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STABILITY AND *IN VITRO* ABSORPTION OF CAPTOPRIL, ENALAPRIL AND LISINOPRIL ACROSS THE RAT INTESTINE

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Abstract—*In vitro* absorption of three angiotensin converting enzyme (ACE) inhibitors, captopril, enalapril and lisinopril, and their stabilities in aqueous buffer as well as their resistance to intestinal and dermal tissue homogenates were investigated. The results demonstrate that the spontaneous oxidation of captopril, enalapril and lisinopril followed first-order degradation kinetics in McIlvaine's citrate–phosphate buffer. The degradation rates for enalapril and lisinopril were much slower than that for captopril. With the former two ACE inhibitors, the first-order rate constants of breakdown in the presence of dermal homogenate were not significantly different from the control values. Intestinal homogenate increased the decomposition of both of these inhibitors when compared to the enzyme-free control systems. On the other hand, the first-order rates of disappearance of captopril in the presence of both dermal and intestinal homogenates were lower than in the enzyme-free system. The extent of reduction was proportional to the amount of homogenate added. This suggests that tissue homogenates prevent the oxidation of captopril to its disulphide dimer. Transport experiments show that the amounts of ACE inhibitors transferred from solution on the mucosal side increased linearly with incubation time over the 2 hr of study. The rates of transfer from the mucosal side to the serosal side had the following rank order: captopril > enalapril > lisinopril roughly in the ratio 1:1.13:1.27. Addition of harmaline caused a significant reduction in the transfer rate of captopril compared to the control system, which strongly suggests that captopril is transported by a sodium-dependent carrier-mediated process across intestinal tissue.

Key words: stability; ACE inhibitors; carrier-mediated absorption

ACE† is a dipeptidyl depeptidase capable of cleaving the decapeptide angiotensin I to produce the potent, pressor octapeptide angiotensin II [1]. Angiotensin converting enzyme inhibitors have been developed to prevent the *in vivo* generation of angiotensin II and thereby reduce peripheral vasoconstriction.

In this study, we report on the *in vitro* absorption of three ACE inhibitors, captopril, enalapril and lisinopril, and on their stabilities in both the presence and absence of intestinal and dermal tissue homogenates. The skin was investigated because of the possible development of transdermal delivery systems for the ACE inhibitors.

MATERIALS AND METHODS

Materials. Captopril and captopril disulphide were provided by Squibb & Sons (Princeton, NJ, U.S.A.); enalapril maleate by Merck & Co., Inc. (Enfield, U.K.) and lisinopril provided by Imperial Chemical Industries plc (Macclesfield, U.K.). Butyl-*p*-hydroxylbenzoate, ethyl-*p*-hydroxylbenzoate, harmaline and ouabain octahydrate were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Ibuprofen was provided by Gibco Limited (Paisley, U.K.). Acetonitrile for HPLC was purchased from Lab-Scan Ltd (Dublin, Ireland). Orthophosphoric acid (A.R. grade) and perchloric acid (60–62% $HClO_4$) were obtained from BDH Ltd (Poole, U.K.). All chemicals used were of analytical reagent grade or the highest purity available.

Preparation of tissue homogenate. The Wistar rats weighing 250–320 g were allowed free access to water prior to the experiments. Dermal (dorsal) and intestinal (distal ileum) tissues were isolated from the freshly-killed animals and underlying muscles and fat were carefully removed where appropriate. The resulting tissues were used without further dissection of layers.

The tissues were dried using tissue paper, weighed and placed in a ground-glass homogenizing tube with an appropriate volume of ice-cold physiological saline to produce a 10% (w/v) tissue homogenate suspension. Homogenization was done at 10,000 rpm and at 4–8°. The homogenate was centrifuged at 5° for 15 min. The supernatant was stored at −18° until required for analysis. The protein content of the intestinal homogenate was diluted to the same level with that of dermal homogenate determined by the method described by Lowry *et al.* [3].

Preparation of Krebs–Ringer solution. Krebs–

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† Abbreviations: ACE, angiotensin converting enzyme; Acetyl-Pro, acetyl-proline; AUFS, absorbance units full scale; Ca^{2+}-Mg^{2+}-ATPase, calcium magnesium adenosine triphosphatase; Gly-Pro, glycyl-proline; GSH, glutathione; Na^+-K^+-ATPase, sodium potassium adenosine triphosphatase; 2,4-DNP, 2,4-dinitrophenol.

