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## ACKNOWLEDGMENTS

Part of this investigation was presented at the 3rd International Conference on Pharmaceutical Technology, Paris, 1983.

# Gastric and Intestinal Absorption of Captopril in Acutely and Chronically Treated Rats: Comparison with Salicylic Acid

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Received December 12, 1983, from the *University of Melbourne, Department of Medicine, Clinical Pharmacology and Therapeutics Unit, Austin Hospital, Heidelberg, Victoria, 3084, Australia.* Accepted for publication April 4, 1984.

**Abstract** □ The gastric and intestinal absorption of captopril, an orally active angiotensin-converting enzyme inhibitor was determined using rat *in situ* gastric pouch and intestinal loop techniques and compared with the absorption of another acidic drug, salicylic acid, whose absorption has been well established from both gastric and intestinal sites. Captopril absorption was determined at two initial intraluminal concentrations in acute (untreated) rats and in rats that had been chronically treated with captopril. Salicylic acid absorption was determined at one concentration in acute rats. During the 40-min experimental period, captopril absorption at the 4.6 mM dose from the gastric pouch was 17.0 ± 1.8% and 17.9 ± 5.4% in acute and chronically treated rats, respectively, and 33.6 ± 9.2% and 23.7 ± 7.6%, respectively, from the intestinal loop. At the 11.5 mM dose the captopril absorption in 40 min was 13.7 ± 2.7% and 17.3 ± 4.2% from the gastric pouch of acutely and chronically treated rats, respectively, and 17.8 ± 4.2% and 22.9 ± 3.3%, respectively, from the intestinal loop. As similar fractions of the different administered doses were absorbed from the respective gastric and intestinal sites in both acutely and chronically treated rats, the absorption process of captopril appears to be principally by passive diffusion and unaffected by chronic administration of captopril. In comparison, salicylic acid was absorbed more rapidly and to a greater extent from both the gastric and intestinal preparations. The percent of salicylic acid absorbed into the plasma at the 11.5 mM dose was 44.8 ± 4.4% and 65.3 ± 5.3% from the gastric and intestinal preparations, respectively. It is concluded that gastric absorption of captopril does occur, but its relative importance to intestinal absorption is dependent on the transit time from the gastric to intestinal site.

**Keyphrases** □ Captopril—*in situ* absorption, comparison with salicylic acid, rats □ Salicylic acid—*in situ* absorption, comparison with captopril, rats

Captopril, 1-[(2*S*)-3-mercapto-2-methylpropionyl]-pyrrolidine-2-carboxylic acid is the first orally effective, antihypertensive drug in a new class of angiotensin-converting enzyme inhibitors. This drug contains a reactive thiol group postulated to be necessary for its binding to the $Zn^{2+}$ of the angiotensin-converting enzyme (1). However, this functional group also reacts to form low molecular weight disulfide conjugates of captopril in tissues such as liver and kidney (2, 3). Captopril also forms covalent links with plasma proteins *via* disulfide linkages with thiol-containing residues (4) and this may explain the extensive tissue binding of captopril (3). Possible significant alterations in the pharmacokinetics of captopril, particularly following chronic treatment, could result from tissue uptake saturation and binding processes.

Several studies on the disposition of captopril in humans and laboratory animals (3, 5–8) have found an early appearance of captopril in the plasma following oral administration with peak drug levels usually occurring within 1 h. As captopril is a structural derivative of the amino acid proline, there exists the possibility that it could be a substrate for an amino acid transport process that rapidly transports captopril across the gut wall. Additionally, the acidic nature of the drug would, according to the pH partition hypothesis (9), allow passive absorption from the highly acidic gastric site prior to entry into the intestinal absorptive sites. Recently, a clinical study with captopril observed much higher blood levels of the drug following chronic treatment than acute captopril administration, suggesting an increase in the rate and extent of absorption and bioavailability (5). However, no studies to date have directly examined whether changes in the absorption rate occur during chronic therapy.

The definition of the sites and processes involved in the absorption of captopril into the systemic circulation has an important place in the description of the disposition of captopril. In this study, the recently described *in situ* gastric pouch and *in situ* intestinal loop preparations (10, 11) have been utilized to investigate captopril absorption at these sites using acute (untreated) rats and rats chronically treated with captopril over 40 d. These techniques offer the advantage of allowing direct determination of drug traversing the gut wall and also enable the quantitation of any metabolites that may be produced that contribute to a "first pass" effect from either the gastric or intestinal sites. For comparison, we have included in the study the acidic drug salicylic acid, which is known to be readily absorbed from both sites (12).

