were taken up by the liver and not preferentially by the kidney. It is unlikely, therefore, that the liver–kidney transfer of cadmium demonstrated in this work is due to the release of Cd/cysteine type complexes from the necrotic liver cells. It is more probable that Cd–metallothionein is released (from the necrotic liver cells) and is subsequently filtered and reabsorbed by the kidney. It has been proposed [5] that the renotoxic effect of Cd–metallothionein is due to the lysosomal degradation of the protein releasing the toxic Cd^{2+}. The lack of kidney damage observed in the cadmium/aflatoxin animals may be due to the fact that the endogenous thionein can bind any Cd^{2+} released from the metallothionein which has been reabsorbed by the kidney and thereby prevent a toxic effect.

In summary, aflatoxin-induced liver damage produces in cadmium-treated rats a loss of hepatic cadmium concomitant with an increase in renal cadmium levels.

It remains to be established whether or not this rapid cadmium transfer is a common effect produced by hepatotoxins in general or is peculiar to aflatoxin-induced liver damage. It is therefore of interest that a recent study has suggested that the induction of zinc–metallothionein protects against CCl_4-induced liver damage [14]. This finding may explain why Webb and Etienne [5] did not produce a cadmium transfer with CCl_4 or retrorsine and the protective effect observed in this study. Currently, other hepatotoxins, particularly those used in industry, e.g. CS_2 and beryllium, are being investigated. If transfer is a general response to such agents, clinically this could be important not only to those individuals currently exposed to cadmium but also to those who have been previously exposed. Thus, for example, an individual may have been industrially exposed to cadmium without any obvious symptoms. At a later stage the individual may suffer hepatic injury (such as alcohol-induced cirrhosis), resulting in a subsequent transfer of cadmium to the kidney, which if it reached the critical concentration (200 p.p.m.) would lead to renal damage.

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Toxicology Unit,
M.R.C. Laboratories,
Woodmansterne Road,
Carshalton,
Surrey, U.K.

KELVIN CAIN
BEATRICE GRIFFITHS

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Effect of 5-hydroxytryptamine on free amino acid composition of stomach and plasma, and on protein synthesis in the stomach of rats

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5-hydroxytryptamine (5-HT) is distributed throughout the body and is known to be involved in regulating many physiological processes. In stomach and duodenum, where it is present in large amounts, the amine is thought to play a role in the regulation of gastric secretory activities [1–3]. Besides this, the amine is found to affect protein synthesis in gastrointestinal tissues. We have recently demonstrated that a single injection of 5-HT markedly reduces amino acid incorporation into total protein of the stomach, small intestine and colon *in vivo* [4, 5]. The mechanism of inhibitory action of 5-HT is, at present, unknown. However, our earlier observation of higher acid-soluble radioactivity in the stomach during the period of lower incorporation after 5-HT injection [5] indicates that the 5-HT-mediated inhibition of protein synthesis in the stomach is not due to diminished uptake of the precursor amino acid by the tissue. To determine further whether 5-HT would affect the amino acid pool, the concentration of free amino acids in both plasma and stomach was analyzed following a single injection of the amine. The incorporation of [^{3}H]-leucine into total protein of the stomach and the specific radioactivity of the precursor pool were also measured after 5-HT injection. We observed that a single injection of 5-HT caused a marked (60.5 per cent) reduction in [^{3}H]-leucine incorporation into total protein of the stomach *in vivo*, with no change in specific radioactivity of the precursor amino acid. Furthermore, 1 hr after 5-HT administration, the concentrations of free amino acids in plasma (excepting aspartic acid) were decreased, whereas in the stomach they were found to be increased (except for aspartic acid, proline, threonine and glutamic acid) when compared with the corresponding control.

Adult male Wistar rats (200–250 g) were fasted for 24 hr before they were injected (i.p.) with either 0.9% NaCl (saline) or 20 mg/kg 5-hydroxytryptamine creatinine sulfate (Sigma Chemical Co., MO) in saline, and were decapitated

Table 1. Effect of 5-hydroxytryptamine on the concentration of free amino acids in plasma and stomach*

