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Plasma Levels of Spirolactones in the Dog

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Abstract □ A fluorometric micromethod for the determination of spironolactone (I) and some of its possible metabolites was utilized to follow plasma concentrations after intravenous administration of I, canrenone (II), and potassium canrenoate (III). Spironolactone (I) was eliminated from plasma of female dogs, with a half-life of less than 10 min., and was partially converted to II and III. The γ-lactone ring of II equilibrated with the γ-hydroxycarboxylic acid of III, resulting in similar plasma levels of II 2 hr. following equimolar intravenous doses of II and III. Canrenone (II) represented the predominant component in plasma. Plasma concentrations of II, following an equimolar intravenous dose of I, were significantly smaller (~40%). Thus, I was only partially dethioacetylated to II.

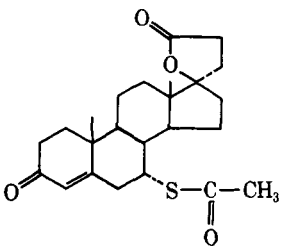
Keyphrases □ Spirolactones—plasma levels after intravenous administration, fluorometric analysis, dogs □ Spironolactone—plasma levels after intravenous administration, fluorometric analysis, dogs □ Canrenone and potassium canrenoate—plasma levels after intravenous administration, fluorometric analysis, dogs □ Plasma levels—spironolactone, canrenone, and potassium canrenoate after intravenous administration, dogs □ Spectrophotofluorometry—analysis, spirolactones

The class of spirolactones, synthesized by Cella and Tweit (1), were found to be potential competitive antagonists of mineralocorticoids (2, 3). One of these, spironolactone (I), is used as a potassium-sparing diuretic due to its high peroral activity. In spite of many studies on the pharmacological activity and metabolic and pharmacokinetic behavior of this compound (4–12), the major metabolic fate and the active principle of I remain unclear. Canrenone (II) was identified as a major metabolite (4, 5), which also possesses pharmacological properties similar to those of its parent compound (1, 6). Since a sensitive fluorometric assay was available for II but not for I (7), most pharmacokinetic studies were based on this method.

The plasma levels of II, obtained after high oral doses of I (100–1000 mg.), were in the low nanogram range in humans (4–12). This may be explained partially by a poor absorption of I due to its low solubility in water (20 mcg./ml.). Pharmaceutical formulations have been devised which apparently lead to a 10-fold enhanced GI absorption, using polysorbate 80 (8, 9, 13) or micronized powder (10, 14). The greater absorption could be correlated with increased dissolution rates in water (13).

The plasma levels of I in man were estimated to be in the range of one-fifth of the levels of II (7); however, in another study, I could not be detected at all (15). Similarly, contradictory results were reported for the appearance of I in urine (15, 16). No conclusive evidence could be presented as to the pharmacologically active principle (17).

The time course of drug action also has to be considered. Compound I shows a slow cumulative action on sodium excretion in the urine (12) and a maximum effect between 4 and 14 hr. following a single dose. Other reports indicated that it may take longer than 24 hr. for maximum effect. It was reported that II disappears from the plasma with a half-life of 4–5 hr. in man (18, 19); another report indicated 8 hr. (20). Neither of these values may represent the terminal log-linear phase of the biological half-life. Half-life studies of II have been undertaken with potassium canrenoate (III) in man (18–20). Compound III was reported to convert rapidly to II (18–20) and to the ester glucuronide of III as a major metabolite in man (20).



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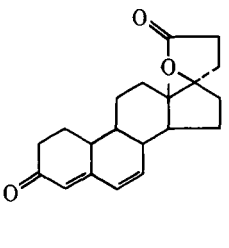

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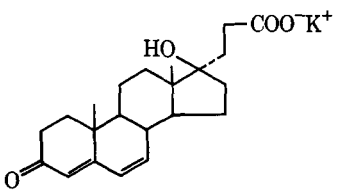