## EXPERIMENTAL SECTION

**Materials**—Captopril[1], captopril disulfide dimer[1], and salicylic acid[2] were used in the experiments. For the measurement of captopril using a gas chromatographic-mass spectrometric (GC–MS) assay, 3-(3-mercapto-2-methyl-propionyl)-4-thiazolidine carboxylic acid[3] (YS-980) was used as an

[1] E. R. Squibb & Sons Pty. Ltd., N.J.
[2] Pharmacopoeial purity.
[3] Courtesy of Dr. J. Iwao, Santen Pharmaceutical, Osaka, Japan.

0022-3549/84/1200-1755$01.00/0

internal standard (3). Other chemicals required for the GC–MS assay were obtained as described previously (3) and all other chemicals were analytical reagent grade.

***In Situ* Gut Preparations**—Male Wistar–Kyoto rats (215–300 g) that had access to food and water *ad libitum* were used for both gastric and intestinal absorption experiments. The gastric pouches were prepared as previously described (10). Briefly, following anesthetizing the animal with pentobarbital sodium (50 mg/kg ip), a jugular vein cannula was implanted for administering heparin (3000 IU/kg) and for whole blood replacement. A cannula for the collection of systemic blood samples was placed into the carotid artery. The esophagus, pylorus, pyloric blood vessels, and the branches of the gastroepiploic blood vessels were ligated. Gastric contents were removed *via* a small incision made in the rat forestomach and the pouch was rinsed clean with saline. Remaining fluid was removed with an absorbent tissue. After transposition of the stomach, and exposing the left gastric vein, a 21-gauge needle attached to a 15-cm length of polyethylene tubing (0.75-mm i.d., 1.45-mm o.d.) was inserted into the vein to allow collection of the venous blood draining the pouch. The drug was then administered *via* a syringe tied into the incision made earlier in the forestomach.

A slight modification of the method previously described by Worland and Ilett (11) was used for preparing the *in situ* intestinal loops. Slightly larger sections (length, 8–10 cm) of jejunum were selected and the same size needle (21 gauge) and cannula length (15 cm) employed for the gastric pouches were used to cannulate the vein leading away from the *in situ* loop. Blood samples were collected from the *in situ* gut preparation over 4-min intervals for a total of 40 min for all experiments.

To maintain blood flow through the *in situ* gut preparations, heparinized whole blood replacement was given *via* the jugular vein at 0.7 mL/min using a peristaltic pump[4]. The blood collected immediately prior to the experiment from donor rats was continually mixed during the replacement by a gently oscillating reservoir.

The stomach and intestine were able to adjust the pH of the administered drug in saline solutions to the physiological relevant values (~1.0 and 6.3, respectively) within a 5-min period.

Captopril was prepared at concentrations of 4.6 or 11.5 mM in normal saline (0.9% NaCl) within 2 min of being administered in a 0.5-mL volume to the isolated gut preparations. These concentrations of captopril were chosen as they approximate doses of 2 mg/kg and 5 mg/kg which are known to be pharmacologically active when given orally to rats (1). Salicylic acid was given in a 0.5-mL volume in normal saline only at a concentration of 11.5 mM.

**Rat Treatment**—Rats were treated with 1 mg/kg of captopril by gavage twice daily for 40 d prior to the absorption experiment; the last dose was given 14 h prior to the experiment. The male Wistar–Kyoto rats used in the study were 250–300 g at the time of the experiment.

**Determination of Captopril and Salicylic Acid Levels**—For the assay of free captopril using GC–MS, an aliquot of the blood sample (200 μL) was taken immediately after sample collection and added to glass extraction tubes containing 2 mg of *N*-ethylmaleimide (20 mg/mL solution in water). Internal standard (4 μg of 1-mg/mL solution in acetone) was included. The determination of total captopril (free captopril and disulfide conjugates) was obtained following reduction of disulfide bonds using the reducing agent dithiothreitol (3). Samples were also assayed for the *S*-methyl metabolite of captopril (3, 13). At the completion of the experiment, the gut tissue used for either the gastric pouch or the intestinal loop was rinsed with saline, blotted, and then finely minced in 5 volumes of phosphate buffer (0.05 M, pH 7.4). It was then homogenized with a homogenizer[5] for 45 s and an aliquot was taken to assay total captopril. Salicylic acid levels were determined from plasma collected from the *in situ* gut preparations using HPLC as described (10).

