animal. We now find that the mere removal of rats from a cage can cause rapid and considerable increases of UFA and alteration of the binding and amount of tryptophan in the plasma of their remaining

cage-mates. This paper describes a study of these changes and of their prevention by antilipolytic drugs.

Methods

Male Sprague-Dawley rats, 160-180 g (Carworth Europe, Alconbury, Huntington), caged in groups of either three of four, were kept in an acoustically lagged housing at 22° C ± 2 and fed a diet of Oxoid 41B *ad libitum*. At least four days after their arrival in the laboratory when body weight had risen to 180-200 g, food but not water was removed from some of the cages at 16 h 00 min and both fed and food-deprived groups were killed by guillotine the next day, between 16 h 00 min-17 h 00 min, in sequence at 2 min intervals, cage by cage. Animals were usually killed in the room containing their housing but out of sight of the remaining rats. Rats which had been singly housed on their arrival in the laboratory were also deprived of food for 24 h and killed at 2 min intervals. In additional groups of fasted rats, nicotinic acid (50 mg/kg as a suspension in 0.9% w/v NaCl solution (saline)) or propranolol (1 mg/kg) were injected intraperitoneally 1 h before the animals were killed. Control rats were injected with saline (2.5 ml/kg).

Brains were removed and plasma samples prepared as previously described (Curzon, Joseph & Knott, 1972).

Analytical methods

Tryptophan was determined by the method of Denkla & Dewey (1967) in whole plasma and also in plasma ultrafiltrates prepared by centrifuging 1.0 ml plasma in a CF50 Diaflo membrane cone (Amicon) at 800 *g* for 30 min at room temperature. Tryptophan was determined in packed red cells by a modification of the approach of Denkla & Dewey. In preliminary experiments it was found that initial protein precipitation and extraction as used in the plasma tryptophan method led to very low recoveries from red cells. A modified method was therefore developed (Kantamaneni, unpublished work). Approximately 1 g of packed red cells was weighed, homogenized with 1 ml deionized water using an X-1020 homogenizer (International Laboratory Apparatus Gmb, Göttingen, Germany) and 20 ml acid butanol added. After agitation of the suspension with a vortex mixer and centrifugation, 5 ml of the supernatant was back-extracted into 2.0 ml 0.1 N HCl after addition of 10 ml heptane. To 0.2 ml of the acid phase, 2.0 ml 10% w/v trichloroacetic acid was added. After centrifugation for 5 min, 0.1 ml 1.8% v/v formaldehyde and 0.1 ml 10% w/v trichloroacetic acid containing 3 mM Fe Cl_3 was added to 1.0 ml supernatant. The mixture was heated in a boiling water bath and the tryptophan fluorophor measured as in the method of Denkla & Dewey (1967). Heating was begun immediately after adding the Fe Cl_3.

Plasma UFA was determined by the method of Laurell & Tibbling (1967). Brain tryptophan and tyrosine and plasma tyrosine, were measured as previously described (Curzon *et al.*, 1972). Plasma corticosterone was determined by the method of Mattingly (1962) scaled down for use with 0.25 ml plasma samples.

Results

Effect of removal of cage-mates on rat plasma unesterified fatty acid and on tryptophan disposition

Fed grouped rats. Plasma UFA, total tryptophan and free tryptophan concentrations did not show significant relationships with order of removal from cages (Figure 1). However the combination of non-significant upward and downward trends in plasma free and total tryptophan respectively led to a small but significant increase of % free tryptophan with order of removal. Brain tryptophan was unaffected by order of removal.

