Absorption, Distribution, and Metabolic Fate of Seclazone [7-Chloro-3,3a-dihydro-2*H*,9*H*-isoxazolo(3,2-*b*)(1,3)benzoxazin-9-one]

JEROME EDELSON[▲], J. F. DOUGLAS, and B. J. LUDWIG

Abstract ☐ The absorption, distribution, and metabolic fate of seclazone was studied in the rat, dog, and rhesus monkey. Seclazone is readily absorbed by all species. The half-life of blood radioactivity following oral administration of seclazone-9-^{14}C was 10, 8.5, and 6 hr. in the rat, beagle hound, and rhesus monkey, respectively. Studies in the rat, using seclazone-9-^{14}C, indicated that about one-half of the radioactivity was excreted in the urine within 20 hr. Significant amounts of ^{14}C were also found in the carcass, liver, and GI tract. Only trace amounts of unchanged drug were voided. Seclazone undergoes metabolic cleavage of the oxazine ring, yielding 5-chlorosalicylic acid which is excreted in the free form and as conjugates with glucuronic acid and glycine. Similar studies using seclazone-2-^{14}C showed that the carbon chain of the isoxazoline ring is cleaved to malonic acid and carbon dioxide.

Keyphrases ☐ Seclazone, radiolabeled—absorption, distribution, metabolism in rats, dogs, rhesus monkeys ☐ 7-Chloro-3,3a-dihydro-2*H*,9*H*-isoxazolo(3,2-*b*)(1,3)benzoxazin-9-one, radiolabeled—absorption, distribution, metabolism in rats, dogs, rhesus monkeys ☐ 5-Chlorosalicylic acid—major metabolite of seclazone

Seclazone[1] [7-chloro-3,3a-dihydro-2*H*,9*H*-isoxazolo-(3,2-*b*)(1,3)benzoxazin-9-one] is a new compound which possesses uricosuric and anti-inflammatory activity in animals and man (1, 2). This report describes studies on the absorption, distribution, and metabolic fate of seclazone in the rat, dog, and rhesus monkey.

EXPERIMENTAL

Preparation of 7-Chloro-3,3a-dihydro-2*H*,9*H*-isoxazolo(3,2-*b*)(1,3)-benzoxazin-9-one-9-[14]C (Seclazone-9-^{14}C)—A mixture of 1.0 g. (3.0 mc.) of 5-chlorosalicylic acid-carboxyl-^{14}C, 2.0 ml. of methanol, and 0.25 ml. of concentrated sulfuric acid was stirred in a centrifuge tube fitted with a condenser and heated in an oil bath at 70° for 18 hr. Ice water was added to the reaction mixture, and the solid which formed was sedimented by centrifugation. This solid was sequentially washed with cold water, cold saturated sodium bicarbonate solution, and cold water. Ten milliliters of an aqueous solution containing 750 mg. hydroxylamine hydrochloride and 865 mg. sodium hydroxide was added, and the mixture was stirred at room temperature until solution occurred (about 2 hr.). The resulting hydroxamic acid was precipitated by the addition of 10 ml. of 12 *M* hydrochloric acid. The solid obtained by centrifugation was washed with cold water and dried *in vacuo* over phosphorus pentoxide.

A solution containing 540 mg. anhydrous hydrogen chloride dissolved in 5 ml. of glacial acetic acid was added to the dried hydroxamic acid in the reaction tube, followed by the addition of 0.45 ml. of freshly distilled acrolein. This reaction mixture was stirred for 3 hr. in an oil bath at 50°. Ice water was added and the solid which formed was separated by centrifugation, washed with cold water, and dried *in vacuo*. The solid in the centrifuge tube was reacted with 10 ml. of cold 1 *M* sodium hydroxide, and the mixture was agitated in ice for 30 min. The product was sedimented by centrifugation and sequentially washed with cold water, cold 5% (w/v) sodium bicarbonate solution, and cold water. The dried residue yielded 600 mg. of seclazone-9-^{14}C, m.p. 149–150°, specific activity 4.61 × 10⁶ d.p.m./mg.

