Absorption, Distribution, Metabolism, and Excretion of Naproxen in Various Laboratory Animals and Human Subjects

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Abstract □ The comparative absorption, distribution, metabolism, and excretion of naproxen [(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid], a potent systemic anti-inflammatory agent, was studied in several species including man. Blood, urine, and fecal analyses were performed on specimens collected at several times after either oral ingestion or rapid intravenous administration of a radioactive dose. Naproxen was found to be rapidly absorbed in all species and, once in the blood, was eliminated with half-lives ranging from 2 to 35 hr. The human plasma half-life ranged from 10 to 17 hr. Evaluation of the distribution of radioactivity in various tissues and organs of the rat indicated that no unusual amount was retained in the animal 24 hr. after administration, nor did there seem to be preferential localization. Estimated volumes of distribution indicated that a large fraction of the drug is held in the blood, much like salicylates are. Virtually all of the drug present in the blood of humans was determined to be unchanged naproxen, while the rat and the monkey showed minor amounts of transformation products. With the exception of the dog, all species excreted naproxen and its metabolic transformation products predominantly in the urine. In the dog the preferred route was fecal. In the human, 94% of the intravenously administered radioactivity appeared in the urine after 5 days, while 1–2% appeared in the feces. Following 14 days of continuous exposure to the drug, the three human subjects displayed no evidence of induction of metabolizing enzymes.

Keyphrases □ Naproxen—absorption, distribution, metabolism, and excretion in animals, man □ (+)-6-Methoxy-α-methyl-2-naphthaleneacetic acid—absorption, distribution, metabolism, and excretion in animals, man □ Metabolism—naproxen, animals, man

The chemistry of naproxen [(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid[1]], a potent systemic anti-inflammatory agent, was reported by Harrison *et al.* (1). Rooks (2) described its biological activities. Naproxen was found to be 0.7, 5.5, and 11 times as active as indomethacin, aspirin, and phenylbutazone, respectively, in inhibiting the carrageenin-induced inflammation of the rat paw. Its analgesic activity was found to be approximately 7 times that of aspirin in a phenylquinone-induced writhing assay, and a dose-related antipyretic activity was observed in rats with yeast-induced pyresis. No significant cardiovascular or CNS activity was noticed. This report summarizes results of a comparative study of the absorption, distribution, metabolism, and excretion of naproxen in several species including man.

MATERIALS AND METHODS

The tritiated naproxen[2] was prepared by the Javorsky and Gorin (3) exchange method with isotopic water. Proof of structure was accomplished by mass spectrometry and NMR spectra of the deuterated analog. Additional details of the radiochemical synthesis and purification will be published elsewhere (4). The specific activity of the tracer compound was 92 mc./mmole.

Intravenous solutions were prepared by dissolving the unlabeled drug in phosphate buffer solutions at pH 9.0. The radioactive material, dissolved in approximately 0.2 ml. of ethanol, was then added to the buffer solution. Oral doses were provided either as solutions or in capsules filled with the loose powder. The finely divided powder was obtained as a coprecipitate from an ethanolic solution of a mixture of the labeled and nonlabeled naproxen by removing the solvent under a stream of nitrogen. Homogeneity was subsequently verified by sampling and assaying portions of the solid. The dried material was then forced through a standard sieve to obtain an average particle diameter of approximately 150 μ, which was verified by examining powder samples under the microscope.

EXPERIMENTAL

Animal Experiments—The rat experiments employed Sprague-Dawley males weighing 450–500 g. Six animals were used, three being dosed orally and three being dosed by the intravenous route. In the dog experiments, six beagles weighing approximately 9 kg. were given nonradioactive drug by both the oral and the intravenous routes. These animals were male and female, while in the radioactive experiment three females received the drug by rapid intravenous injection. The guinea pig study employed 30 male and female animals, each weighing about 0.8 kg. Three animals of this group received radioactive naproxen along with the nonradioactive drug. For the monkey experiment, three female rhesus monkeys weighing about 5 kg. and one male weighing about 9 kg. were used. In the minipig experiments, four young, sexually mature female pigs weighing between 29 and 32 kg. were used.