Fasted grouped rats Plasma UFA progressively and significantly increased with order of removal and total tryptophan progressively and significantly fell so that its mean concentration in the last rats in each cage was about two-thirds of that found for the intially removed rats killed 6 min earlier. Free tryptophan showed an upward trend though this was not significant while % free tryptophan rose significantly. Brain tryptophan was unaffected by order of removal. Mean values for plasma UFA, free tryptophan, % free tryptophan and brain tryptophan of 1st, 2nd, 3rd and 4th killed fasted rats were greater than those for corresponding groups of fed animals though not all of these differences were significant (see legend to Figure 1). Although plasma UFA concentration of 1st killed fasted rats was not significantly different from that of 1st killed fed rats, standard deviations were large and numbers of determinations small. However, when 1st killed fasted rats were compared with 1st, 2nd, 3rd and 4th killed fed rats combined, then significant differences were found (fasted, 0.662 ± 0.090 mEq/1, $n = 8$; fed, 0.495 ± 0.140 mEq/1, $n = 14$:

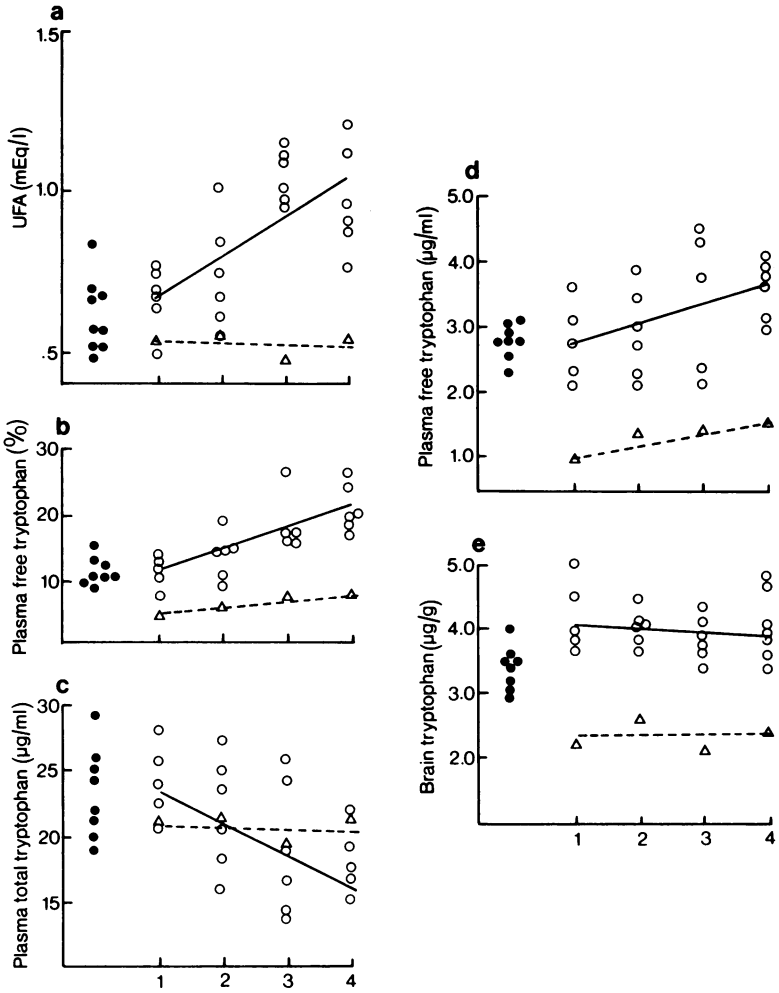

Figure 1 Effect of order of removal of rats from cage on plasma unesterified fatty acid (UFA) concentrations and plasma and brain tryptophan concentrations. *X* axis shows order of killing. Animals were killed at 2 min intervals. (○) Fasted rats housed 4/cage, individual values are shown; regression lines for these points are unbroken. (△) Fed rats housed 4/cage, each point is the mean of three observations; the best fit line is broken. Standard deviations are not shown to improve clarity. (●) Fasted singly housed rats, each point shows an individual value.