**Data Analysis**—The BMDP statistical software package (14) was used for the analysis of variance (ANOVA, one- and two-way). A significant difference was assumed at the $p < 0.05$ level in all statistical tests. The results are expressed as the mean ± *SEM* of four experiments.

## RESULTS AND DISCUSSION

Captopril has previously been observed to be rapidly absorbed into the systemic circulation following oral administration (3, 5–8), and the gastric absorption of captopril, an acidic drug ($pK_a = 3.7$)[6] could be expected to contribute to its early appearance in systemic blood samples. In addition to passive absorption of captopril from either the gastric or intestinal regions, there is the possibility of an active absorption process in the small intestine principally intended for amino acids, but capable of transporting captopril as it is a structural derivative of the amino acid proline. The experiments reported herein have utilized *in situ* gastric pouch and intestinal loop preparations to directly determine the absorption of captopril from both the gastric and intestinal sites as represented by these *in situ* gut preparations. Although these preparations can only approximate the real situation, as the animal is anesthetized and the absorption site is necessarily restricted to either the stomach or a section of jejunum, they do allow a direct comparative measurement of drug absorption and drug metabolism across the gut wall from the respective regions.

**Table I—Percent of Administered Captopril Dose in Effluent Venous Blood from *In Situ* Gut Preparations**[a]

| Initial Intraluminal Concentration, mM | Gastric Pouch | | Intestinal Loop | |
|---|---|---|---|---|
| | Total Captopril | Disulfide Conjugates | Total Captopril | Disulfide Conjugates |
| 4.6 | | | | |
| Acute | 17.0 ± 1.8 | 5.4 ± 2.7 | 33.6 ± 9.2 | 3.1 ± 1.8 |
| Chronic | 17.9 ± 5.4 | 1.7 ± 1.4 | 23.7 ± 7.6 | 0.4 ± 0.2 |
| 11.5 | | | | |
| Acute | 13.7 ± 2.7 | 1.5 ± 1.0 | 17.8 ± 4.2 | 1.9 ± 1.4 |
| Chronic | 17.3 ± 4.2 | 6.7 ± 4.9 | 22.9 ± 3.3 | 1.1 ± 1.0 |

[a] Taken over 40 min. Results presented as mean percent ± *SEM* ($n = 4$).

The percentages of captopril doses absorbed into the blood draining the *in situ* gut preparations are shown in Table I. No significant differences between the acutely and chronically treated rats were observed in either the gastric or intestinal preparations at either initial intraluminal concentrations of 4.6 or 11.5 mM. Also, the extent of disulfide conjugates appearing as metabolites from the gastric or intestinal sites at either initial concentration of captopril were not different. Captopril (detection limit, 1 ng) was not detected in systemic blood samples obtained at the completion of the experiment (40 min) indicating that the collected blood from the *in situ* gut preparation contained only absorbed drug and its metabolite produced during the absorption from the tissue or within the blood draining the tissue. A similar percentage of administered dose, to either the gastric or the intestinal preparation, was absorbed at the two concentrations; this suggests that the predominant absorption mechanism is a passive process at these doses. The significant absorption from the gastric preparation suggests that gastric absorption contributes to the early appearance of captopril in the plasma observed after oral administration of the drug (3, 5–8).

The extent of disulfide formation was small and variable. Earlier work on the biotransformation of captopril in blood by Wong *et al.* (15) has shown that, following the addition of captopril to whole rat blood and immediately attempting to stabilize the free drug with *N*-ethylmaleimide, ~17% of added captopril was covalently bound to whole blood components. Similar results were obtained by Park *et al.* (4). In our experiments the sample collection period from the gastric pouch and intestinal loop was 4 min and it would seem plausible that the extent of disulfide formation observed has occurred entirely