This product gave a single radioactive zone by TLC in chloro-

form–methanol (97:3), and its IR spectrum was identical to that of authentic seclazone.

Preparation of 7-Chloro-3,3a-dihydro-2*H*,9*H*-isoxazolo(3,2-*b*)-(1,3)-benzoxazin-9-one-2-[14]C (Seclazone-2-^{14}C)—A solution of 540 mg. of anhydrous hydrogen chloride in 5 ml. of glacial acetic acid was placed in a centrifuge tube, and 1.05 g. of 5-chlorosalicylohydroxamic acid was added. A solution of 829 mg. (3.0 mc.) of the diethyl acetal of 3-chloropropionaldehyde-3-^{14}C in 5 ml. of benzene was transferred to the centrifuge tube. The reaction mixture was vigorously stirred in a water bath at 54° for 2.5 hr. and allowed to stand overnight at room temperature. The benzene was removed *in vacuo*, and a solid was precipitated by the addition of ice water. After centrifugation, the solid pellet was washed with cold water, dried, and suspended in 20 ml. of cold 1 *M* ammonia, and the suspension was stirred for 1.5 hr. in an ice water bath. The product, seclazone-2-^{14}C, was sedimented by centrifugation in the cold, washed with water, and dried. The yield was 250 mg. of seclazone-2-^{14}C, m.p. 145–146°, specific activity 5.25 × 10⁶ d.p.m./mg.

The product was homogeneous as determined by TLC in two different solvent systems.

Preparation of 5-Chlorosalicyluric Acid—A mixture of 69 g. of 5-chlorosalicylic acid, 100 g. of thionyl chloride, 4 ml. of *N,N*-dimethylformamide, and 400 ml. of benzene was warmed to 50° for 1.5 hr. and then heated to 80° for an additional 1.5 hr. The solvent was removed *in vacuo*, and the residue was added to a solution of 30 g. of glycine and 18 g. of sodium hydroxide in 100 ml. of water. Additional sodium hydroxide was added to maintain alkaline conditions, pH 9–10. After stirring the mixture overnight, the oily residue was removed and discarded. The supernatant solution was acidified with concentrated hydrochloric acid, which precipitated the 5-chlorosalicyluric acid. The purified compound was obtained by recrystallization from ethyl acetate–hexane. The yield was 7.5 g., m.p. 203–204°.

Anal.—Calc. for $C_9H_8ClNO_4$: C, 47.02; H, 3.52; N, 6.13. Found: C, 47.12; H, 3.55; N, 6.10.

Absorption and Blood Concentration—Six male Sprague–Dawley rats, weighing between 150 and 200 g., each received by stomach tube a solution of 4.08 mg. of seclazone-9-^{14}C dissolved in 0.5 ml. of polyethylene glycol 400. Blood samples were taken from the tail vein at appropriate intervals and were assayed for radioactivity by liquid scintillation counting as previously described (3).

A female rhesus monkey, weighing 3.9 kg., was given a capsule orally containing 105.6 mg. of seclazone-9-[14]C. Blood samples taken from the jugular vein at suitable intervals were assayed for radioactivity.

Table I—Blood Radioactivity after Oral Administration of Seclazone-9-^{14}C to Six Rats

Time after Administration, hr.	Mean Radioactivity[a], d.p.m./ml. × 10⁴
0.25	3.88 ± 0.30
0.50	6.09 ± 0.55
1.0	7.51 ± 0.71
1.5	7.17 ± 0.37
2.0	7.60 ± 0.50
3.0	7.63 ± 0.48
4.0	6.67 ± 0.47
5.0	6.00 ± 0.44
6.0	5.85 ± 0.39
7.0	5.12 ± 0.40
24.0	1.51 ± 0.14
26.0	1.41 ± 0.15
28.0	1.38 ± 0.16
30.0	1.18 ± 0.19
48.0	0.30 ± 0.05

[a] Values are given with their standard error.