Correlations with order of killing: (a) Plasma UFA: fasted rats, $r = 0.6744$, $n = 24$, $P < 0.001$; fed rats, $r = 0.0271$, $n = 12$, NS. (b) Plasma % free tryptophan: fasted rats, $r = 0.7309$, $n = 22$, $P < 0.001$; fed rats, $r = 0.6083$, $n = 12$, $P < 0.05$. (c) Plasma total tryptophan: fasted rats, $r = -0.5612$, $n = 23$, $P < 0.01$; fed rats, $r = -0.1411$, $n = 12$, NS. (d) Plasma free tryptophan: fasted rats, $r = 0.4195$, $n = 22$, NS; fed rats, $r = 0.5540$, $n = 12$, NS. (e) Brain tryptophan: fasted rats, $r = -0.1054$, $n = 24$, NS; fed rats, $r = -0.0251$, $n = 12$, NS. Values for fasted and fed rats compared by Student's *t* test are given below.

Order of removal	*Total tryptophan*	*Free tryptophan*	*% Free tryptophan*	*UFA*	*Brain tryptophan*
1st	NS	<0.01	<0.001	NS	<0.001
2nd	NS	<0.02	<0.02	NS	<0.001
3rd	NS	<0.05	<0.001	<0.001	<0.001
4th	NS	<0.001	<0.001	<0.001	<0.02

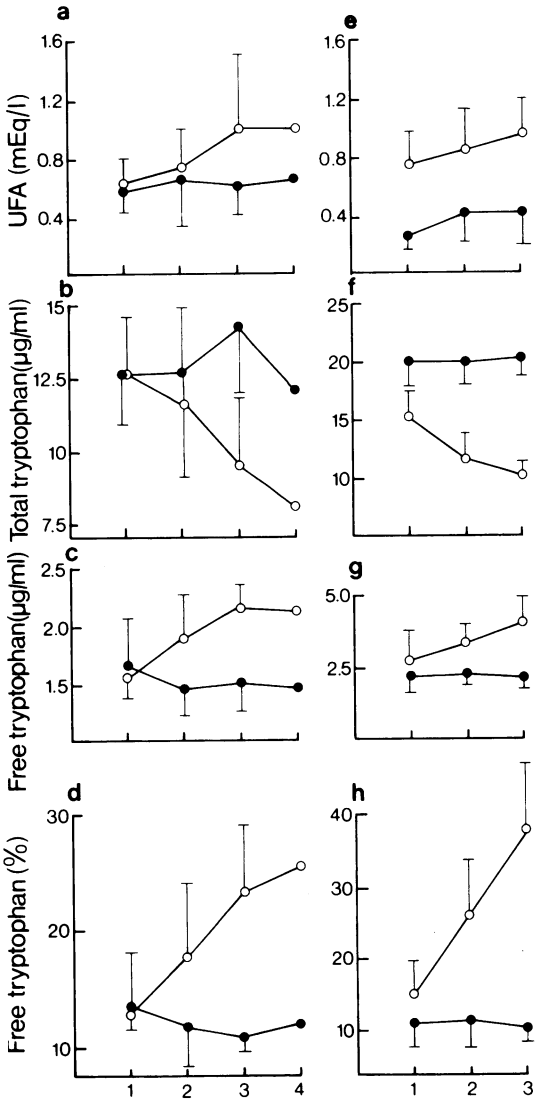

Figure 2 Effect of antilipolytic drugs on the changes of plasma unesterfied fatty acid (UFA) and trytophan concentrations following removal of cage-mates of fasted rats. *X* axis shows order of killing. Animals were killed at 2 min intervals. (a)-(d) Effects of propranolol, (○) saline; (●) propranolol. Rats were housed either 3 or 4/cage. Each point is the mean of 6 observations except the last point which represents the mean of 2 animals only. Vertical lines represent one s.d. Propranolol (1 mg/kg) was given intraperitoneally and rats were killed 1 h after injection. (e)-(h) Effects of nicotinic acid, (○) saline; (●) nicotinic acid. Rats were housed 3/cage. Each point is the mean of 6 observations. Nicotinic acid (50 mg/kg) was given intraperitoneally and rats were killed 1 h after injection. Vertical lines represent one s.d.

$P < 0.02$). As plasma UFA of fed rats showed no sign of a relationship with order of killing, this statistical treatment seems reasonable.

