These findings suggest that the direction of a pH gradient across the membrane is an important factor influencing the direction of net folate flux. It seems likely, therefore, that $^{14}CH_3H_4PteGlu_1$ permeates through the membrane by a process that does not translocate electric charge. This postulated mechanism of coupled H^+ copermeation or OH^- exchange is analogous to lactate and β-hydroxybutyrate transfer [13–15] and indicates further similarities between these erythrocyte membrane transport systems. Previous studies in our laboratory showed that $^{14}CH_3H_4PteGlu_1$ transfer, like lactate transport, is sensitive to sulfhydryl reagents and amino reactive agents but is resistant to proteolytic enzymes [10]. We also found that several amphipathic drugs known to inhibit anion transport, such as ethacrynic acid, sulfinpyrazone and dipyridamole, also impair folate transport [16]. These findings, taken together with the present studies, indicate that the red cell membrane carrier systems for folate compounds and for other, smaller organic anions are remarkably similar.

In summary, we measured the effects of external pH on influx, efflux and net steady-state levels of $^{14}CH_3H_4PteGlu_1$. Initial rates of uptake were inversely proportional to external pH. Lowering the pH of the suspending medium increased influx by enhancing the affinity of the carrier: the apparent K_m values at pH 6, 7.2 and 8.1 were 0.14, 0.25 and 0.63 μM respectively. In contrast, V_{max} was independent of external pH. Efflux rate constants at pH 6, 7.5 and 8.5 were 0.034, 0.059 and 0.067/min respectively. Consequently, lowering the external pH increased steady-state levels of $^{14}CH_3H_4PteGlu_1$.

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Distribution of pepstatin and statine following oral and intravenous administration in rats. Tissue localisation by whole body autoradiography

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Pepstatin A is a potent inhibitor of pepsin activity with a K_i of the order of 10^{-11} M, and is one of several low molecular weight protease inhibitors isolated by Umezawa *et al.* [1] from the culture filtrates of various strains of *Streptomyces* (Fig. 1). In addition to pepsin, pepstatin A also inhibits many other acid proteinases, in particular renin (K_i 10^{-7} M) and cathepsin D (K_i 5×10^{-10} M). It has been advocated as a possible chemotherapeutic agent for the treatment of duodenal ulceration [2], hypertension [3] and inflammation [4]. We have used whole body autoradiography to compare the distribution *in vivo* of the relatively insoluble radiolabelled analogue, pepstatinyl-[^{14}C]glycine, which has a similar K_i value to pepstatin A [5], with that of the soluble derivative [^{3}H]acetylstatine which also inhibits pepsin but has a K_i value 10^6-fold less than pepstatin A [6].

Materials and methods

Pepstatin A was a kind gift from Dr. Jan Muller (H. Lundbeck & Co., Copenhagen-Valby, Denmark). [1-^{14}C]Glycine (40 mCi/mmole) and [^{3}H]acetic anhydride (10 Ci/mmole) were from Amersham International (Amersham, U.K.). Cremophor EL (polyoxyethylated castor oil) was from Blagdon, Campbell Chemicals Ltd. (Croydon, U.K.).

Pepstatinyl-[^{14}C]glycine was prepared by the method of Knight and Barrett [5]. The specific radioactivity of pepstatinyl-[^{14}C]glycine (15.5 mg) was estimated to be 5 mCi/mmole. An R_f value of 0.8 was obtained for pepstatinyl-[^{14}C]glycine by thin-layer chromatography [1] and this agreed closely with a value of 0.76 for unmodified pepstatin A [1]. The radiolabelled analogue was homogeneous since [1-^{14}C]glycine did not move from the origin

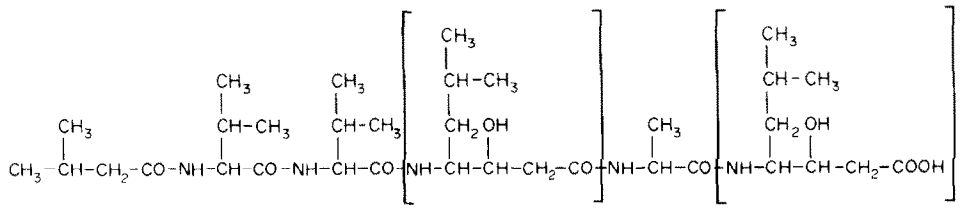

Fig. 1. Structure of pepstatin A. Square brackets indicate the two statine residues.

and no other radiolabelled material was detected.