During all experiments the animals were restrained in metabolism cages provided with means for collection of urine and fecal samples. The animals were fasted at least 12 hr. prior to the start of the experiment, with water being allowed *ad libitum* during that period. The experiment was initiated either by rapid intravenous injection of the drug solution or by oral administration in gelatin capsules. In the rat

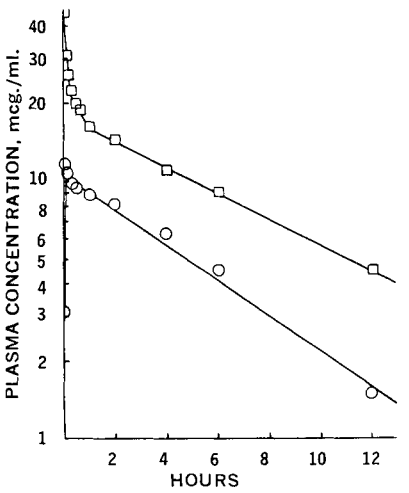

Figure 1—*Naproxen plasma concentrations in rats following intravenous* (□) *and oral* (○) *administration of 3 mg./kg. of* ^{3}*H-labeled naproxen.*

[1] This name is the correct *Chemical Abstracts* index name, but this compound was identified previously in the chemical and biological literature as *d*-2-(6′-methoxy-2′-naphthyl)propionic acid.

[2] The authors wish to thank Dr. W. Hafferl and Mr. A. Hary of Syntex Research, Palo Alto, Calif., for providing the radioactive compound.

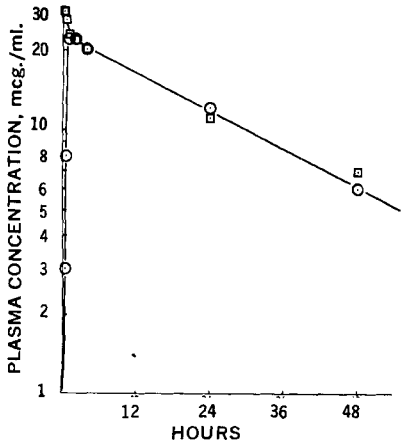

Figure 2—*Naproxen plasma concentrations in beagle dogs following intravenous (⊡) and oral (⊙) administration of 2.5 mg./kg.*

experiment the oral dose was administered in an aqueous solution by gavage. The rats received doses of 3 mg./kg. containing 27.4 μc. of tritium label either orally or intravenously. The dogs were injected with 2.5 mg./kg. along with 400 μc. of tritium-labeled compound. The monkeys, guinea pigs, and minipigs were administered doses of 5 mg./kg. of naproxen.

In the rat and the minipig experiments, rapid blood sampling was made possible by jugular vein cannulation; blood sampling in the dog, guinea pig, and monkey was achieved by jugular vein puncture at the appropriate times. The blood specimens were drawn into heparinized Vacutainers[3] and centrifuged, and the plasma was promptly frozen. Urine samples were collected in metabolic pans at frequent intervals during the first 24 hr. of the experiment, followed by single daily collections for the remainder of the experiment. A few drops of toluene were placed in each sample prior to storing in the frozen state. Fecal samples were obtained daily and kept frozen until analyzed. Urine and fecal collections were made only on the guinea pigs receiving the radioactive material.

Human Studies—The human studies were done on one group of subjects receiving the test compound orally and another receiving the material intravenously. In the oral experiments, six healthy male volunteers, ranging in weight from 59.0 to 77.2 kg. (130 to 170 lb.), were given single 100-, 200-, or 300-mg. doses of naproxen in loosely packed gelatin capsules. The average particle diameter of the drug powder was determined by microscopic examination to be 150 μ. Blood samples (10 ml.) were obtained by vein puncture at 1, 2, 4, 6, 12, 18, 24, 48, and 72 hr. after ingestion of the oral dose. Urine and fecal samples were not analyzed in this group.

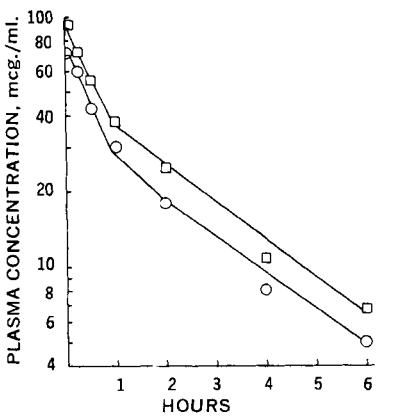

Figure 3—*Plasma concentrations in rhesus monkey 402 following intravenous administration of 5 mg./kg. of ^{3}H-labeled naproxen. Key: □, concentration calculated from plasma radioactivity; and ○, concentration of naproxen.*

[3] Becton-Dickinson Co., Rutherford, N. J.