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[1] Seclazone previously has been referred to in the literature as W-2354.
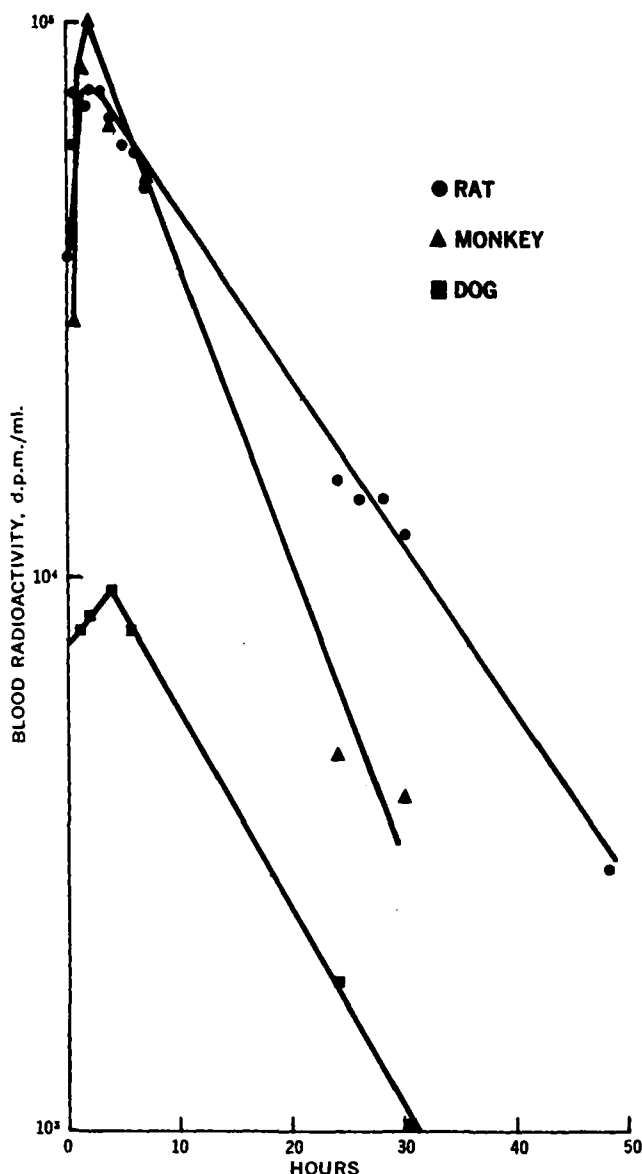
Figure 1—*Blood radioactivity after oral administration of seclazone-9-^{14}C to three species. Dose: rat, 1.07 × 10^6 d.p.m./kg., 23.4 mg./kg.; monkey, 3.54 × 10^6 d.p.m./kg., 27.1 mg./kg.; and dog, 1.95 × 10^7 d.p.m./kg., 42.8 mg./kg.*

A male beagle hound, weighing 12 kg., was given a capsule containing 515.4 mg. of seclazone-9-^{14}C. At appropriate time periods, blood samples were taken from the vein in the toe nail and their plasma radioactivity was measured.

Tissue Distribution in the Rat—A male Sprague-Dawley rat, weighing about 200 g., received by stomach tube 1.7 mg. of seclazone-9-^{14}C dissolved in 1.0 ml. of polyethylene glycol 400. The animal was placed in a metabolism chamber for 20 hr. and then sacrificed. The excreta, soft tissue organs, and the remainder of the carcass were assayed for radioactivity as previously reported (3).

A second rat, weighing about 200 g., received orally a solution containing 2.35 mg. of seclazone-2-^{14}C in 1.0 ml. of polyethylene glycol 400. After 24 hr., the animal was sacrificed and its tissues were processed and analyzed for radioactivity. The carbon dioxide of respired air was trapped in 2.5 M sodium hydroxide, and the radioactivity of an aliquot of this solution was measured.