Amino acids	Plasma (μmoles/ml)		Stomach (μmoles/g)	
	Control	5-HT	Control	5-HT
Aspartic acid	0.030 ± 0.003	0.030 ± 0.005	1.18 ± 0.19	1.20 ± 0.05
Threonine	0.347 ± 0.035	0.196 ± 0.039 (−44)	0.86 ± 0.06	0.83 ± 0.03
Serine + glutamine	0.648 ± 0.107	0.543 ± 0.101 (−16)	3.08 ± 0.10	3.32 ± 0.02 (+8)
Glutamic acid	0.126 ± 0.008	0.088 ± 0.034 (−30)	3.72 ± 0.05	2.52 ± 0.14 (−32)
Proline	0.203 ± 0.035	0.106 ± 0.015 (−48)	0.44 ± 0.01	0.40 ± 0.01 (−9)
Glycine	0.206 ± 0.016	0.169 ± 0.049 (−18)	1.65 ± 0.11	1.85 ± 0.29 (+12)
Alanine	0.367 ± 0.033	0.254 ± 0.044 (−31)	1.90 ± 0.11	2.11 ± 0.17 (+11)
½ Cystine	0.052 ± 0.011	0.040 ± 0.010 (−23)	0.03 ± 0.01	0.07 ± 0.03 (+133)
Valine	0.169 ± 0.010	0.117 ± 0.005 (−31)	0.43 ± 0.03	0.53 ± 0.02 (+23)
Methionine	0.040 ± 0.004	0.020 ± 0.009 (−50)	0.28 ± 0.02	0.38 ± 0.02 (+36)
Isoleucine	0.085 ± 0.003	0.063 ± 0.008 (−26)	0.31 ± 0.02	0.36 ± 0.01 (+16)
Leucine	0.142 ± 0.008	0.096 ± 0.006 (−33)	0.60 ± 0.06	0.79 ± 0.03 (+32)
Tyrosine	0.048 ± 0.004	0.039 ± 0.005 (−19)	0.31 ± 0.02	0.43 ± 0.03 (+39)
Phenylalanine	0.055 ± 0.001	0.043 ± 0.008 (−22)	0.28 ± 0.02	0.41 ± 0.01 (+46)
Lysine	0.495 ± 0.040	0.352 ± 0.120 (−29)	0.79 ± 0.03	0.87 ± 0.05 (+10)
Histidine	0.060 ± 0.008	0.044 ± 0.007 (−27)	0.20 ± 0.01	0.24 ± 0.01 (+20)
Arginine	0.114 ± 0.016	0.069 ± 0.008 (−39)	0.36 ± 0.01	0.45 ± 0.01 (+25)

* Each value represents the mean ± S.E.M. of three experiments. Figures in parentheses represent percentage increase or decrease, compared to the respective control.

1 hr later. L[4,5-^{3}H]-leucine (10 μCi/100 g; 52 Ci/mmole, Radiochemical Centre, Amersham, U.K.) was injected (i.p.) 30 min before being killed. Blood was obtained through the neck wound into heparinized centrifuge tubes. Blood from 2–3 rats was pooled, and plasma was recovered by centrifugation. The stomach (fundic area) was dissected out, washed thoroughly in cold saline, and was immediately frozen on solid CO_2.

For determination of protein specific radioactivity (c.p.m./mg protein) of the stomach, about 100 mg of tissue was homogenized in 5 ml 10% (w/v) trichloroacetic acid (TCA). Precipitated proteins were collected by centrifugation and were treated the same way as reported earlier [5, 6]. The supernatant was saved for the determination of precursor pool radioactivity. The concentration of protein was measured by the method of Lowry *et al.* [7].

The concentration of free amino acids in plasma and stomach was determined on deproteinized filtrates in a Beckman Auto Amino acid Analyzer 120-C, as reported earlier [6].

The present dose (20 mg/kg) of 5-HT was chosen on the basis that it produced maximal inhibition of protein synthesis in the stomach [5]. Such a dose has also been shown to inhibit pepsin secretion [8]. One hour after 5-HT injection, the incorporation of [^{3}H]-leucine into total protein of the stomach *in vivo* was found to be decreased by 62 per cent (c.p.m./mg protein; control = 1850 ± 65, and 5-HT = 730 ± 30, N = 6; $P < 0.001$). On the other hand, the TCA-soluble radioactivity (data not shown), as well as the free leucine concentration in the stomach (Table 1), were found to be higher in the 5-HT-treated groups than in the control. Thus, when the specific radioactivity of the leucine pool in the stomach was calculated by dividing the amount of TCA-soluble radioactivity by the concentration of leucine in the tissue, the values were found to be the same for both groups (μCi/μmole; control = 0.136 and 5-HT = 0.139). The results suggest that the inhibition of protein synthesis by 5-HT, at least in the stomach, is not due to either diminished availability of leucine or lower specific radioactivity of the precursor pool in the tissue.

To evaluate whether 5-HT would affect amino acid composition of the stomach, the concentration of free amino acids in the tissue was measured. Plasma free amino acids were also determined to correlate the changes in tissue amino acid levels with plasma. It was observed that, except for aspartic acid, threonine, glutamic acid and proline, the concentrations of all other amino acids in the stomach were increased (8–133 per cent) after administration of 5-HT (Table 1). The maximal increment was observed for ½ cystine. In the stomach, the concentrations of proline and glutamic acid were decreased by 9 and 32 per cent, respectively, and aspartic acid and threonine showed no apparent changes following 5-HT treatment (Table 1). Contrary to the observation in the stomach, the concentrations of all amino acids in plasma (with the exception of aspartic acid) were decreased (16–50 per cent) after 5-HT administration (Table 1). The highest reduction (50 per cent) was noted for methionine.