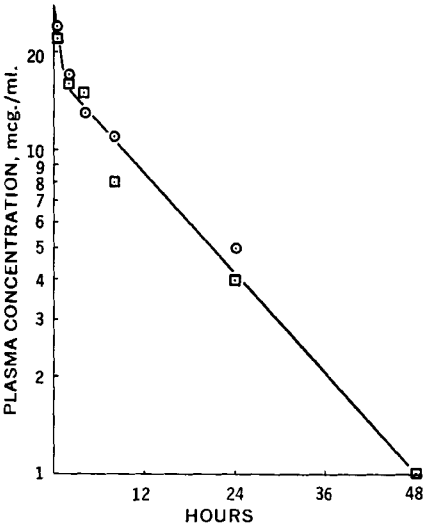

Figure 4—*Day 1, Subject A: plasma concentrations following intravenous administration of 1.2 mg./kg. of ^{3}H-labeled naproxen. Key: ⊡, concentration calculated from plasma radioactivity; and ⊙, concentration of naproxen.*

The intravenous study employed three healthy volunteers, two males and one female whose weights were 77.2, 86.3, and 49.9 kg. (170, 190, and 110 lb.), respectively. The dose injected intravenously consisted of 93 mg. drug containing 30 μc. tritium label dissolved in 10 ml. of phosphate buffer. Following the initial intravenous dose, the three volunteer subjects received 100-mg. oral doses of naproxen from Day 2 through Day 14. A second intravenous injection, formulated as already described, was administered on Day 15. Following each of the radioactive intravenous injections, 10-ml. blood samples were drawn at 0.5, 2, 4, 8, and 24 hr. Urine specimens were pooled for the time periods 0–1, 1–3, and 3–6 hr. and at 6-hr. intervals thereafter. Fecal samples were obtained daily. In both the oral and intravenous studies, the volunteers fasted 12 hr. preceding the experiment with fluids allowed *ad libitum* during this period.

Analytical Procedures—Duplicate plasma samples of 0.1 ml. each were pipeted into 15 ml. of a scintillation fluid, which was prepared by dissolving 260 mg. of 1,4-bis[2-(methyl-5-phenyloxazolyl)]-benzene, 13 g. of 2,5-diphenyloxazole, 208 g. of naphthalene in 600 ml. of methanol, and 1 l. each of toluene and dioxane. Urine samples were counted in exactly the same way. All samples were corrected for quenching.

The radioactive fecal and rat tissue samples were homogenized, dried, and combusted using conventional techniques (5). In an attempt to evaluate the biological stability of the ^{3}H-label, urine and fecal samples from the dog experiments were analyzed for tritiated water in the following experiments:

1. A 5-ml. urine sample was distilled at 100°.
2. A 5-g. fecal sample was diluted with 5 ml. of water, and the slurry was distilled at 100°.

The vapors were condensed, and the distillate was collected and counted as already described.

Unlabeled naproxen in plasma was assayed by a GLC technique. The method of analysis, which is to be described in detail elsewhere[4], involves extraction of a 1-ml. acidified plasma sample with ethyl acetate. This was followed by extraction of the ethyl acetate phase with aqueous sodium bicarbonate solution. The naproxen was isolated from the acidified sodium bicarbonate solution with diethyl ether, and the ethereal solution was treated with diazomethane to form the methyl ester. Quantitation was achieved by GLC using flame-ionization detection. The methyl ester of 6-methoxy-2-naphthylacetic acid was employed as an internal standard. A 1.22-m. (4-ft.) long, 3-mm. i.d. glass column, packed with 3.8% silicone elastomer (SE-30) on 80–100-mesh Diatoport S, was used under the following conditions: column temperature, 170°; injection temperature, 200°; detector temperature, 240°; and carrier gas (helium) flow rate, 70 ml./min.

[4] L. J. Throop and R. J. Leibrand, to be published.

Calculations of Kinetic Parameters—Semilog plots of the plasma concentration data following intravenous administration of naproxen yielded biphasic curves in all of the experiments. Generally, curves of this shape are fitted to an equation of the form $C_p = Ae^{-\lambda t} + \beta e^{-\alpha t}$ derived from the two-compartment open-model system (6, 7). However, in treatment of the plasma data being reported here, the initial "distributional" phase was ignored and the simple one-compartment open model was used as a basis for discussion.

The apparent volume of distribution (6), V_d, was calculated by dividing the dose in milligrams per kilogram of body weight (7) by the apparent initial plasma concentration, C_p^0, in milligrams per milliliter of unchanged naproxen. C_p^0 was obtained by extrapolating the terminal linear portion of the log C_p *versus* time curve to zero time. The plasma half-life was determined graphically from the same segment of the curve. Areas, in square inches, were obtained from the linear plasma concentration *versus* time plots by plainimetry.