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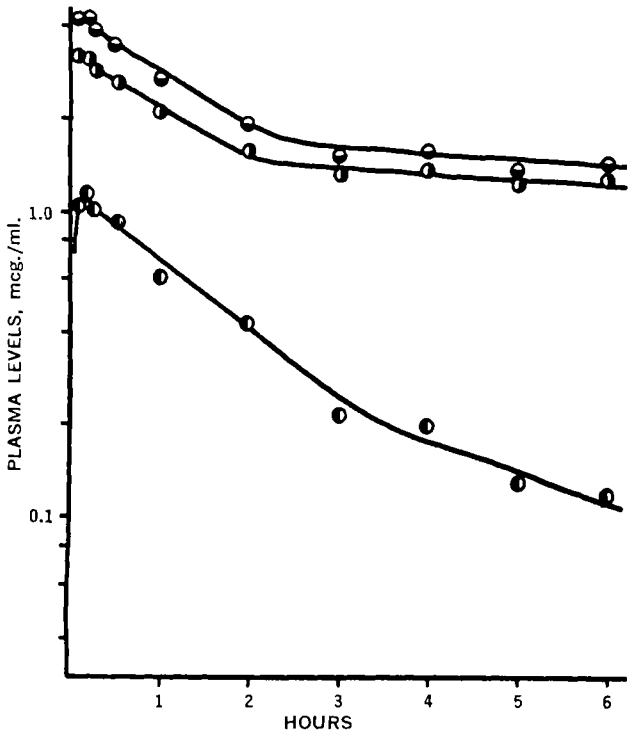

Figure 1—*Plasma levels after an intravenous dose of 82 mg. II (equivalent to 100 mg. I) to Dog 16526. Key: ◑, II; ◐, III; and ◒, II + III. All plasma values are expressed as micrograms of II.*

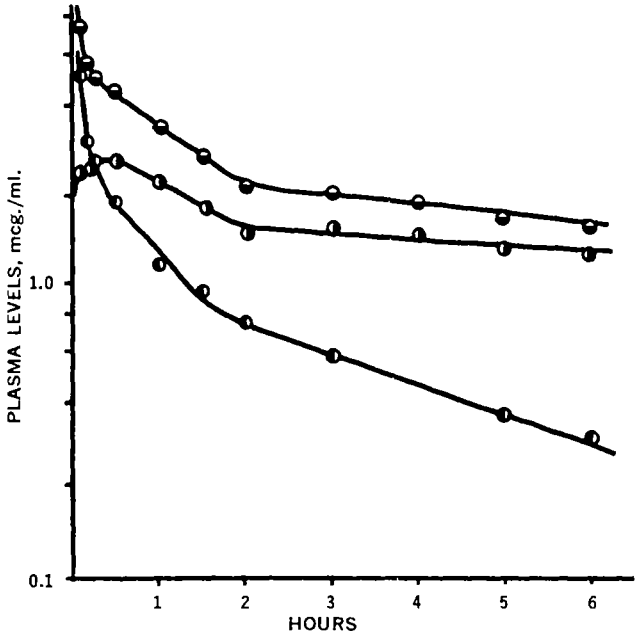

Figure 2—*Plasma levels after an intravenous dose of 95 mg. III (equivalent to 100 mg. I) to Dog 16526. Key: ◑, II; ◐, III; and ◒, II + III. All plasma values are expressed as micrograms of II.*

In the present study, the kinetic relationships between I, II, and III are presented. Dogs were used as the experimental animals, since this species has been shown to be valuable in demonstrating the antimineralocorticoid activity and the absorption rates from various dosage forms (4).

EXPERIMENTAL

Animal Procedures—Mongrel female dogs, weighing 18–20 kg. each, were prepared by cholecystectomy, ligation of the lesser pancreatic duct, insertion of a Thomas cannula into the duodenum opposite the opening of the common bile duct, and insertion of another Thomas cannula into the stomach. Experiments were begun no sooner than 3 weeks after surgery. Following an 18-hr. fast, the dogs were placed in sling harnesses. A polyethylene tube[1] was inserted 5–6 cm. into the common bile duct through the open duodenal cannula. Specimens of hepatic bile were collected at 15–30-min. intervals by gravity drainage during each experiment. A polyethylene tube[2] was inserted into a leg vein for infusion of 0.9% NaCl plus 0.5% sodium taurocholate[3] to compensate for the loss of bile acids; this infusion was given at 66 ml./hr. throughout each experiment, using a calibrated peristaltic pump[4]. All test drugs were given 1 hr. after taurocholate infusion was started. Compounds I and II were administered into the femoral vein in a polyethylene glycol 400 solution by a rapid infusion over 3–4 min. Compound III was injected intravenously in an aqueous solution.