Isolation of Metabolites—Pooled 24-hr. dog urine (1300 ml.) from three male beagles, who had each been given 50 mg./kg. of nonradioactive seclazone by capsule, was continuously extracted with carbon tetrachloride for 18 hr. The organic phase was separated, washed with 50 ml. of water, dried over sodium sulfate, and

evaporated to dryness *in vacuo*. The residue was crystallized from ethyl acetate, m.p. 149-150°. This solid was identified as seclazone.

The urine remaining from the initial extraction was acidified to pH 1 with concentrated hydrochloric acid and continuously extracted with carbon tetrachloride for an additional 18 hr. The organic phase was separated, washed with 50 ml. of acidified water, dried over sodium sulfate, and evaporated to dryness *in vacuo*. The resulting solid, recrystallized from methanol and then from chloroform, was identified as 5-chlorosalicylic acid, m.p. 171-172°.

Inverse Isotope Dilutions—Inverse isotope dilution experiments were carried out on the urine of animals that had received labeled seclazone. A typical procedure is described below:

1. A male Sprague-Dawley rat, weighing about 200 g., received by stomach tube 1.7 mg. of seclazone-9-^{14}C dissolved in 1.0 ml. of polyethylene glycol 400. Urine was collected for 24 hr. and its radioactivity was measured. Isotope dilutions were carried out on separate aliquots of urine as follows:

(a) Nonradioactive 5-chlorosalicylic acid (1000 mg.) was added to the first 1.0-ml. aliquot, followed by sufficient methanol to give a clear solution. Upon the addition of water, 5-chlorosalicylic acid precipitated. The solid was removed and repeatedly recrystallized from methanol-water to constant specific activity, m.p. 174-176°.

(b) Nonradioactive 5-chlorosalicyluric acid (1000 mg.) was added to a second 1.0-ml. aliquot of urine, and the mixture was diluted with water and heated until homogeneous. Upon cooling, crystalline 5-chlorosalicyluric acid separated and was removed by filtration. It was recrystallized from ethyl acetate-hexane to constant specific activity, m.p. 202-204°.

(c) Nonradioactive seclazone was added to another aliquot of urine. Sodium carbonate solution was added to adjust the pH to 9-10, and the mixture was extracted three times with 10 volumes of chloroform. The chloroform phase was evaporated to dryness. The residue, consisting of crude seclazone, was recrystallized from ethyl acetate to constant specific activity, m.p. 149-150°.

(d) The metabolites occurring in the urine in a combined form were quantitated following conversion to the free form by hydrolysis. An aliquot of urine was treated with an equal volume of concentrated hydrochloric acid, and the mixture was heated on a steam bath for 10 min. Nonradioactive carrier was then added, and the inverse isotope dilution was carried out as described for the free metabolites. Conjugated urinary end-products were calculated by difference.

Similar studies were performed on the urine of a dog and monkey that had received 515 and 106 mg. of seclazone-9-^{14}C, respectively.

2. A male Sprague-Dawley rat, weighing about 200 g., received by stomach tube 2.35 mg. of seclazone-2-^{14}C dissolved in 1.0 ml. of polyethylene glycol 400. Urine was collected for 24 hr. and assayed for radioactivity.

(a) Nonradioactive malonic acid (1000 mg.) was added to a 3.0-ml. aliquot of urine, and the mixture was warmed gently until a clear solution was obtained. A few drops of concentrated hydrochloric acid were added, and the mixture was extracted twice with 15 ml. of ethyl acetate. The organic phase was dried

Table II—Tissue Distribution of ^{14}C in the Rat after Oral Administration of Seclazone

	Radioactivity Recovered, Percent of Dose	
Specimen	Seclazone-9-^{14}C[a]	Seclazone-2-^{14}C[b]
Urine	52.1	58.9
Carcass	34.6	5.98
Liver	4.9	1.24
GI tract, including feces	3.4	4.52
Kidney	0.8	0.21
Lung	0.6	0.07
Heart	0.3	0.04
Spleen	0.1	0.03
Carbon dioxide	0.0	27.2
Recovery	96.8	98.2

[a] Animal sacrificed 20 hr. after drug administration. [b] Animal sacrificed 24 hr. after drug administration.