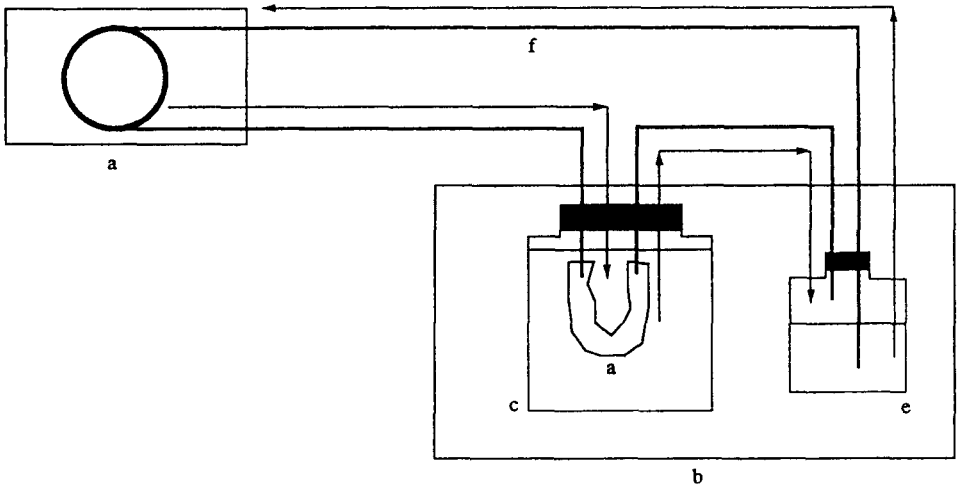

Fig. 1. A line diagram of the device used for continuous absorption studies: a, peristaltic pump; b, water bath; c, drug reservoir; d, everted intestinal tissue; e, sampling reservoir; f, Teflon tubing.

Ringer solution was prepared by the method of Kojima *et al.* [2].

Resistance of captopril, enalapril maleate and lisinopril to intestinal and dermal tissue homogenates and their stabilities in aqueous buffer. One millilitre of supernatant from fresh rat dermal or intestinal homogenate (1 mL) was incubated at 37° with 50 mL of ACE inhibitor solution (1.5 mM for enalapril maleate and lisinopril and 0.4 mM for captopril) in 0.2 M citrate–phosphate buffer with a pH of 7.4 in a shaking water bath. Triplicate aliquots (1000 μL) were withdrawn at various time intervals and added to glass tubes containing 1 mL of acidic internal standard solution.

The stability of the three ACE inhibitors, in the absence of enzymes was monitored at 37° and pH 7.4 using McIlvaine's citrate–phosphate buffer. One millilitre of saline was added to 50 mL of 1.5 mM ACE inhibitor (captopril, enalapril maleate, lisinopril) and the residual concentrations of the three ACE inhibitors were measured.

Continuous flow transport system. Everted segments of ileum, isolated from adult rats, were used in all absorption experiments. Male Wistar rats weighing 250–320 g were starved for 16 hr prior to initiation of the experiments. In each experiment, the distal portion of the ileum, about 13 cm long, was excised from the freshly killed rat and the connecting fat and mesentery were carefully removed. The isolated tissues were blotted dry on tissue paper and weighed. Each segment was everted with a glass rod (250 mm long, 2.5 mm diameter) by pushing the ileal end of the gut into the lumen. Mucosal fat and fluid were washed away with saline. The everted intestines were slipped off the glass rod and placed in Krebs–Ringer solution at room temperature.

A multichannel peristaltic pump was connected to Teflon tubings (1.5 mm outer diameter, 0.8 mm inner diameter) and a segment of everted intestine as shown in Fig. 1. Five millilitres of Krebs–Ringer buffer were added to the inner compartment of the intestinal loop which was then placed in a sealed wide-necked glass jar containing 100 mL of a solution of the appropriate drug in Krebs–Ringer solution. Both the sampling reservoir and the drug reservoir were maintained at 37° in a thermostatic water bath.