Fasted singly housed rats. Mean values for all of the above parameters were comparable with those found for the first killed members of fasted groups of rats. Singly housed fed rats were not studied in this experiment but in a separate experiment it was found that singly housed fasted rats had significantly ($P < 0.001$) higher brain tryptophan concentration (4.97 ± 0.33 μg/g wet wt., $n = 5$) than did singly housed fed rats (3.59 ± 0.27 μg/g wet wt., $n = 6$). (Results ± one s.d.)

Modifications of experimental procedure. The possibility that the plasma changes in fasted grouped rats were artefactual or related to trivial aspects of the experimental situation were investigated with animals housed in different enclosures or removed from cages by one investigator and killed by another investigator in a different room. In addition, chemical determinations were made in random order. Results of all experiments were consistent with those shown in Figure 1.

Erythrocyte tryptophan. It was thought possible that the fall of plasma tryptophan concentration of fasted rats on removal of cage-mates was simply due to movement of newly freed tryptophan into red cells either *in vivo* or during the separation of plasma. However red cell tryptophan did not rise (Table 1).

Prevention by antilipolytic drugs of changes of plasma unesterified fatty acid and tryptophan following removal of cage-mates of fasted rats

Propranolol. Injection of the β-adrenoceptor blocking agent, propranolol (1 mg/kg i.p.), 1 h before killing prevented the changes of both plasma UFA and tryptophan following removal of cage-mates of fasted rats (Figure 2). Plasma UFA and tryptophan concentrations of initially removed rats were unaffected by propranolol and brain tryptophan concentration was similar to that of fasted rats injected with saline (Table 2).

Nicotinic acid. Injection of nicotinic acid (50 mg/kg i.p.) 1 h before killing similarly prevented or opposed the effects of removal of cage-mates of fasted rats (Figure 2). In agreement with previous experiments (Curzon & Knott, 1974), the plasma UFA concentration of initially

removed nicotonic acid-treated rats was significantly lower and total tryptophan concentration significantly higher than values for saline-treated fasted controls. Plasma UFA concentrations of subsequently removed nicotinic acid-treated rats were not significantly higher than values for initially removed rats while plasma tryptophan concentrations remained similar to those of the initially removed animals and as before (Curzon & Knott, 1974) brain tryptophan was significantly lower than in saline-treated fasted control rats (Table 2).

Other effects of fasting, removal of cage-mates and antilipolytic drugs

Plasma tyrosine. To determine whether the plasma tryptophan changes of fasted rats on previous removal of cage-mates were specific to this amino acid, plasma tyrosine determinations were also made on animals caged in groups of three, killed at 2 min intervals (Table 3). Values were not significantly related to order of removal. In addition, injection of propranolol (1 mg/kg i.p.) 1 h before killing was without effect on plasma tyrosine concentration although nicotinic acid (50 mg/kg) caused moderate but significant increases. Percentage tyrosine increases were comparable with those of tryptophan (Figure 2).

Plasma corticosterone. Removal of cage-mates of fasted rats was associated with increased plasma corticosterone concentration in the remaining rats (Table 4). Plasma corticosterone concentrations were positively and significantly correlated with both order of removal and plasma UFA concentra-

Table 1 Effect of removal of cage-mates on plasma and red cell tryptophan of 24 h fasted rats

Order of removal	*Plasma total tryptophan (μg/ml)*	*Plasma free tryptophan (μg/ml)*	*Red blood cell tryptophan (μg/g)*
1st	13.04 ± 2.39 (8)	1.82 ± 0.27 (8)	6.22 ± 0.50 (8)
2nd	10.81 ± 2.18 (8)	2.48 ± 0.58 (8)	6.64 ± 2.41 (8)
3rd	10.21 ± 1.18 (8)*	2.75 ± 0.35 (8)	6.84 ± 0.97 (8)

Animals were caged in groups of 3. Results are expressed as means ± one s.d. Numbers of determinations shown in parentheses. Results compared by Student's *t* test, *, significantly less than values for first removed rats $P < 0.02$.