Table III—TLC[a] R_f Values of Urinary End-Products of Seclazone

Compound	Solvent System A[b]	Solvent System B[c]
Seclazone	0.60	0.68
5-Chlorosalicylic acid	0.47	0.47
5-Chlorosalicyluric acid	0.35	0.16
5-Chlorosalicylic acid glucosyluronide	—	0.06

[a] Silica gel. [b] Benzene–methanol–glacial acetic acid (180:32:16). [c] n-Butanol saturated with 2 N ammonia.

Table IV—Quantitation of Seclazone-9-[14]C Urinary End-Products in the Rat, Dog, and Monkey

Urinary End-Product	Percent of Urinary Radioactivity[a] Rat 1	2	Dog	Monkey
5-Chlorosalicylic acid				
Free	70.5	63.2	75.4	66.0
Bound[b]	25.7	25.6	22.3	21.2
5-Chlorosalicyluric acid				
Free	1.3	0.2	0.3	6.0
Bound[b]	n.d.[c]	n.d.	0.4	0.6
Seclazone				
Free	n.d.	0.97	0.6	0.03
Bound[b]	n.d.	n.d.	n.d.	n.d.
Total radioactivity recovered	97.5	90.0	99.0	93.8

[a] Values obtained by inverse isotope dilution. Animals received the following oral doses of drug: rat 1, 1.7 mg.; rat 2, 55 mg.; dog, 515 mg.; and monkey, 106 mg. [b] Values obtained by difference, before and after acid hydrolysis. [c] None detected.

over anhydrous sodium sulfate, and hexane was added to the ethyl acetate solution to precipitate malonic acid. The solid was separated and recrystallized from ethyl acetate–hexane to constant specific activity, m.p. 133–134°.

(b) Nonradioactive succinic acid (1000 mg.) was added to a 2.0-ml. aliquot of urine, and the mixture was diluted with a few milliliters of water and heated until homogeneous. After concentrated hydrochloric acid was added to adjust the pH to 1–2, the solution was cooled and allowed to stand. The crystals of succinic acid which separated were removed by filtration and recrystallized from ethyl acetate–hexane to constant specific activity, m.p. 185–186°.

RESULTS AND DISCUSSION

Absorption and Blood Concentration—Plasma radioactivity was observed within 1 hr. following oral administration of 9-labeled seclazone to the rat, dog, or rhesus monkey, indicating that the drug was readily absorbed in each of these three species. The elimination of ^{14}C from the bloodstream appeared to follow first-order kinetics. The half-lives of radioactivity in the blood of the rat, beagle hound, and rhesus monkey were approximately 10, 8.5, and 6 hr., respectively (Fig. 1). The means plus the standard errors obtained for six rats are given in Table I.

Tissue Distribution in the Rat—A distribution study of radioactivity following oral administration of seclazone-9-^{14}C showed that the ^{14}C was principally excreted in the urine within 20 hr. Significant amounts of ^{14}C were also found in the carcass, liver, and GI tract at that time (Table II).

A similar study with seclazone-2-^{14}C showed that radioactivity from the 2-carbon atom was also largely eliminated in the urine, since almost 60% of the radioactivity of the administered dose was voided in the urine within 24 hr. Moreover, over one-fourth of the ^{14}C was exhaled as respiratory carbon dioxide, indicating that the carbon chain of the isoxazoline ring was cleaved and oxidized in the body. The distribution of the residual radioactivity is shown in Table I.

