tion would be expected to take place by interaction of the lone electron pair on the nitrogen atom and the π electrons of the carbonyl group. This would impart a partial positive charge on the nitrogen atom which would in turn decrease the nucleophilic character of both nitrogen atoms and thus stabilize the molecule. Alternatively the resistance to reduction could be due to a steric effect of the carbonyl group but this seems less likely as the hydrazide grouping is known to be usually planar along all 4 atoms.

These experiments indicate that under in vitro conditions the intestinal microflora is able to effect extensive reductive fission of all the hydrazines examined, but the extent to which the intestinal microflora participates in vivo can in fact only be assessed by investigations in intact animals, as the rate of uptake of the administered compound from the gut, the period of contact between the compound and the microflora and the physical and chemical conditions obtaining in the lumen of the intestine are clearly important factors.

Table 3. Chromatographic characteristics of dansyl derivatives of the amine metabolites and other compounds

Dansyl derivative	R_f-value in solvent system A	B	C	D
Methylamine	0.17	0.14	0.26	-
Aniline	0.28	0.20	0.38	0.44
Benzylamine	0.25	0.27	0.80	-
1-Phenethylamine	0.27	0.31	-	0.77
Isopropylamine	0.40	0.22	0.60	-

Dansyl derivatives were detected on TLC plates by their fluorescence under $UV_{365\,nm}$ irradiation. All separations were carried out on silica gel G layers employing the solvents indicated: A, benzene-acetone (98:2 by vol.); B, chloroform-benzene (1:1 by vol.); C, chloroform; D, benzene-triethylamine (4:1 by vol.).

Table 4. Chromatographic characteristics of substituted hydrazides and related compounds

Compound	R_f in solvent system E	F	G	H	I	Detection
Iproniazid	0.75	0.95	-	-	-	1
Isoniazid	0.55	0.62	-	-	-	1
Nialamide	0.15	0.95	0.0	0.0	-	1
Isocarboxazid	-	-	0.80	0.77	0.70	2
Iproclozide	0.95	-	0.60	0.65	-	2
Tersavid	0.65	-	0.65	0.85	0.65	2
Isonicotinamide	0.40	0.80	-	-	-	1
Isonicotinic acid	0.05	0.55	-	-	-	1
Benzylhydrazine	-	-	0.40	-	0.15	2

All separations except that employing solvent F were carried out on silica gel G layers. Separations employing solvent F were carried out on cellulose powder layers.
E, chloroform-acetone-diethylamine (5:4:1 by vol.); F, propan-2-ol-water (85:15 by vol.); G, 1,2-dichloro-ethane-ethylacetate-formic acid (3:1:1 by vol.); H, benzene-1,4-dioxane-acetic acid (90:25:4 by vol.); I, chloroform-ethylacetate-formic acid (5:4:1 by vol.). 1, nitroprusside reagent[15]; 2,4-dimethylamino-cinnaldehyde reagent[16].

Although it has been reported by Schwarz[13] that small amounts of methylamine are detectable in the urine of rats dosed with procarbazine, free phenylethylamine and benzylamine were not detected among the urinary metabolites of [^{14}C] mebanazine and [^{14}C] benzylhydrazine respectively in the intact rat[14] although the possible formation of benzylamine as an intermediate in the formation of either benzoyl glucuronide or of benzoylglycine, the major urinary metabolite of benzylhydrazine, cannot be ruled out.

The demonstration that intestinal microorganisms under favourable conditions are capable of degrading hydrazines to their corresponding primary amines may be of particular significance in relation to intestinal diverticulosis in man since this condition is known to result in areas of intestinal stasis which are characterized by considerable proliferation of the bacterial population.

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Separation and quantitative determination of imipramine and desipramine from rat biological samples by high pressure liquid chromatography

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Summary. Imipramine and its metabolite, desipramine, have been determined in several rat biological samples by high pressure liquid chromatography. The method allows one to detect 15 ng in column of both drugs with a lower sensitivity limit of 20 $\mu g\,l^{-1}$.