It is evident that for most amino acids the 5-HT-mediated reduction in plasma is accompanied by a concomitant rise in the stomach, indicating a shift in amino acid pool from plasma to stomach. Such an observation suggests further that the 5-HT-mediated inhibition of protein synthesis in the stomach is not the result of a diminished supply of amino acids in the tissue. The amine is known to exert a powerful vascular effect, and in the dog the 5-HT-mediated inhibition of gastric secretion is attributed to decreased blood supply [9]. On the other hand, in rats 5-HT is found to dilate gastric mucosal blood vessels [10], which suggests that the increased free amino acid content observed in the stomach of 5-HT-treated rats could be the result of a higher blood flow in the tissue. Whether changes in endogenous 5-HT level would also affect protein synthesis and amino acid composition in the stomach remains to be evaluated.

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Institute of Medical Biochemistry, University of Aarhus, Dk-8000 Aarhus C, Denmark.

ATIF M. NAKHLA
ADHIP NANDI MAJUMDAR*

* To whom reprint requests should be addressed.

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Progesterone provokes a selective rise of monoamine oxidase A in the female genital tract

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We reported earlier a striking increase in human endometrial monoamine oxidase (MAO) activity during the second half of the menstrual cycle [1] which correlated with peak plasma progesterone activity. On giving progesterone to female rats, we were, similarly, able to record a substantial increase in uterine (but not hepatic) MAO activity [2]. Since these experiments were performed, Johnston's classification of MAO [3] into A and B forms, on the basis of selective inhibition by clorgyline, has been widely accepted. The A form which, by definition, is relatively sensitive to clorgyline, selectively oxidizes the neurotransmitter monoamines, 5-hydroxytryptamine (5-HT) and noradrenaline; the relatively clorgyline-resistant MAO B acts on phenylethylamine (PEA) and benzylamine (Bz) whilst tyramine and dopamine can be oxidized by both forms [4]. However, these specificities depend to some extent on substrate concentration, tissue and species [5, 6]. In the light of this new information, we thought it interesting to re-examine the progesterone effect to determine whether both forms of the enzyme are affected equally or whether one or other predominates. We now report highly selective increases in MAO A activity in certain organs of the human and rat female genital tract.

Endometrial biopsy fragments were obtained by curettage from 15 normal subjects (age range 20–30 yr), under examination for a variety of minor gynaecological disorders. All had normal menstrual cycle length (28 ± 2 days) and none was on hormone treatment. The specimens were placed immediately on dry ice and later transferred to storage at −20°. For the animal experiments six female Wistar rats (150–180 g) were injected subcutaneously with progesterone, 3.3 mg/kg, at the same time of day, for three consecutive days. Six control rats were injected with physiological normal saline (0.9% w/v), 0.25 ml subcutaneously, and 0.75 ml intraperitoneally for three consecutive days. This routine was followed to replicate that of the previous study [2]. All animals were killed on the fourth day by dislocation of the neck. Liver, uterus, ovary and adrenal glands were dissected out, freed from connective tissue and stored at −20°. All tissue was homogenized in 50 mM potassium phosphate buffer, pH 7.4, and made up as a 10% (w/v) suspension. MAO was assayed radiometrically as previously described [7]. Protein was estimated by the method of Lowry *et al.* [8], using bovine serum albumin as standard.

Figure 1 shows MAO activity using three different substrates, 5-HT, DA and PEA, in endometrial biopsy samples taken from women at different stages of the menstrual cycle. It is clear that activity, when measured with 5-HT and DA, increased markedly during the course of the cycle, whereas activity with PEA was relatively constant. Table 1 shows mean activity from five samples obtained during the first week of the cycle compared with five samples from the fourth week. Both 5-HT and DA-oxidizing activity increased about 7-fold and the difference between first and fourth-week values was highly significant ($P < 0.01$). PEA-oxidizing activity was low throughout, and did not increase significantly from early to late cycle.

Table 2 shows findings after progesterone injection into rats. The drug caused large and significant increases in 5-

Table 1. Monoamine oxidase activity in human endometrium samples obtained during the first and fourth week of the menstrual cycle*

	No. of samples	Substrates		
		5-HT	DA	PEA
1st week	5	7.2 ± 0.7	4.3 ± 0.5	3.6 ± 0.3
4th week	5	51.9 ± 1.8†	48.7 ± 1.8†	7.2 ± 0.8‡

* Activities are expressed as nmoles product formed per mg protein per 30 min at 37°.
† $P < 0.01$ using Wilcoxon rank sum test; fourth week different from first week.
‡ Difference not significant.