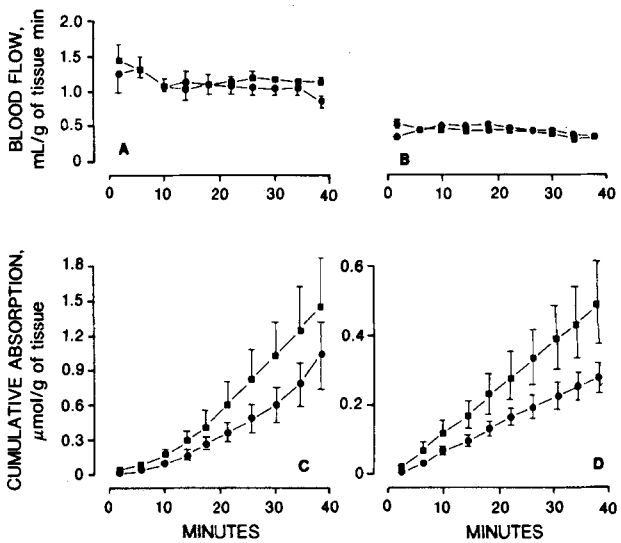


**Figure 1**—*Blood flow from* in situ *gut sections of rats receiving a single dose of captopril and the cumulative absorption of captopril at 4.6 mM (●) and 11.5 mM (■) initial intraluminal concentration for the intestinal (A, C) and gastric regions (B, D). Results are mean* ± SEM (n = 4)*; error bars are not shown if less than symbol size.*

[4] Manostat, N.Y.
[5] Polytron PT-10; Kinematica, Lucerne, Switzerland.
[6] E. R. Squibb & Sons Pty. Ltd. representative, personal communication.

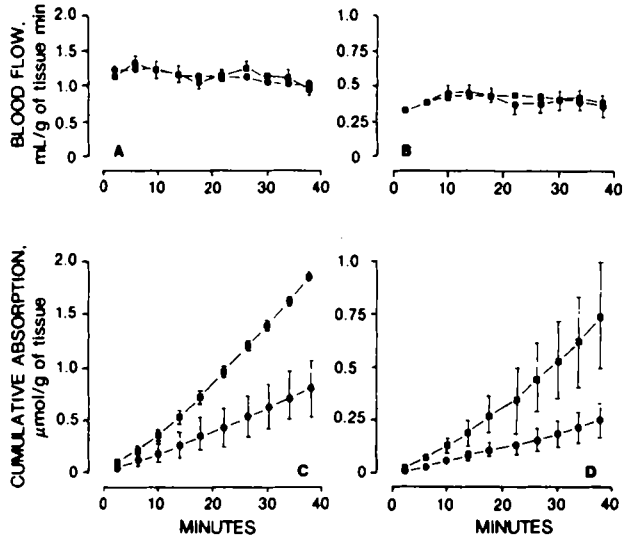


**Figure 2**—*Blood flow from* in situ *gut sections of rats chronically treated with captopril and the cumulative absorption of captopril at 4.6 mM (●) and 11.5 mM (■) initial intraluminal concentrations for the intestinal (A, C) and the gastric regions (B, D). Results are mean ± SEM (n = 4); error bars are not shown if less than symbol size.*

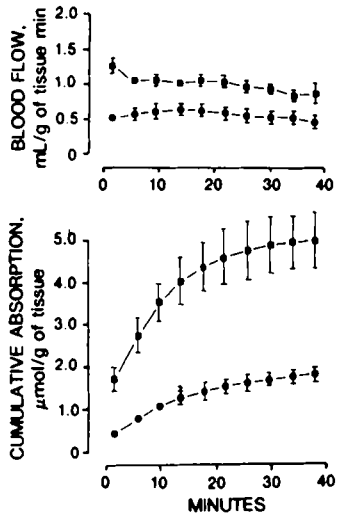


**Figure 3**—*Blood flow from the* in situ *gastric (●) and intestinal (■) preparations and the cumulative absorption of salicylic acid at 11.5 mM initial intraluminal concentration into the plasma. Results are mean ± SEM (n = 4); error bars are not shown if less than symbol size.*

within the blood and not the gut wall. The *S*-methyl metabolite of captopril was not detected in the blood draining from either the gastric or intestinal preparation. This metabolite has been detected in human and animal studies following oral administration of captopril (3, 13, 16), but does not appear to be produced from either the gastric or intestinal site during the absorption process.

Blood flow through the intestinal tissue has been demonstrated to be an important factor in the absorption of drugs (17-20) particularly when movement of the drug across lipoidal barriers is not the rate-limiting step. In order to eliminate possible changes in the absorption rate that may have resulted from variable blood flow, replacement blood was delivered at a constant rate throughout the experiment. The blood flow rates from the gastric pouch and intestinal loop preparations that had been given captopril are shown in the upper panels of Figs. 1 and 2. The blood flow rates did not vary significantly during the experiments and were not affected by either the dose of captopril or the chronic treatment with captopril. On the standardized basis of blood flow per gram of wet weight tissue, the blood flow from intestinal loops was two- to threefold greater than that of the gastric pouches. The weights of the intestinal segments used for the captopril (and salicylic acid) experiments were not significantly different between groups (overall mean weight, 0.74 g). Similarly, the weights of the gastric pouches (overall mean weight 1.59 g for the captopril and salicylic acid experiments) were not significantly different between groups as tested by an ANOVA (one-way).