The transport rates were determined by recirculation for at least 2 hr after initiation of the experiment, taking care that sink conditions still prevailed.

At predetermined time intervals 500 μL samples were withdrawn from the sampling reservoir and added to glass tubes containing 0.5 mL of acidic internal standard solution, for analysis by HPLC. At the same time, an equal volume of buffer at the same temperature was added to the reservoir to keep the solution volume constant. During the experimental runs the sample ports were covered to prevent any evaporation of the solvent. Appropriate concentration corrections were made for the volume discarded at each sampling point.

Determination of intestinal transfer rate of captopril, enalapril and lisinopril using the continuous flow transport system. The initial concentration of captopril, enalapril maleate or lisinopril used in each case was 1.5 mM in Krebs–Ringer buffer with a pH of 7.0. For captopril, a 100 μL solution was also sampled from the donor reservoir at time intervals to monitor the disappearance of drug and check mass balance.

HPLC analysis. Captopril, enalapril and lisinopril as well as the disulphide captopril dimer were measured by HPLC. The HPLC system consisted of a reciprocating pump (LKB, 2150 HPLC pump, Bromma, Sweden), a 15 cm × 4.6 mm C_{18} column (S5 ODS2, Phase Separation Ltd, U.K.), a 20 μL sample loop with conventional injection valve and a UV variable-wavelength detector (LKB, 2151 variable wavelength monitor, Bromma, Sweden). Data acquisition and integration were performed with an integration software package (Data trans-

lation with integration software, softron, Kontron Instruments, Grafelfing, Germany).

For assaying captopril and its oxidation dimer, chromatography was performed at a wavelength of 210 nm with a sensitivity of 0.16–0.32 AUFS and pre-filtered (0.45 μm) mobile phase consisting of acetonitrile/water/phosphoric acid (30:70:0.1) delivered at a flow rate of 1 mL/min. Ethyl-*p*-hydroxylbenzoate was chosen as an internal standard. The internal standard solution contained 0.163 mmol of ethyl-*p*-hydroxylbenzoate and 0.3 M perchloric acid (an enzyme reaction stopper). The resulting solution with a pH of 0.96 prevented further oxidation of captopril for at least 2 days.

For analysis of enalapril, the eluate was monitored at 220 nm with an AUFS of 0.08–0.16. The pre-filtered mobile phase consisted of acetonitrile/water/phosphoric acid (40:60:2.0) and was delivered at a flow rate of 1 mL/min. Mobile phase containing 0.185 mM of butyl-*p*-hydroxylbenzoate and 0.3 M perchloric acid, was used as the internal standard solution. The pH of the resulting solution was 0.88.

For determination of lisinopril, the mobile phase consisted of acetonitrile/water/phosphoric acid (40:60:1.5) delivered at 1 mL/min. The peak detection was at 220 nm with sensitivity settings ranging from 0.08 to 0.16 AUFS. Ibuprofen (0.032 mg/mL) dissolved in mobile phase solution, pH 0.90, containing 0.3 M perchloric acid, was used as the internal standard.

Inhibition of transport of captopril by harmaline and ouabain. After the intestinal tissues have been incubated in 1.5 mM of captopril in Krebs–Ringer buffer at pH 7.0 for 1 hr, the tissues were removed and placed in a fresh captopril solution (1.44 mM) which also contained 1 mM of harmaline or 1 mM of ouabain. Further samples were taken at intervals from the receiver compartment and analysed by HPLC. The experiment was repeated three times with each inhibitor.

Statistical analysis. Rate constants were calculated by linear regression and differences assessed by Analysis of Variance followed by Neuman–Keul's multiple range test if appropriate [4]. A P value ≤ 0.05 is taken as being significant.

RESULTS

Stability of the ACE inhibitors

With the HPLC assay, captopril (retention time, Rt = 2.66 min) was resolved from its oxidation dimer (Rt = 3.75 min) and ethyl-*p*-hydroxylbenzoate (Rt = 9.35 min). For enalapril assay, the retention times were enalapril (4.40 min), enalapril breakdown product (2.25 min) and butyl-*p*-hydroxybenzoate, the internal standard (3.60 min). For lisinopril, the retention times were lisinopril (3.50 min), lisinopril breakdown product (2.25 min) and ibuprofen (4.50 min).