An intravenous catheter[5] was inserted into the contralateral femoral vein for collecting blood samples into heparinized syringes. The blood was centrifuged, and the plasma was collected and frozen for subsequent analysis.

When urine was collected, the dog was lightly anesthetized with thiamylal intravenously and a catheter[6] was inserted into the bladder through the urethra and held in place with the 5-ml. balloon. Urine samples and gastric fluid were collected at 1-hr. intervals. For complete recovery of urine, the urinary bladder was rinsed each time with 40 ml. 0.9% NaCl solution.

Analytical Procedures—The fluorescence of I and its metabolites in sulfuric acid was used as the analytical method to determine the plasma concentrations. The analytical procedures were reported previously (21).

RESULTS AND DISCUSSION

The results reported here are restricted to those obtained from plasma concentrations. In precursor–successor experiments, equimolar doses of spironolactone (I, 100 mg.), canrenone (II, 82 mg.), and potassium canrenoate (III, 95 mg.) were given intravenously to female dogs to investigate the relation between the plasma levels of these compounds, with emphasis on rapid metabolic steps occurring during the first 6 hr. postadministration. Bile, gastric fluid, and urine were also collected in some experiments. The data for absorption and excretion using these sample collections will be presented in a subsequent paper (22).

The plasma levels of II and III resulting from an intravenous dose of 82 mg. II are shown in Fig. 1. The rapid initial dilution of II was not apparent due to the infusion time of 3–4 min. The elimination of II from the plasma represented a biexponential decay curve over 6 hr. Maximum plasma levels of the hydrolytic product III peaked after 10 min., and it was then eliminated from plasma by rate processes that differ in magnitude significantly from II (Fig. 1).

An equimolar dose of III was given intravenously to the same dog at a different time (Fig. 2). The elimination of III from plasma seemed to follow a triexponential decay curve, with a much smaller initial volume of distribution when compared to II. Canrenone (II) was rapidly formed, with maximum plasma levels 30 min. after the administration of III. The plasma concentrations of II were similar after 2 hr. following equimolar intravenous doses of II and III. It is apparent that a rapid equilibrium between II and III occurs, suggesting an enzymatic mechanism, since II and III are relatively stable in plasma for several days. Considerable differences in terminal half-lives were found in various dogs, as demonstrated in Fig. 3 (Dog 06584). The half-lives of II were estimated to range from 3.5 hr. (Dog 06580) to about 8 hr. (Dog 16526) in the first 6 hr.

Figures 4 and 5 demonstrate plasma concentration curves after rapid intravenous infusion (3–4 min.) of 100 mg. I in polyethylene glycol 400. Both II and III rapidly appeared in the plasma as metabolic products. After 1 hr., II and III were the only fluorescent species

[1] Intramedic, P. E. 190.
[2] Intramedic P. E. 50.
[3] Maybridge Chemical Co.
[4] Harvard Apparatus Co., Milds, Mass.
[5] Intracath R, C. R. Bald, Inc., Murray Hill, N. J.
[6] 10F Foley.

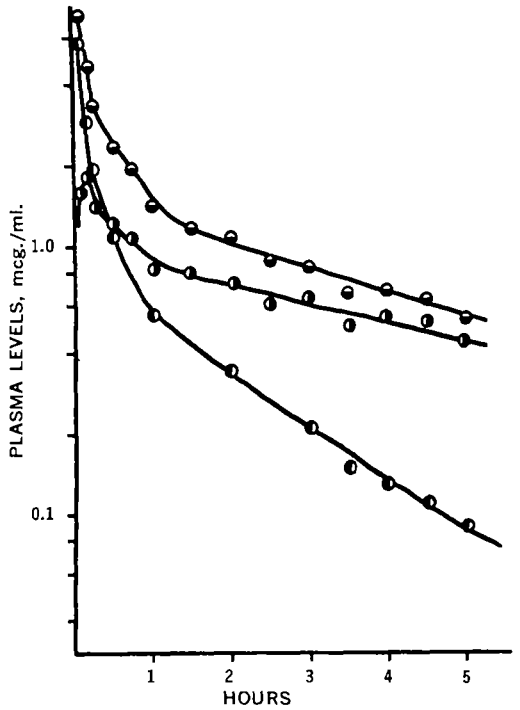

Figure 3—*Plasma levels after an intravenous dose of 95 mg. III to Dog 06584. Key: ◑, II; ◐, III; and ◒, II + III. All plasma values are expressed as micrograms of II.*