Additional experiments with rats showed that these animals excreted similar levels of radioactivity during the 24-hr. period after drug administration. The ^{14}C voided, as percent of dose, for two

animals given seclazone-9-^{14}C was 52.0 and 62.1%, while the urinary content of two rats given 2-labeled drug was 52.0 and 53.3%.

Identification, Isolation, and Quantitation of Urinary End-Products—Four radioactive substances were detected by TLC in the urine of animals that had received seclazone-9-[14]C. Three of these compounds were identified by isolation and/or isotope dilution as seclazone, 5-chlorosalicylic acid, and 5-chlorosalicyluric acid. In addition, evidence was obtained indicating that 5-chlorosalicylic acid was also present in the urine in the form of a glucuronide conjugate. The R_f's of these compounds in two different solvent systems are shown in Table III.

Unchanged seclazone and 5-chlorosalicylic acid, isolated from the urine of three beagle hounds, were identified by their TLC properties, melting points, and IR spectra.

Quantitation of the various metabolites of seclazone-9-^{14}C occurring in the urine of the three animal species is summarized in Table IV.

The major urinary end-product in the three species examined—rat, dog, and monkey—is 5-chlorosalicylic acid, resulting from hydrolysis of the ingested compound. Seclazone is almost completely broken down in the animal body since only insignificant amounts of unchanged drug were excreted in the urine. Essentially all of the urinary radioactivity could be accounted for as 5-chlorosalicylic acid, either free or in the form of its conjugates with glycine and glucuronic acid.

In a single experiment on radioactive dog urine, evidence was obtained which indicated that the "bound" 5-chlorosalicylic acid occurs chiefly as the glucuronide. In this study the glucuronides were separated using the basic lead salt method of Kamil et al. (4). These were found to contain the same amount of radioactivity as that present in the bound 5-chlorosalicylic acid (approximately 20% of the urinary ^{14}C). The glucuronide was hydrolyzed by hydrochloric acid and β-glucuronidase to yield 5-chlorosalicylic acid, but it was
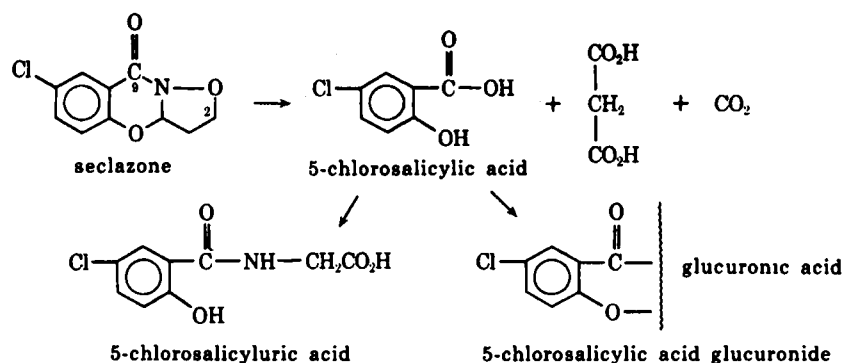
Scheme I—*Metabolic fate of seclazone*

not hydrolyzable by aryl sulfatase. The identity of the hydrolysis product was confirmed by inverse isotope dilution and TLC. The nature of the glucuronide bonding has not been determined.

Inverse isotope dilutions on the urine of three rats that had received seclazone-2-^{14}C indicated the presence of malonic acid in an amount that accounted for 2–14% of the urinary radioactivity. In addition, succinic acid, a known metabolite of malonic acid, was present and represented 1–3% of the urinary radioactivity. The presence of these two compounds suggests that the carbon dioxide-^{14}C present in respired air (Table I) originates either from the direct decarboxylation of malonic acid or from succinic acid through the tricarboxylic acid cycle. In any event, the radioactive carbon dioxide arises through metabolic cleavage of the isoxazoline ring of seclazone.