The uncatalysed oxidation of captopril followed first-order degradation kinetics in the buffered solution. The disulphide oxidation product was the only detectable decomposition product indicating that the amide linkage of captopril was stable in the pH 7.4 buffer at 37° over the study period.

Enalapril and lisinopril also showed first-order degradation kinetics in a citric acid–phosphate buffer at pH 7.4 and 37° (Fig. 2). The degradation rate for lisinopril was smaller than that of enalapril which in turn degraded much more slowly than captopril (Table 1).

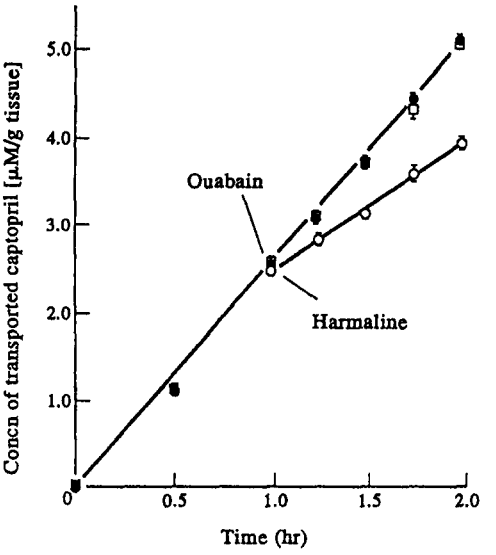

Fig. 2. Effect of inhibitors on captopril transport across the rat intestine wall. Each point was mean ± SD of three experiments with two injections. □, control*; ◆, ouabain*; ◇, harmaline*. Star means the total amount of captopril and its disulphide.

Resistance of three ACE inhibitors to tissue enzymes

Lisinopril and enalapril both showed first-order decomposition in the presence of both dermal and intestinal tissue homogenates but not significantly different from the control values. Intestinal homogenate, on the other hand, increased the decomposition of both of those inhibitors when compared to the enzyme-free control systems (Table 1).

The captopril data is particularly interesting in that the first-order rates of disappearance in the presence of both dermal and intestinal homogenates were actually lower than in the enzyme-free system and the decrease in rate became significantly more pronounced with increasing amount of tissue homogenate (Table 1).

Kinetics of transfer of captopril, enalapril and lisinopril across the everted rat intestine

The amounts of ACE inhibitors transferred from donor mucosal solution increased linearly with incubation time over the 2 hr of study. Table 2 lists the apparent zero-order transfer rate constants for captopril, enalapril and lisinopril across the everted tissues. The rates of transfer from the mucosal side to the serosal side was found to have the following rank order: captopril > enalapril > lisinopril roughly in the ratio 1:1.13:1.27. The rate constants were normalised per unit weight on the assumption that weight and surface area are linearly related.

Table 1. A comparison of the rate constants for oxidation of captopril in homogenate-free McIlvaine's citrate–phosphate buffer and in the buffer containing tissue homogenates (pH 7.4 and 37°) (N = 6)

Medium	Rate constant (hr^{-1}) Captopril	Enalapril	Lisinopril
Buffer	37.243×10^{-3}	2.822×10^{-3}	1.013×10^{-3}
Intestinal homogenate (1 mL)	29.929×10^{-3}	3.333×10^{-3}	1.555×10^{-3}
Intestinal homogenate (2 mL)	22.437×10^{-3}		
Dermal homogenate (1 mL)	27.276×10^{-3}	2.916×10^{-3}	1.145×10^{-3}
Dermal homogenate (2 mL)	19.993×10^{-3}		

Table 2. The rate constants for transport of captopril, enalapril and lisinopril from mucosal to serosal compartments in Krebs–Ringer solution at pH 7.4 and at 37°

ACE inhibitor	Rate constant (μM/g tissue/hr)	SD
Captopril	2.45	0.01 (N = 6)
+Ouabain	2.45	0.05 (N = 3)
+Harmaline	1.51	0.09 (N = 3)
Enalapril	2.17	0.08 (N = 6)
Lisinopril	1.92	0.09 (N = 6)

Mechanisms of absorption of ACE inhibitors

To investigate further the absorption of ACE inhibitors, the possibility of carrier-mediated absorption of captopril was studied. In the present study, ouabain and harmaline were utilized to identify possible carrier-mediated transport of captopril.