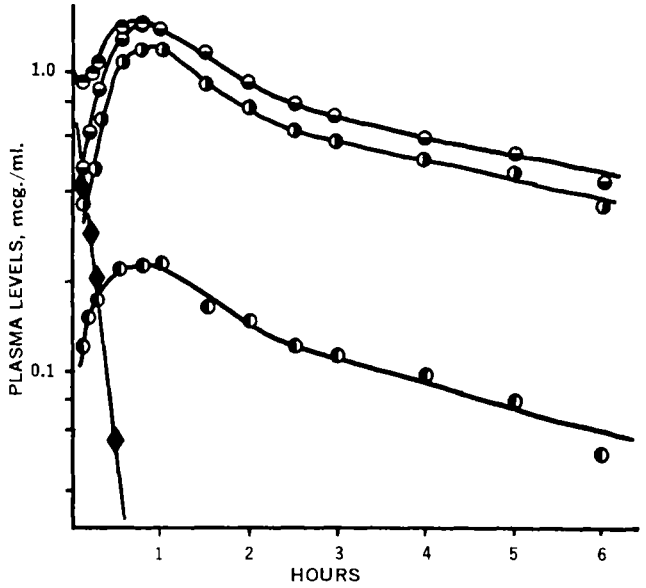

Figure 5—*Plasma levels after an intravenous dose of 100 mg. I to Dog 15871. Key: ◑, II; ◐, III; ◒, II + III; ◓, total fluorescent concentration, and ◆, (◓ − ◒) ~ I. All concentrations are expressed in micrograms of II.*

detectable within the sensitivity of the assay. Figures 4 and 5 include the data for the "total fluorescence" (see *Reference 21*) and the sum of II and III. Beyond the 1st hr., both curves proved to be identical. It can be assumed that the differences between the two assays in the 1st hr. after administration of I can be accounted for in part as unchanged I. A plot of these differences (Figs. 4 and 5) results in an estimation of the half-life of I to be less than 10 min. Therefore, it is not likely that I contributes significantly to the pharmacological activity, since the latter does not peak before 10 hr. postadministration. Doses of 50, 100, and 200 mg. of I resulted in equivalent concentration ratios of II in plasma.

The data suggest that I is converted rapidly to II. However, this may not be the exclusive metabolic pathway. Figure 6 is a plot of the concentration of II which results after injection of molar equivalents of I and II into the same dog at different times. The concentration of II resulting from injection of I is much less (~40%) than the level obtained by direct injection of II. Therefore, an alternate pathway of elimination of I other than conversion to II appears to be present.

The pharmacokinetic pattern of I–III was different in the dog when compared to man. No polar metabolites, such as the ester glucuronide of III, could be detected in plasma, which was found to be a major metabolite in human plasma (20).

It is evident that I represents a metabolically labile compound. It is, therefore, doubted that I itself may have access to the active site to a considerable degree. In dogs, metabolites other than II may contribute to the pharmacological activity induced by the administration of I.

For the estimation of unknown metabolites, tritium-labeled I and III were used; this study will be reported (22).

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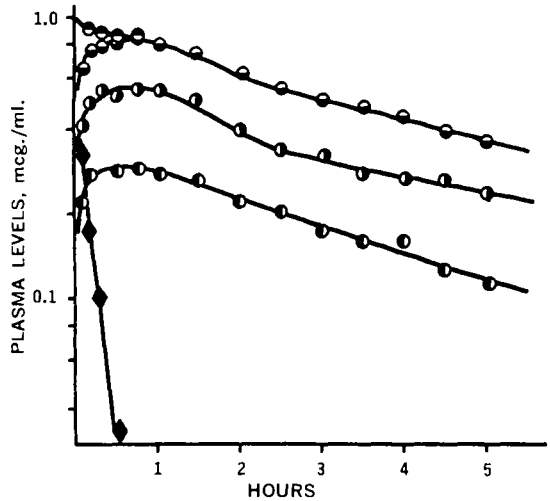