Statine was isolated from 500 mg of 6 N HCl acid-hydrolysed pepstatin A by preparative reverse-phase high-pressure liquid chromatography [7]. The purified statine was identified by high-voltage paper electrophoresis at pH 1.9 (formic acid : acetic acid : water, 25 : 87 : 88) and amino acid analysis. The yield was estimated to be about 20% (42 mg) of the available statine. Fourteen milligrams of statine was acetylated with 25 mCi [^{3}H]acetic anhydride and separated from by-products by ion-exchange chromatography on Dowex 1- × 8 (acetate form). The specific radioactivity of *N*-[^{3}H]acetylstatine (9 mg) was estimated to be 66.5 mCi/mmole. The ^{3}H-labelled material was homogeneous by thin-layer chromatography [1] and by high voltage paper electrophoresis at pH 6.5.

Whole-body autoradiography. Male Wistar rats (130 g) were starved overnight prior to experimentation. Pepstatinyl-[^{14}C]glycine (15.5 mg) was solubilised in 8.5 ml ethanol and 1.2 ml Cremophor EL. The ethanol was removed under N_2 and the Cremophor EL/pepstatinyl-[^{14}C]glycine was resuspended in 7.8 ml water. Four millilitres of the suspension was added to 0.25 ml 2 M HCl and 3 rats each received approximately 16 μCi (2.2 mg) of pepstatinyl-[^{14}C]glycine intragastrically using an oral-dosing needle. The animals were sacrificed by carbon dioxide inhalation at 1, 2 and 6 hr after administration and immediately frozen by immersion in hexane chilled to −70° with dry-ice. Of 0.5 M Na phosphate buffer, pH 7.3, 0.1 ml was added to the remaining material and 3 rats each received 8 μCi (1.1 mg) of pepstatinyl-[^{14}C]glycine by slow infusion into their tail veins over a 3 min period. The animals were sacrificed at 15, 30 and 60 min after administration and frozen as described. Nine milligrams of *N*-[^{3}H]acetylstatine was solubilised in 8 ml H_2O and divided into 4 ml aliquots. The experimental design was identical to that described for pepstatinyl-[^{14}C]glycine except that each animal received approximately 330 μCi (1 mg) of *N*-[^{3}H]acetyl-statine either intragastrically (in 0.1 M HCl) or intravenously (in 10 mM Na phosphate buffer, pH 7.3). All carcases were stored at −20°. Whole body autoradiography was carried out essentially as described by Ullberg [8].

In a separate experiment bile was collected for 2 hr from a 200 g male Wistar rat after intravenous administration of 0.7 μCi (0.1 mg) of pepstatinyl-[^{14}C]glycine in 10% (w/v) Cremophor EL. Samples were analysed together with pepstatinyl-[^{14}C]glycine, and the unlabelled glycine and taurine conjugates of cholic acid and deoxycholic acid by thin-layer chromatography using chloroform : ethanol : acetic acid : water (12 : 8 : 4 : 1) as the solvent system. Another rat received an intravenous dose of 2 μCi pepstatinyl-[^{14}C]glycine (0.3 mg) and urine was collected by needle puncture of the bladder 2 hr later and the recovery of ^{14}C-labelled material estimated.