As Fig. 2 shows, addition of harmaline caused a significant reduction in the transfer rate of captopril compared to the control system. Ouabain, on the other hand, produced no detectable change. Both ouabain and harmaline were added to the captopril-containing solution after a 1 hr incubation. Table 2 lists the rate constants for captopril transfer in all three systems.

DISCUSSION

Our observations on the breakdown of the ACE inhibitors are consistent with the results reported earlier by Timmins *et al.* [5], Lee and Notari [6] and Ip and Brenner [7].

Lisinopril is much more stable in the buffer solution than captopril and enalapril (Table 1). This is not surprising since lisinopril, unlike enalapril, is not an ester prodrug and is indeed excreted entirely unchanged in the urine [8].

Larmour *et al.* [9] reported significant conversion of enalapril to enalaprilic acid (enalaprilat) by human liver homogenates but relatively little conversion by lung, spleen, heart, brain or kidney homogenates and serum. Ulm *et al.* [8], on the other hand, reported that 94% of an orally administered dose of enalapril maleate was recovered in urine and faeces with a bioavailability, based on urinary recovery of 61%. The enalaprilic acid to enalapril concentration ratio in urine was 0.70. Enalapril activation to enalaprilat by human enzymes is therefore by no means complete *in vivo*. This is consistent with the modest increased rate of hydrolysis found in the presence of rat intestinal enzymes in the present study. The data would suggest that absorption rates through the intestinal tract is fast enough to ensure that enzymic hydrolysis is incomplete.

The bioavailability of lisinopril is of the order of 26–27% [8, 10, 11]. Using a radioimmunoassay, Ulm *et al.* [8] reported essentially quantitative recovery when urinary and faecal lisinopril were pooled. This is different from our data using rat enzymes. Significant decomposition was found with intestinal enzymes. With the slow achievement of maximum plasma concentration *in vivo* (6–8 hr), one would have expected significant lisinopril metabolism in humans [10, 11]. That this was not so, would suggest possible differences in selectivity of rat and human enzymes thereby making prediction in humans, from animal data, difficult. The differences could, of course, also be attributed to difficulties in scaling [12].

In whole blood or plasma of rats, dogs and humans captopril is primarily oxidized to its dimer or to form disulphide bonds with other substances such as glutathione, cysteine, peptides and proteins [13, 14]. Following administration of [^{14}C]captopril to mildly hypertensive patients, 46% unchanged captopril, 45% captopril-L-cysteine mixed disulphide and a small amount of captopril dimer (about 6%) were observed in urine of man [15, 16]. In the present study, intestinal or dermal homogenate leads to the formation of only the disulphide oxidation dimer.

The results would indicate that the susceptibility of captopril to auto-oxidation to disulphides is similar to exogenous thiols such as pencillamine and endogenous thiols such as cysteine and glutathione. In biological systems, disulphides can be reduced to thiols either by thiol disulphide interchange or by reduction, catalysed by a reductase requiring NADH or NADPH [17]. *In vitro* studies have shown that thiols such as glutathione and cysteine release protein-bound captopril by displacement with formation of glutathione and cysteine mixed disulphides with captopril [18]. Dithiothreitol, on the other hand, releases protein-bound captopril by reduction of disulphide bonds and the subsequent liberation of captopril [18]. Other reports indicate

that the mechanism of this reduction involved both enzymic and nonenzymatic processes [19, 20]. Intravenous administration of cysteine-captopril disulphide to spontaneously hypertensive rats and beagle dogs [21], or oral administration of captopril disulphide dimer to rats [22] leads to extensive conversion to captopril and consequent inhibition of ACE. Those authors concluded that captopril disulphide conjugates may act as prodrugs. More recent data shows that, at pH 7.4, the initial rate of reduction and the equilibrium constant for reduction of captopril disulphide are, respectively, 267 times larger and 875 times larger than those for penicillamine (a thiol drug) [23].