Table 2 Effects of propanolol and nicotinic acid on brain tryptophan of 24 h fasted rats

Injected	*Brain tryptophan (μg/g)*
Saline (12)	3.68 ± 0.37
Propranolol, 1 mg/kg (12)	3.59 ± 0.45
Saline (18)	4.23 ± 0.88
Nicotinic acid, 50 mg/kg (18)	3.33 ± 0.58*

Injections were made i.p. and rats killed 1 h later. Volumes injected were 2.5 ml/kg body wt. Animals were caged in groups of 3 and results on 1st, 2nd and 3rd removed rats combined. Results are expressed as means ± one s.d. Numbers of determinations shown in parentheses. Results compared by Student's *t* test. * $P = 0.001$.

Table 3 Effects of removal of cage-mates and of propranolol and nicotinic acid on plasma tyrosine of fasted rats

	Order of removal		
	1st	*2nd*	*3rd*
Injected	*Plasma tyrosine (μg/ml)*		
Saline	13.92 ± 1.60 (8)	14.94 ± 3.51 (4)	15.72 ± 3.73 (8)
Propranolol, 1 mg/kg	13.60 ± 1.48 (4)	12.80 ± 1.11 (4)	14.10 ± 1.90 (3)
Nicotinic acid, 50 mg/kg	17.74 ± 3.05 (4)*	20.83 ± 3.09 (4)*	20.02 ± 1.99 (4)

General conditions as in Table 2.
* $P < 0.05$.

tion. Treatments with propranolol and nicotonic acid 1 h before killing had markedly different effects on corticosterone concentrations. Thus, while propranolol-treated rats had concentrations not significantly different from those of saline-treated fasted control animals, nicotinic acid caused a large increase of plasma corticosterone.

Discussion

The previous finding that fasting for 24 h leads to increased brain trytophan (Curzon, Joseph & Knott, 1972; Knott & Curzon, 1972) has been confirmed in both singly and group-housed rats. Results also show that in fasted rats lipolytic mechanisms are readily enhanced by environmental disturbance so that the mere removal of rats from cages is followed by large and rapid increases of plasma UFA in their former cage-mates. Increased plasma UFA within minutes of exposure to stress situations occurs in human subjects (Court, Dunlop & Leonard, 1971; Taggart & Carruthers, 1971; Nimmo, Kirby & Lassers, 1973) while rats have been previously reported to show plasma UFA changes following minimal environmental disturbance (Barrett, 1964).

Two factors are probably responsible for the lack of increase of plasma UFA on removal of cage-mates of fed rats. Firstly, the starved animals were much more obviously responsive to disturbance and therefore sympathetic activity may have increased much more in these animals than in fed rats on removal of cage-mates. Secondly, similar increases of sympathetic activity have greater lipolytic effect in starved than in fed rats (Brodie, Krishna & Hynie, 1969).

In circumstances such as fasting (Knott & Curzon, 1972) and experimental hepatic coma (Curzon, Kantamaneni, Winch, Rojas-Bueno, Murray-Lyon & Williams, 1973) or following the administration of many drugs (Curzon & Knott, 1974; Knott & Curzon, 1975), the weakened binding of plasma tryptophan to albumin which results from an increase of plasma UFA appears to lead to increased brain tryptophan. Furthermore, a group of uninjected rats showed significant positive correlations between free trytophan in plasma and trytophan concentration in brain regions (Curzon, Kantamaneni, Fernando, Woods & Cavanagh, 1975). Therefore it seemed possible that the rapid plasma UFA increase on removal of cage-mates or on other brief disturbances could lead to similar transient brain tryptophan changes. Present findings do not indicate this. The rapid increases of plasma UFA, though they led to liberation of tryptophan from albumin binding as shown by large increases of percentage free tryptophan, were also associated with a decrease of plasma total tryptophan so that absolute concentrations of plasma free tryptophan only increased relatively slightly and brain tryptophan did not increase within the brief period over which measurements were made. The fall of plasma total tryptophan presumably resulted from a shift of the newly freed tryptophan into intracellular compartments other than the brain. Thus rapid transient changes of plasma UFA on brief stress, unlike the more prolonged changes in previoulsy studied circumstances, do not appear to cause comparable brain tryptophan changes.