Results and discussion

A previous report describing the distribution of tritiated pepstatin A after oral administration to mice demonstrated that about 1% of the dose was absorbed from the alimentary tract although it was not clear whether the tritium-label remained associated with pepstatin A [1]. In this study orally administered pepstatinyl-[^{14}C]glycine remained in the alimentary tract over the 6 hr period of the experiment. There was no delineation of the gastric or intestinal mucosae in any of the animals suggesting very little absorption of the inhibitor. The lungs of all three animals receiving intravenous pepstatinyl-[^{14}C]glycine were strongly labelled (Fig. 2) which was consistent with this relatively insoluble material coming out of solution and becoming lodged in the pulmonary bed [9]. Inhibitor that had not been trapped in the lungs was primarily cleared by the liver and kidneys and the isotope was concentrated in the bile ductules and renal medulla. In rats not used for autoradiography approximately 20 and 25% of an intravenous dose of pepstatinyl-[^{14}C]glycine was excreted in bile or urine, respectively, within 2 hr of administration. This represented the excretion of intact inhibitor since thin-layer chromatography confirmed that the [^{14}C]glycine had remained covalently attached to pepstatin A during bile excretion and 0.3 ml aliquots of urine which contained an estimated 2 μmoles of pepstatinyl-[^{14}C]glycine completely inhibited pepsin hydrolysis of casein. The apparent secretion of pepstatinyl-[^{14}C]glycine into the lumen of the stomach after intravenous administration (Fig. 2) was therefore almost certainly artefactual as bile was found in the stomachs of all three animals. This observation was supported by the fact that no isotope could be recovered from the stomach if the duodenum was occluded prior to administration of the inhibitor.*

N-[^{3}H]acetylstatine remained in the lumen of the alimentary tract after oral administration and most of it could be found in the large intestine after 6 hr. By contrast this compound was widely distributed to most tissues when it was administered intravenously, but concentrations above blood level were only seen in the liver, kidney, bladder and gastric mucosa (Fig. 3). Some secretion across the stomach wall may have occurred since the mucosa was clearly delineated at 15 min whereas the lumen did not contain ^{3}H isotope until 30 min after administration.

The use of pepstatin A or one of its analogues appears to offer a promising approach in the management of acute gastric erosions which may be due to the inappropriate intramucosal autoactivation of pepsinogen [10]. The exceptional K_i value of pepstatin A (10^{-11} M) would suggest that only relatively small amounts of the inhibitor need be localised in the gastric mucosa in order to achieve significant inhibition, but our study has shown that there was little or no absorption of either pepstatinyl-[^{14}C]glycine or *N*-[^{3}H]acetylstatine. The synthesis of pepstatin analogues which are more water-soluble but retain K_i values comparable to pepstatin A may prove more effective in penetrating the gastric mucosa since they would be more amphipathic in character. The K_i value does not appear to be severely affected by modification of the C-terminal statine residue [5] and the addition of a hydrophilic ligand at this site may be advantageous. For example, pepstatinyl-arginine has been reported to be 50 times more water-soluble than pepstatin A and has been shown to be effective in the treatment of hypertension in animals [3].

* T. F. Ford, unpublished observations.