In recent years, different analytical techniques have been developed for the qualitative and quantitative determination of imipramine and desipramine in biological samples. The techniques so far used can be distinguished in spectrophotometric[1], spectrophotofluorimetric[2], autoradiographic[3], isotopic[4], TLC[5–7] and gas chromatographic[8,9]. In this work we have employed high pressure liquid chromatography (HPLC), in order to separate and detect imipramine and its metabolite desipramine in biological samples; this technique presents, in addition to the advantages of a high specificity and sensitivity (in the range of ng), a considerable simplicity and rapidity of execution.

Materials and methods. Male pellet-fed Wistar rats weighing 190–210 g, were injected i.p. with a standard dose of

50 mg/kg of imipramine hydrochloride in 0.5 ml of water. The animals were killed by decapitation at various times after the administration of the drug, and 4 of them were used at each time point. At decapitation 5 ml of blood were collected in a heparinized tube and centrifuged in order to obtain the plasma. Moreover brain, liver, kidney and heart were immediately dissected and homogenized as a whole in a glass homogenizer in 2 vol. of HCl 0.01 N and aliquots corresponding to 1 g of the different tissues were used for extraction. The biological samples were adjusted to pH 12 with NaOH 1.5 N, extracted 3 times with 4 vol. of heptane containing 1.5% isoamyl alcohol by shaking 30 min. in a mechanical shaker and centrifuging (5 min, 3000 rev. min^{-1}). The organic phases were dried under 'vacuum' and dissolved in 500 µl of chromatographic mobil phase[10].

The recovery was 87% for imipramine and 78% for desipramine. Standard solutions of imipramine hydrochloride and desipramine hydrochloride (obtained respectively from Ciba-Geigy, Saronno, Italy and from Chiesi Farmaceutici, Parma, Italy) were prepared by dissolving the compounds in the mobile phase to give concentrations of 1 mg/ml calculated as base.

The tests were carried out by means of a Series 4000 Varian aerograph liquid chromatograph, equipped with UV-visible Beckman DU detector and with a constant pressure pump. A Micropak SI 10 column of a 50 cm length and of an internal diameter of 2.2 mm was used. Samples up to 8 µl were injected by 1, 5, 10 µl Hamilton syringes using the stop flow technique.

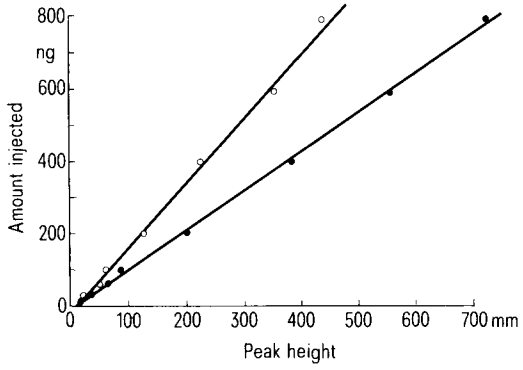

Fig. 1. Standard curves of imipramine (●) and desmethylimipramine (○).

The volume of the microcell was 60 µl. The mobile phase was constituted by chloroform-n-propanol-32% ammonia (100:100:2, v/v/v). The flow rate was 0.33 ml/min under a pressure of 1000 psi. All measurements were carried out at room temperature (25 °C). Detection was by UV absorbance at 256 nm. The retention times were 7.4 min for imipramine and 16.3 min for desipramine.