Figure 4—*Plasma levels after an intravenous dose of 100 mg. I to Dog 12219. Key: ◑, II; ◐, III; ◒, II + III; ◓, total fluorescent concentration; and ◆, (◓ − ◒) ~ I. All concentrations are expressed in micrograms of II.*

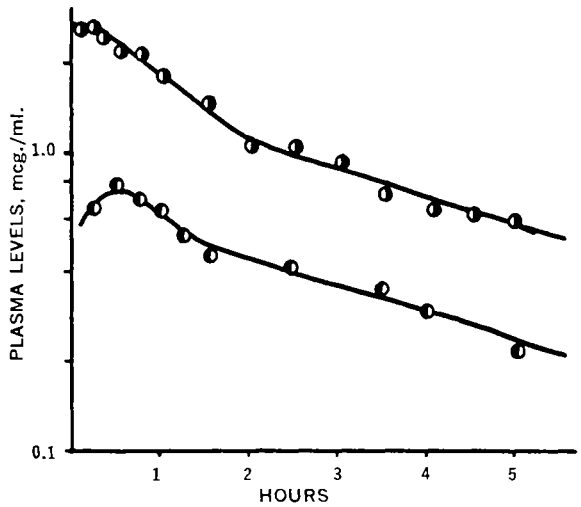

Figure 6—*Plasma levels of II after independent intravenous doses of 100 mg. I and 82 mg. II (equivalent to 100 mg. I) to Dog 13115. Key: ◑, II after administration of II; and ◐, II after administration of I.*

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Disposition of Tritium-Labeled Spirolactones in the Dog

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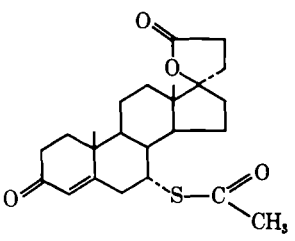
Abstract □ The disposition and metabolism of spironolactone (I), canrenone (II), and potassium canrenoate (III) in dogs were followed by fluorescence methods and by tritium label of I and III. Spironolactone was rapidly converted to nonfluorogenic, possibly reduced, metabolites, which were concentrated in the bile fluid (35% of the dose within 6 hr.). The 4,6-dienone, III, followed a similar metabolic pathway to nonfluorogenic metabolites; however, it was eliminated at a slower rate than I. Urinary excretion was rather low (about 2% of the dose in 6 hr.), and gastric cycling was virtually absent. A preliminary pharmacokinetic model in the dog is suggested. Equimolar amounts of I and II were given perorally and intravenously for absorption studies. The bioavailability to the central compartment was about 40% following oral doses, whereas the absorption amounted to about 80% when judged by the biliary excretion after oral and intravenous doses. The difference is explained by the effective hepatic clearance following oral administration.

Keyphrases □ Spirolactones, fluorescent and tritium labeled—disposition, metabolism, dogs □ Spironolactone, fluorescent and tritium labeled—disposition, metabolism, dogs □ Canrenone and potassium canrenoate, fluorescent and tritium labeled—disposition, metabolism, dogs □ Absorption, spirolactones—disposition, metabolism, dogs □ Biliary excretion—spirolactones, dogs □ Radiolabeled spirolactones—disposition, metabolism, dogs □ Spectrophotofluorometry—analysis, spirolactones

The structure-activity relationships among the diuretic steroidal 17-spirolactones have been studied. The compound most potent when given orally was found to be spironolactone (I) (1–5), which is metabolized to canrenone (II) by dethioacetylation. Canrenone (II) was shown to be in rapid equilibrium with its open-chain analog, III, in dogs (6). *In vitro* studies with the small gut mucosa of rats revealed an effective hydrolytic cleavage of the γ-lactone ring of II at this site (7).

Earlier workers (4) suggested that either I or a metabolite of I other than II may also contribute to the pharmacological effect. However, no significant difference in activity between I and the potassium salt of canrenoic acid, III, was found following oral and



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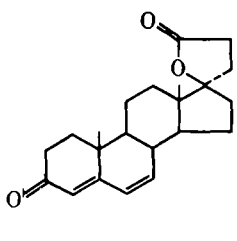

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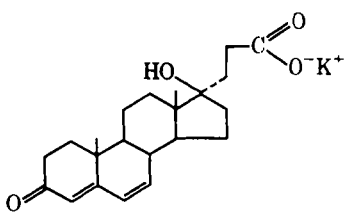

III