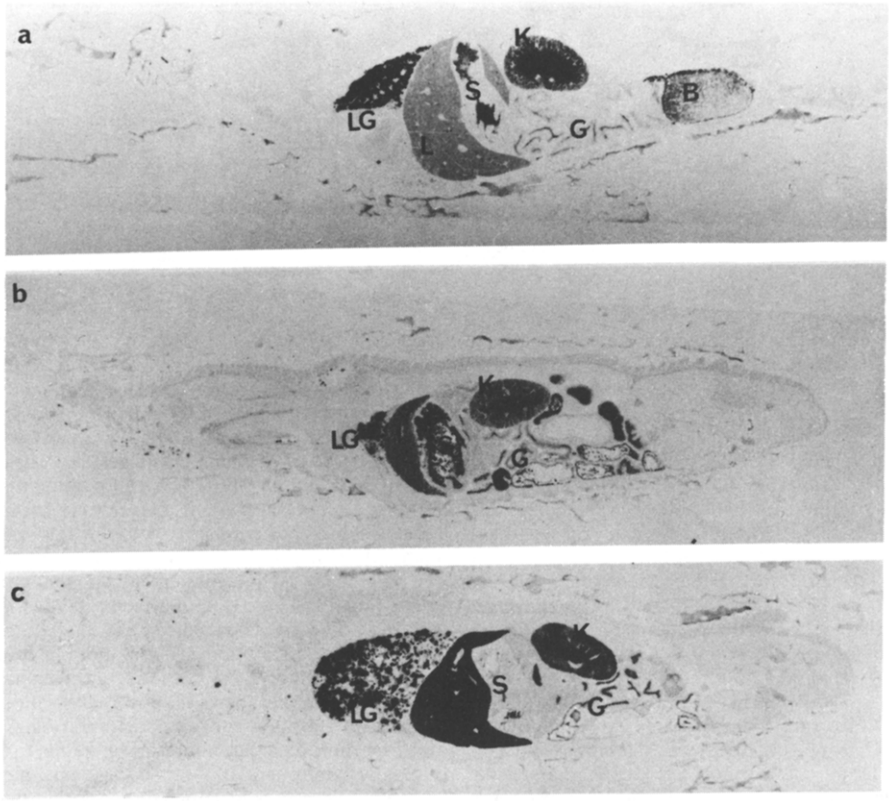

Fig. 2. Autoradiography after intravenous administration of pepstatinyl-[^{14}C]glycine. Animals were sacrificed at (a) 15 min, (b) 30 min, (c) 1 hr after dosing. Tissues were identified as L, liver; K, kidney; S, stomach; LG, lung; B, bladder; G, gut.

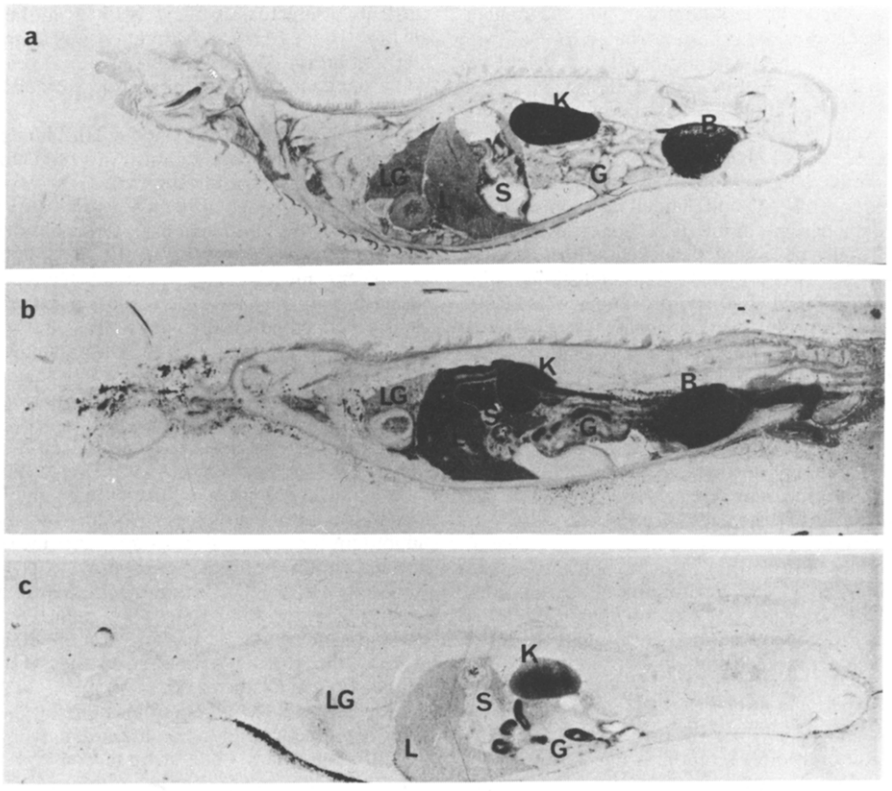

Fig. 3. Autoradiography after intravenous administration of *N*-[^{3}H]acetylstatine. Animals were sacrificed at (a) 15 min, (b) 30 min, (c) 1 hr after dosing. Tissues were identified as in Fig. 2.