The lower panels of Figs. 1 and 2 show the cumulative absorption of captopril during the experiments using the acutely and chronically treated rats at the two doses. The graphs are plotted on a standardized basis of per unit wet tissue weight to show differences in the capacity for absorption between the gastric and intestinal tissue. On this standardized basis, the intestinal tissue absorbed a significantly greater amount of captopril than the gastric tissue (ANOVA, two-way, $p < 0.001$) and the magnitude of the difference in the amount of captopril absorbed was similar to the difference observed in blood flow from the two sites. The back extrapolation of the terminal phase of the intestinal absorption plots from the acutely treated rats suggests a lag phase for absorption in the early period of the experiment. This phase was not as prominent in the corresponding gastric absorption and intestinal absorption plots obtained from the chronically treated rats.

**Table II—Gut Tissue Concentrations of Total Captopril** [a]

| Initial Intraluminal Concentration, mM | Gastric Pouch | Intestinal Loop |
|---|---|---|
| 4.6 | | |
| Acute | 381.3 ± 78.0 (24.5%)[b] | 1515.6 ± 199.6 (49.3%) |
| Chronic | 278.5 ± 46.8 (23.3%) | 1390.0 ± 278.6 (42.7%) |
| 11.5 | | |
| Acute | 389.2 ± 78.0 (10.9%) | 1192.8 ± 238.4 (15.0%) |
| Chronic | 597.0 ± 194.5 (16.7%) | 1374.6 ± 382.4 (17.2%) |

[a] In nmol/g of tissue wet weight, determined at the completion of the absorption experiments. Results presented as mean ± *SEM* ($n = 4$). [b] Values in parentheses represent the amount in the tissue as a percentage of the dose administered.

Gut tissue concentrations of total captopril were determined at the completion of the experiment. The total captopril concentrations in the gut walls from either the gastric or the intestinal tissue were not different after different doses. This suggests a saturation of tissue uptake of captopril and is perhaps more clearly shown as a percentage of administered dose. At the higher administered dose a lower percentage of the dose is found within the gut wall (Table II).

The results of this study demonstrate that in rats the rate of captopril absorption from both the gastric and intestinal sites is unchanged after chronic captopril treatment, supporting a previous report that indicated pretreatment of mice for a minimum of 14 d did not affect the extent of absorption or bioavailability of captopril as assessed by urinary excretion (21). However, these rodent studies do not conform with a report of captopril kinetics in hypertensive patients (5) in which greater plasma levels of the drug were achieved after chronic usage, suggesting a possible increase in the bioavailability of captopril.

As captopril is an acidic drug, it was of interest to compare the absorption characteristics as determined by these *in situ* gut preparations with salicylic acid, a drug whose absorption has been well studied (12, 17). The cumulative absorption of salicylic acid on the standardized basis of unit weight from the gastric and intestinal *in situ* preparations is shown in the lower panel of Fig. 3 with the respective blood flows shown in the upper panel. Significant, but small differences in blood flow were found by an ANOVA (two-way) when comparing blood flow from the intestinal loop after receiving either captopril at the two doses (acutely and chronically treated rats) or salicylic acid at the 11.5-mM dose. This was also true for blood flow from the gastric pouch. Blood flow through the intestine was less following salicylic acid administration relative to captopril ($p < 0.001$) but greater from the gastric pouch after salicylic acid than captopril ($p < 0.0001$). During the 40-min experimental period, 44.7 ± 4.4% and 65.3 ± 5.0% of the salicylic acid added to the lumen was absorbed into the plasma draining through the *in situ* stomach and intestinal preparations, respectively. The majority of this was absorbed in the first 12 min of the experiment and a lag phase for salicylic acid absorption was not evident from the absorption profiles with the collection times shown in Fig. 3. The salicylic acid absorption profiles differ markedly from those observed with captopril. This difference resulted from a greater absorption rate for salicylic acid from the gastric and intestinal loop. Twelve minutes after administration of salicylic acid ~25 and ~46% of the administered dose to the gastric and intestinal sites, respectively, had been absorbed into the plasma. In comparison, captopril absorption was only 3.3 and 2.3% of the same molar dose from the *in situ* gastric and intestinal loop preparations in 12 min. Thus for salicylic acid, the large changes in the amounts and the concentration within the gut lumen would result in a decrease in the driving force for absorption across the gut wall. For captopril, although the absorption process at the doses given suggests passive absorption, the absorption rate was not observed to decrease during the experiment. This can be expected as the intraluminal captopril concentrations would not be altering as rapidly nor to the same extent as salicylic acid within the time of the experiment.