Table 4 Effects of removal of cage-mates and of propranolol and nicotinic acid on plasma corticosterone of fasted rats

		Order of removal			
	Injected	*1st*	*2nd*	*3rd*	*1st, 2nd, 3rd combined*
		Plasma corticosterone (μg/100 ml)			
Expt 1.	–	32.7 ± 12.7 (8)	36.3 ± 10.2 (8)	46.1 ± 12.1 (8)	
Expt 2.	Saline	12.1 ± 5.0 (4)	22.4 ± 7.7 (3)	20.6 ± 9.0 (4)	18.0 ± 8.1 (11)
	Propranolol, 1 mg/kg	14.3 ± 8.3 (4)	19.7 ± 15.7 (4)	10.8 ± 7.1 (3)	15.3 ± 10.9 (11)
Expt 3.	Saline	22.3 ± 4.3 (4)	16.8 ± 9.5 (4)	29.6 ± 10.4 (4)	22.8 ± 9.4 (12)
	Nicotinic acid, 50 mg/kg	42.2 ± 12.7 (4)	50.0 ± 4.7 (4)	50.5 ± 18.6 (4)	47.3 ± 13.1 (12)*

General conditions as in Table 2.
Expt 1. Relationship between corticosterone concentration and order of removal: $r = 0.4460$, $n = 24$, $P < 0.05$. Relationship between corticosterone and UFA concentrations (latter values not shown): $r = 0.5583$, $n = 24$, $P < 0.01$.
* $P < 0.001$.

Another plasma amino acid, tyrosine, did not show similar changes to those of tryptophan. This finding is consistent with the fall of plasma tryptophan being related to its liberation from albumin, as tryptophan is the only amino acid present in plasma in both protein-bound and free states (McMenamy *et al.*, 1957). Also the prevention of the rapid plasma tryptophan changes by the antilipolytic drugs propranolol and nicotinic acid is consistent with the changes being caused by an increase of plasma UFA.

Although the β-adrenoceptor blocking agent, propranolol, prevented the increase of plasma UFA on removal of cage-mates, it did not decrease plasma UFA in initially removed rats; nicotinic acid decreased UFA even in these rats. This observation indicates that sympathetic changes were responsible for the rapid UFA increase on removal of cage-mates but not for the underlying increase of plasma UFA on fasting and agrees with the results of Brodie *et al.* (1969) who showed that the increase of UFA caused by fasting is unaffected by β-receptor blockade but is opposed by nicotinic acid which acts directly on fat cells by reducing the level of cyclic AMP.

Unlike propranolol, nicotinic acid decreased brain tryptophan concentration, in agreement with earlier work on fasted rats (Curzon & Knott, 1974; Fernando, Joseph & Curzon, 1975). The roles of the decrease of plasma UFA and the increase of corticosterone in these tryptophan changes require further investigation.

These results demonstrate how readily the disposition of tryptophan is influenced by a brief and largely psychological disturbance. They also suggest that propranolol and nicotinic acid and its derivatives may be useful tools in research on tryptophan metabolism. For example, they could be used to prevent changes mediated by the peripheral sympathetic-UFA mechanism and thus facilitate study of purely centrally provoked changes of brain trytophan and 5-HT metabolism in stress situations. The stabilizing effect of these drugs on plasma tryptophan levels and the influence of nicotinic acid on the availability of tryptophan to the tissues (Curzon & Knott, 1974) may be of interest in relation to the reported alteration of trytophan metabolism in schizophrenic crises (Brune & Himwich, 1962) and the beneficial effect of propranolol in schizophrenia (Yorkston, Zaki, Malik, Morrison & Havard, 1974). Although there have been claims that nicotinamide is beneficial in schizophrenia (Hoffer, 1962) they have not been confirmed (e.g. Ban, 1973).

Finally, the results described here indicate that often disregarded environmental disturbances may be relevant to the design and interpretation of experiments on tryptophan and 5-HT metabolism.

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