In summary, the distribution in rats of two radiolabelled derivatives of the potent pepsin inhibitor, pepstatin A, has been monitored by whole-body autoradiography. There was a very poor absorption of pepstatinyl-[^{14}C]glycine and *N*-[^{3}H]acetylstatine across the gastric and intestinal mucosae after oral administration. Both inhibitors were rapidly cleared from the blood by the liver and kidneys after intravenous administration. Neither inhibitor was localised in the gastric mucosa at a concentration that could be expected to be effective in inhibiting intracellular pepsin activity.

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A new specific inhibitor of monoamine oxidase A

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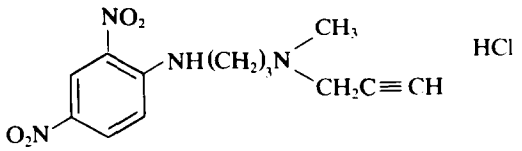
It is now generally agreed that monoamine oxidase [amine–oxygen oxidoreductase (deaminating, flavin-containing), EC 1.4.3.4] (MAO) occurs in two forms differing in substrate specificity and in inhibitor sensitivity. Johnston [1] first showed their existence using the inhibitor *N*-3-(2,4-dichlorophenoxy)propyl-*N*-methylprop-2-ynylamine (clorgyline), which preferentially inhibits oxidation of 5-hydroxytryptamine (5-HT). He called this form MAO A. MAO B, for which preferred substrates include benzylamine (BZA) and 2-phenylethylamine, was shown to be less sensitive to the inhibitor.Tyramine is a good substrate for both forms. Knoll and Magyar [2] described a similar inhibitor, (−)-*N*-methyl-*N*-phenylisopropyl prop-2-ynylamine (deprenyl), with a selectivity opposite to that of clorgyline. Each of these compounds show only partial specificity since either will inhibit their 'non-preferred' form of MAO at higher concentration or on more prolonged time of contact with the enzyme. Earlier studies in this laboratory have produced a number of propynylamines which were for the most part non-selective inhibitors of MAO [3, 4]. In attempts to improve selectivity it has been shown that *N*-desmethyl deprenyl discriminates somewhat better between MAO A and MAO B than does deprenyl, though it is not as potent an inhibitor as the latter [5, 6]. This study reports that the compound N^1-(2,4-dinitrophenyl)-N^2-prop-2-ynyl-1,3-diaminopropane (dinitranyl) is a good irreversible inhibitor of MAO A, as judged by inhibition of 5-HT oxidation, but is apparently without effect on MAO B. The structure of dinitranyl is shown below. Its purity and identity were established by infrared spectroscopy and elemental analysis.



Experimental. Mitochondria from rat liver were prepared by manual homogenization in 0.25 M sucrose (9 vol.) using a Teflon/glass homogenizer, with 6–8 passes of the pestle. Debris was sedimented by centrifuging at 2000 *g* for 10 min. The supernatant was centrifuged at 9000 *g* for 7 min and the pellet was washed twice by resuspension and centrifugation, using first one-half, then one-quarter, of the original volume of sucrose. The pellet was stored frozen in 0.25 M sucrose at a protein concentration of about 30 mg/ml. Protein was measured by the method of Lowry *et al.* [7]. For MAO assay the suspension was diluted with 0.05 M Na/K phosphate buffer, pH 7.5. The assay mixture consisted of 0.85 ml of diluted mitochondrial suspension (approx. 2 mg of protein) and 0.1 ml of buffer. When inhibitor was used the 0.1 ml of buffer was replaced by the same volume of inhibitor solution. Assay tubes were shaken in air at 30° and the reaction was started by adding substrate (0.05 ml),