Intestinal (small and large intestines) homogenates have been reported to be less effective in converting captopril disulphide to captopril, compared with liver and lung tissue preparations [19]. In the present study, intestinal homogenates were also less active in preventing oxidation of captopril to its dimer than dermal homogenates. This is probably because intestinal tissues are maintained to a lesser extent in the reduced form (GSH, NADH and NADPH) intracellularly than dermal, hepatic and lung tissues.

Two widely-used probes, ouabain and harmaline [27–34], were used to investigate the mechanisms of absorption of the ACE inhibitors. Ouabain is a potent inhibitor of active cation transport and Na^+-K^+-ATPase [24] and inhibits binding of ATP and dephosphorylation of the phospho-intermediate by reacting with Na^+-K^+-ATPase on the outer membrane. It does not inhibit other ATPases such as Ca^{2+}-Mg^{2+}-ATPase, anion-sensitive ATPase and K^+-H^+-ATPase [25]. Harmaline interacts with the sodium-binding site of Na^+-K^+-ATPase and blocks the formation of the phosphorylated intermediate by competition with sodium ions in this enzyme [26].

Inhibition of captopril transfer by harmaline was demonstrated in this study. This strongly suggests that captopril is transported by a sodium-dependent carrier-mediated process across intestinal tissue. Ouabain, on the other hand, failed to block the transport of captopril probably due to its weak effect on Na^+-K^+-ATPase in the membrane, compared to harmaline [26, 28]. The difference in inhibitory behaviour of the two compounds may be due to the fact that harmaline inhibits intestinal active transport in two ways: irreversibly binding Na^+-K^+-ATPase, a mechanism shared by ouabain; and reversibly binding Na^+-binding sites competitively, a mechanism which is not shared by ouabain [28].

The intestinal carrier-mediated mode of captopril absorption identified in this study is in agreement with a recent finding by Hu *et al.* [34]. In their studies, captopril was found to compete with its analogues, Gly-Pro and Acetyl-Pro, for absorption using a single-pass perfusion method. Moreover, the metabolic inhibitor 2,4-DNP was found to significantly decrease the absorption of captopril suggesting that captopril absorption requires energy.

REFERENCES

1. Soffer RL, Angiotensin-converting enzyme and the regulation of vasoactive peptides. *Annu Rev Biochem* **45**: 73–94, 1976.
2. Kojima S and Miyake J-I, Factors influencing absorption and excretion of drugs IV. Effect of hypertonic and hypotonic solutions on *in situ* rat intestinal absorption of several drugs. *Chem Pharm Bull* **23**: 1247–1255, 1975.
3. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
4. Elliott AC, Kwikstat condensed version of manual *Bare Bones Edition*. Mission Technologies, PA, version 1.0, 1986.
5. Timmins P, Jackson IM and Wang Y-CJ, Factors affecting captopril stability in aqueous solution. *Int J Pharm* **11**: 329–336, 1982.
6. Lee T-Y and Notari RE, Kinetics and mechanism of captopril oxidation in aqueous solution under controlled oxygen partial pressure. *Pharm Res* **4**: 98–103, 1987.
7. Ip DP and Brenner GS, Enalapril maleate. *Anal Profiles Drug Substances* **16**: 207–243, 1987.
8. Ulm EH, Hichens M, Gomez HJ, Till AE, Hand E, Vassil TC, Biollaz J, Brunner HR and Schelling JL, Enalapril maleate and a lysine analogue (MK-521) disposition in man. *Br J Clin Pharmacol* **14**: 357–362, 1982.
9. Larmour I, Jackson B, Cubela R and Johnston CI, Enalapril (MK421) activation in man: importance of liver status. *Br J Clin Pharmacol* **19**: 701–704, 1985.
10. Mojaverin P, Rocci ML, Vlasses PH, Hoholick C, Clementi A and Ferguson RK, Effect of food on the bioavailability of lisinopril, a nonsulfhydryl angiotensin converting enzyme inhibitor. *J Pharm Sci* **75**: 395–397, 1986.
11. Beermann B, Pharmacokinetics of lisinopril. *Am J Med* **85**: 25–30, 1988.
12. Boxeubaum R and D'Souza RW, Interspecies pharmacokinetic scaling, biological design and neoteny. In: *Advances in Drug Research*, Vol. 19. (Ed. Testy B), pp. 139–191. Academic Press, New York, 1990.
13. Keith K, Wong S-J and Bruce HM, *In vitro* biotransformations of [^{14}C]captopril in the blood of rats, dogs and humans. *Biochem Pharmacol* **30**: 2643–2650, 1981.
14. Kadin H, Captopril. *Anal Profiles Drug Substances* **11**: 80–137, 1982.
15. Kripalani KJ, Meeker Jr. FS, Dean AV, Mckinstry DN and Migdalof BH, Biotransformation of ^{14}C-captopril in hypertensive patients and normal subjects. *Fed Proc* **39**: 307–313, 1980.
16. McKinstry DN, Kripalani KJ, Migdalof BH and Willard DA, The effect of repeated administration of captopril (CP) on its disposition in hypertensive patients. *Clin Pharmacol Ther* **27**: 270–271, 1980.
17. Jocelyn PC, *Biochemistry of the SH Group*, p. 121. Academic Press, London, 1972.
18. Wong KK, Lan S-J and Migdalof BH, *In vitro* biotransformations of [^{14}C]captopril in the blood of rats, dogs and humans. *Biochem Pharmacol* **30**: 2643–2650, 1981.
19. Lan S-J, Weinstein SH and Migdalof BH, Biotransformation of the disulfide of captopril in subcellular fractions of rat tissue. *Drug Metab Dispos* **10**: 306–311, 1982.
20. Yeung JHK, Breckenridge AM and Park BK, Drug protein conjugates-VI. Role of glutathione in the metabolism of captopril and captopril plasma protein conjugates. *Biochem Pharmacol* **32**: 3619–3625, 1983.
21. Kripalani KJ, Dean AV and Migdalof BH, Metabolism of captopril-cysteine, a captopril metabolite, in rats and dogs. *Xenobiotica* **13**: 701–705, 1983.
22. Drummer O and Jarrot B, Captopril disulfide conjugates may act as prodrug: disposition of the disulfide dimer of captopril in the rat. *Biochem Pharmacol* **33**: 3567–3571, 1984.
23. Drummer O, Routley L and Christophidis N,