The metabolic fate of seclazone is summarized in Scheme I.

SUMMARY

Seclazone-9-^{14}C is readily absorbed after oral administration to the rat, beagle hound, and rhesus monkey. The half-lives of blood radioactivity in the three species were found to be 10, 8.5, and 6 hr., respectively. In the rat, about half of the ^{14}C given orally was excreted in the urine within 24 hr. Significant amounts of radioactivity were also found in the carcass, liver, and GI tract. Seclazone is almost completely metabolized to 5-chlorosalicylic acid in each of these three species. The latter compound was excreted either uncombined or conjugated with glucuronic acid or glycine. Studies with seclazone-2-^{14}C suggested that carbons 2, 3, and 3A of seclazone are metabolized to malonic acid.

REFERENCES

(1) F. M. Berger, M. Kletzkin, and H. J. Spencer, Fed. Proc., 31, 578(1972).
(2) D. B. Reisner, B. J. Ludwig, H. M. Bates, and F. M. Berger, U. S. pat. 3,598,814 (1971).
(3) J. Edelson, A. Schlosser, and J. F. Douglas, Arch. Int. Pharmacodyn. Ther., 187, 139(1970).
(4) I. A. Kamil, J. N. Smith, and R. T. Williams, Biochem. J., 50, 235(1951).

Displacement of Sulfonylureas from Human Serum Proteins by Coumarin Derivatives and Cortical Steroids

JOSEPH JUDIS

Abstract ☐ Several coumarin derivatives (ethyl biscoumacetate, phenprocoumon, anisindione, acenocoumarol, and diphenadione) were examined for their abilities to displace sulfonylureas (acetohexamide, chlorpropamide, and tolbutamide) from human serum albumin, utilizing equilibrium dialysis for the estimation of protein binding. All of the coumarin compounds caused reduction in binding of the sulfonylureas, although there was no clearcut pattern with variation of pH. At lower pH values, ethyl biscoumacetate caused the greatest reduction in binding of sulfonylureas; but at pH 8.4, diphenadione was the most potent displacing agent. Acetohexamide was tested for its ability to displace cortical steroids from human serum albumin and human serum fraction α-globulin IV-4, the latter containing transcortin. Of the steroids examined (cortisone, cortisol, prednisone, prednisolone, and corticosterone), the binding of cortisol was the most reduced with human serum albumin as the protein; but if α-globulin fraction IV-4 was the protein in the system, prednisone was most displaced. The data establish that coumarin derivatives are capable of interfering with the binding of sulfonylureas to human serum albumin, and acetohexamide causes reduction of binding of the steroids tested to human serum albumin and α-globulin fraction IV-4. These observations may be the mechanism for interactions between these drugs.

Keyphrases ☐ Sulfonylureas—displacement from human serum proteins by coumarin derivatives and cortical steroids ☐ Serum protein binding, sulfonylureas—displacement by coumarins and cortical steroids ☐ Coumarin derivatives—displacement of sulfonylureas from human serum proteins ☐ Cortical steroids—displacement of sulfonylureas from human serum proteins ☐ Acetohexamide—displacement of cortical steroids from human serum proteins

Reports in the recent literature have cited interactions of sulfonylureas and various drugs including coumarin derivatives (1–9). Although there have been apparently no reports of displacement of cortical steroids by sulfonylureas, compounds such as phenylbutazone have been shown to displace cortical steroids bound to human serum proteins; since competition has been demonstrated between phenylbutazone and sulfonylureas for binding sites on human serum proteins, one could conclude that similar competition should exist between cortical steroids and sulfonylureas in serum protein bindings (10). Sellers and Koch-Weser (11) recently pointed out that, although the clinical importance of metabolic interactions between drugs has become increasingly clear, not enough work has been done to demonstrate the mechanisms of drug interactions by in vitro laboratory experiments including studies of displacement of drugs bound to plasma proteins. Such data would make it possible to predict clinically important drug interactions.