Results and discussion. Standard curves of imipramine and desipramine (figure 1) were obtained injecting 15, 30, 60, 100, 400, 800 ng of the standard solutions. Standard curves were calculated by regression analysis as $y=1.1\ x-6.3$ ($r=1.0$) for imipramine and $y=1.9\ x-25.9$ ($r=1.0$) for desipramine, where y = ng injected, x = peak height (mm) calculated at a sensitivity of 0.02 absorbance unit-fullscale deflection and r = correlation coefficient. In order to estimate the SD on the peak heights, 6 determinations were carried out for each concentration used for the standard curves. SD were of the order of magnitude of 6.5%. The identification of imipramine and of its metabolite desipramine in the different biological samples was accomplished

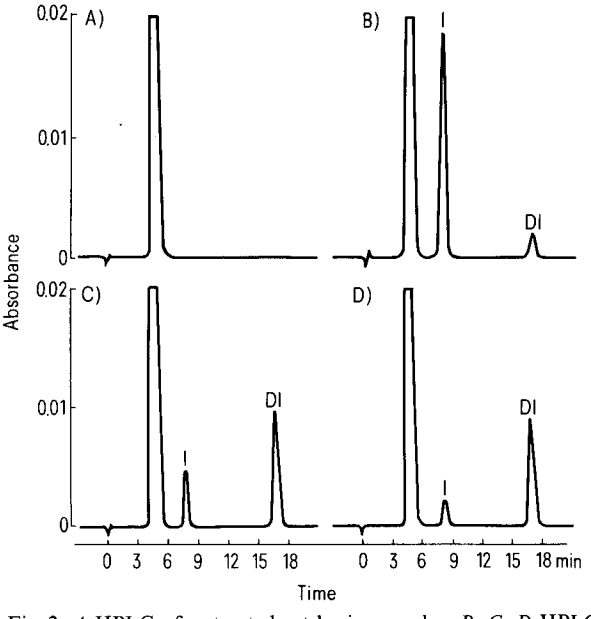

Fig. 2. *A* HPLC of untreated rat brain samples. *B, C, D* HPLC of rat brain samples 15, 120, 360 min after administration of imipramine. I = imipramine peak; DI = desmethylimipramine peak.

Mean tissue concentrations of imipramine (I) and desipramine (DI) at the times of the experiment

	15 min µg/g	nmole/g	DI/I*	120 min µg/g	nmole/g	DI/I*	360 min µg/g	nmole/g	DI/I*
Brain									
I	19.3	68.8	0.1	5.0	17.8	2.1	2.5	8.9	4.5
DI	2.2	8.2		10.1	37.9		10.7	40.1	
Liver									
I	51.6	184.0	1.6	22.2	79.2	2.7	8.1	28.9	3.9
DI	79.6	298.7		57.4	215.4		30.5	114.4	
Heart									
I	-	-	-	2.7	9.6	3.0	1.1	3.9	12.8
DI	-	-		7.8	29.3		13.3	49.9	
Kidney									
I	45.4	161.9	1.2	16.3	58.1	2.1	14.9	53.1	2.5
DI	51.2	192.1		33.0	123.8		35.7	134.0	
Plasma									
I	0.31	1.1	1.8	0.85	3.0	1.7	0.42	1.5	2.4
DI	0.53	2.0		1.34	5.0		0.95	3.6	

* Ratio of molarity.

by comparison with the retention times of the pure standards.

Suitable quantities of the cell effluent, collected corresponding to the chromatographic peaks of the 2 drugs under examination, were tested for homogeneity by qualitative bidimensional TLC using Merck silica-gel precoated glass plates (20 × 20 cm, 0.25 mm).

The 1st development used (12 cm) was benzene : dioxane : ethanol : concentrated ammonia (50:40:5:5, v/v) and the 2nd was ethanol saturated with sodium chloride : acetic acid : water (70:20:5, v/v)[11]. After double development, the plates were dried for 25 min at 110 °C and sprayed, in the order, with diazoted p-nitroaniline and concentrated hydrochloric acid. The chromatogram obtained in this way corresponded perfectly in colour (green) and in R_f-values to that obtained developing in the same manner the pure reference compounds.