Reversibility of disulfide formation, comparison of chemical and enzyme-mediated reduction of penicillamine and captopril disulfides. *Biochem Pharmacol* **36**: 1197–1201, 1987.
24. Skou JC, Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol Rev* **45**: 596–617, 1965.
25. Stekhoveu FS and Bonting SL, Transport adenosine triphosphatases: Properties and functions. *Physiol Rev* **61**: 1–76, 1981.
26. Canessa M, Jaimovich E and Fuente M de la, Harmaline: A competitive inhibitor of Na ion in the Na^+-K^+ ATPase system. *J Memb Biol* **13**: 263–282, 1973.
27. Sepulveda FV and Robinson JWL, Harmaline, a potent inhibitor of sodium-dependent transport. *Biochim Biophys Acta* **373**: 527–531, 1974.
28. Patil SD, Prakash K and Hegde SN, Differential effects of harmaline and ouabain on intestinal glucose transport in the pigeon. *Arch Int Physiol Biochim* **94**: 57–64, 1986.
29. Ganapathy V and Radhakrishnan AN, Sodium-dependent inhibition of amino acid and dipeptide transport by harmaline in monkey small intestine. *Biochem Pharmacol* **29**: 713–716, 1980.
30. Ganapathy V, Mendicino JF and Leibach FH, Transport of glycyl-L-proline into intestinal and renal brush border vesicles from rabbit. *J Biol Chem* **256**: 118–124, 1981.
31. Ganapathy V and Leibach FH, Is intestinal peptide transport energized by a proton gradient? *Am J Physiol* **249**: G153–G160, 1985.
32. Cheeseman CI and Johnston G, Glycyl-L-leucine transport in the rat small intestine. *Can J Physiol Pharmacol* **60**: 1177–1184, 1982.
33. Ganapathy V and Leibach FH, Carrier-mediated reabsorption of small peptides in renal proximal tubule. *Am J Physiol* **251**: F945–953, 1986.
34. Hu M and Amidon GL, Passive and carrier-mediated intestinal absorption components of captopril. *J Pharmacol Sci* **77**: 1007–1011, 1988.