In summary, our results have shown that the gastric absorption of captopril would contribute to the early appearance of captopril in the blood, but the intestine has a greater capacity than the stomach for the absorption of captopril and the relative magnitude of captopril absorption from the gastric site will depend on the transit rate through the stomach. Furthermore, it has been shown that chronic treatment of captopril in the rat does not increase the rate of captopril absorption from either the stomach or intestine. In comparison to salicylic acid, captopril is not as rapidly absorbed from either the stomach or intestine.

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## ACKNOWLEDGMENTS


The authors thank the National Health and Medical Research Council of Australia for their financial assistance.


# Pharmacokinetics of Probucol in Male Rats


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Received July 15, 1983, from the *Department of Pharmacokinetics and Drug Metabolism*, and the *Department of Bioanalytical Chemistry, Merrell Dow Research Institute-Indianapolis Center, Indianapolis, IN 46268-0470.* Accepted for publication April 5, 1984.



**Abstract** □ The bioavailability and pharmacokinetic behavior of 10 mg/kg of [$^{14}$C]probucol in an oil-water emulsion was determined after oral and intravenous administration to rats. The bioavailability of the oral formulation was ~6%. For the first 12-h interval after the intravenous bolus, plasma probucol concentrations increased after an initial decrease. This effect may be attributed to the formulation or precipitation of the drug in the vasculature. The terminal plasma half-life was 6 d. By 7 d, 45 and 4.65% of the labeled intravenous bolus was excreted in the feces and in the urine, respectively. Although most of the labeled dose was excreted in the bile, any enterohepatic recirculation that did occur did not contribute to the atypical plasma concentration *versus* time profile. The tissue distribution of the label and elimination rates in the bile differed between the two routes of administration. Either the total body burden, precipitation of the drug, or the emulsion vehicle may be responsible for the nonlinear distribution and clearance of the intravenous dose.



**Keyphrases** □ Bioavailability—oil-in-water emulsion, tissue distribution, probucol, biliary elimination, rats □ Probucol—bioavailability, oil-in-water emulsion, tissue distribution, biliary elimination


Probucol (I)[1], 4,4′-[(1-methylethylidene)bis(thio)]-bis[2,6-bis(1,1-dimethylethyl)]phenol, is an orally effective hypocholesterolemic drug. Its efficacy has been demonstrated in mice, rats, monkeys, and humans (1), and its chemical structure is unlike that of other hypocholesterolemic agents. There are no published pharmacokinetic studies of probucol in rats to characterize the dose-response of the drug (2) and only recently have aspects of the pharmacokinetic behavior of probucol in humans been presented (3).

The present pharmacokinetic studies were conducted in rats. Long-term toxicity and efficacy studies have been conducted in rats and other rodent and nonrodent species, and the identity of pharmacokinetic parameters that might assist interpretation of the outcome of the dose-response studies was sought.

## EXPERIMENTAL SECTION

**Probucol Formulations**—[Ring(U)-$^{14}$C]probucol[2] with a specific activity of 7.58 or 34.1 μCi/mg was used for distribution and excretion studies. The compound is labeled with statistical uniformity in all positions of each ring. A radiochemical purity of >98% was determined with TLC, autoradiography, and liquid scintillation counting (LSC) techniques. Two TLC systems were used: hexane–benzene (80:20) and hexane–ether (95:5) on silica gel (0.25 mm) plates[3]. Uniformly ring-labeled [$^{14}$C]probucol with a specific activity of 34.1 μCi/mg and a radiochemical purity of 99.5% by HPLC and LSC techniques was used for the biliary excretion studies. Labeled probucol was mixed with nonlabeled probucol which had a chemical purity of >99.5% to provide the intended regimens of oral and intravenous probucol. The specific activities of the resultant mixtures ranged from 1.57 to 8.74 μCi/mg.

Both oral and intravenous formulations of probucol were prepared as oil-

[1] Merrell Dow Research Institute-Cincinnati Center. Available in the U.S. under the trademark Lorelco.

[2] Synthesized by D. F. Gransden and G. A. Roth, The Dow Chemical Co., Midland, Mich.

[3] Silica Gel, F-254; MCB Manufacturing Chemists, Inc., Cincinnati, Ohio.




*0022-3549/84/1200-1758$01.00/0*