Figure 2 shows, as an example, typical liquid chromatograms of brain samples of untreated and imipramine treated rats, and the table reports the mean plasma and tissue concentrations of imipramine and of its metabolite detected at various times after i.p. administration of 50 mg/kg of imipramine.

In the case of plasma 5 ml were used for the extraction and concentrated to 50 µl; considering that the amount we were able to detect was 15 ng in column for both substances (figure 1), the lower sensitivity limit is about 20 µg l^{-1}. However, by using a smaller microcell than that at our disposal, the sensitivity can be further raised by about 5–8 times.

We can therefore conclude that our method is rapid, easy to perform, specific and allows the simultaneous dosage of both imipramine and desipramine; moreover this technique presents a good sensitivity, comparable, in the conditions we adopted, to that obtained by Weder and Bickel[8] by the gas chromatographic assay.

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Effect of the administration of (d-Ala) ^{2}methionine-enkephalin on the serotonin metabolism in rat brain[1]

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Summary. The effect of cerebroventricular injection of [D-Alanine] methionine-enkephalin (DALA), a synthetic analog of met-enkephalin, on the serotonergic system of rat brain has been studied. This opioid peptide caused an increase in 5HT turnover which was particularly evident in the limbic forebrain. This effect was completely antagonized by naloxone pretreatment.

It is now established that intracerebral injection of methionine-enkephalin or leucine-enkephalin, 2 pentapetides extracted from mammalian brain[3,4], gives rise in the rat to symptomatology, such as analgesic and decreased motor activity, closely resembling that elicited by morphine and other narcotic drugs[5,6].

Due to their rapid inactivation by brain endopeptidases[7,8], these peptides have a very short-lasting effect, and it is not possible by the usual methodological approaches to demonstrate modifications in the metabolism of central monoamines of the type correlated with the analgesic activity of morphine[9].

We have recently reported that (D-Ala) ^{2}methionine-enkephalin (DALA), a synthetic analog of methionine-enkephalin made resistant to enzymatic cleavage by substituting D-alanine for glycine in the second position of the peptidic chain[10], gives not only a more powerful pharmacological action, but also a clear stimulant effect on the synthesis of brain dopamine[11,12].

There is evidence that the central serotonergic system is involved in the analgesic effect of morphine in the rat, and administration of this drug is known to increase serotonin (5HT) turnover[9,13]. The objective of the experiments reported here was to determine whether the similarity between morphine and DALA, as regards the pharmacological action and the effect on central dopaminergic system[11,12], also existed as regards the effect on brain 5HT.

DALA was injected into unanaesthetized rats (175–200 g male Sprague-Dawley, Charles River, Calco, Italy) through 2 indwelling polyethylene cannulas implanted in the lateral ventricles 3 days before the experiment. The peptide was dissolved in saline and injected in the volume of 5 µl/ventricle at different concentrations and at different times before killing. The brains were rapidly excised, dissected, frozen, and kept frozen until the assays. Serotonin, its metabolite, 5-hydroxyindolacetic actid (5HIAA) and its precursor tryptophan (TP) were determined fluorimetrically in the whole brain, purified in organic solvent according to the procedure of Curzon and Green[14] and Denkla and Dewey[14,15]. Intraventricular injection of 25 µg of DALA, a dose which induced analgesia, immobility and increased dopamine turnover[6] led to an increase of brain 5HIAA (experiment I). Levels were significantly raised 45 min after the injection, and increased further to 90 min, when they peaked out. After 135 min, 5HIAA levels were still high. A lower dose of the peptide (10 µg) led to an increase of 5HIAA, but this did not reach the level of a statistical significance (experiment II). A higher dose (50 µg), on the other hand, was no more effective than 25 µg (experiment II). None of these experimental conditions changed the brain concentration of 5HT (data not reported).

The DALA-induced rise in 5HIAA appears due more to an increased rate of formation of this metabolite than to DALA-induced inhibition of its efflux from the brain. In