RESULTS AND DISCUSSION

Figures 1–5 illustrate the plasma profiles following intravenous administration of naproxen in the various species. The concentration values in rat plasma (Fig. 1) following both oral and intravenous dosing were arrived at by correcting the ^{3}H-naproxen concentration to 90% of its value[5]. In the dog, minipig, monkey, and human experiments, naproxen concentration was determined by the GLC technique as well as by measurement of total radioactivity. In the case of the guinea pig plasma samples, analysis of naproxen was performed by GLC only.

All of the plasma concentration curves following intravenous administration had two linear phases; and in all species studied when blood samples were drawn early enough, the general shape and the plasma concentrations were remarkably similar. Only the slope of the terminal linear segment of the curve varied among the species. This latter aspect will be discussed later.

In Figs. 4 and 5 are summarized the results obtained in Subject A after intravenous administration of the first dose of radioactive naproxen and after the second intravenous dose, which followed 14 days of daily oral administration of nonradioactive drug. The results from the other two human subjects were essentially identical to those seen in Subject A. The Day 15 experiment (Fig. 5) demonstrated the effect of previous dosing on the plasma curve. The plasma concentrations of unchanged naproxen, as determined by GLC, were above the radioactive concentrations at all times during the experiment, reflecting residuals of the previous doses.

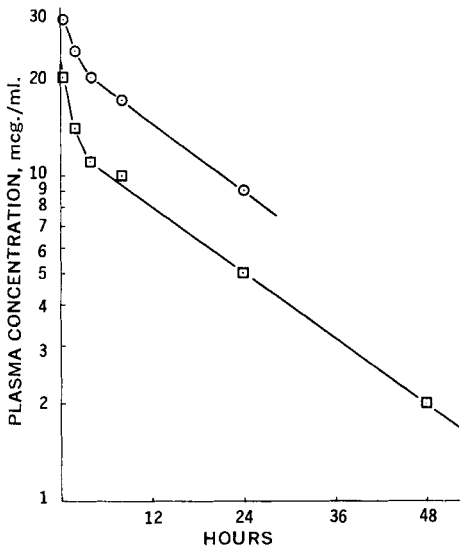

Figure 5—*Day 15, Subject A: plasma concentration following intravenous administration of 1.2 mg./kg. of ^{3}H-labeled naproxen. Key: ⊡, concentration calculated from plasma radioactivity; and ⊙, concentration of naproxen.*

[5] Plasma samples were exhaustively extracted of all residing radioactivity, and this extract was separated using a TLC technique. Ninety percent of the total ^{3}H concentration was found to be unchanged naproxen.

Table I—Radioactivity Levels in Tissues of a Rat 24 hr. after Oral Administration of 3 mg./kg. of ^{3}H-Labeled Naproxen

Tissue	d.p.m./Total Tissue[a]	d.p.m./g. Tissue	Percent of Administered Dose
Spleen	1,050	1,390	0.01
Heart	890	680	0.01
Lung	10,010	2,910	0.03
Liver	36,770	2,000	0.12
Kidney	9,160	2,510	0.03
Digestive system	168,500	8,100	0.50
Feces	478,000	168,280	1.5

[a] Radioactivity determined by combustion techniques on an aliquot of the dried sample.

Half-Life—The species differences in plasma half-lives of naproxen are remarkable. These range from 2 hr. in the rhesus monkey to 35 hr. in the dog, with the value in the human being approximately 14 hr. The species closest to the human in this regard was the guinea pig, with a half-life of 9 hr. The plasma half-life following oral administration was the same as that determined after intravenous administration in the rat, dog, minipig, and human. Moreover, half-lives were unchanged when doses of 2.5 and 5 mg./kg. were given to the rhesus monkey[6]. In the human, half-life values remained unchanged although the dose was doubled and tripled (Fig. 6). This finding distinguished naproxen from the salicylates, which have a potentially dangerous tendency to display increasing half-lives with increasing doses (8). A longer half-life implies a decrease in the relative rate of elimination and an increased likelihood that toxic accumulation of the drug will occur if the dosage regimen is not adjusted accordingly.

Absorption—Following oral administration in the rat, maximum levels of radioactivity were achieved in 10–20 min. (Fig. 1); in the dog (Fig. 2) and minipig, the peak occurred in 1–2 hr. In the dog the drug was absorbed so rapidly that blood levels after oral administration were essentially identical to those seen after intravenous administration. Smith *et al.* (9) reported similar behavior in the dog with a pyrazole carboxylic acid. Whether the GI tract of the dog is consistently more permeable to carboxylic acids than other chemical species is not known.

Earlier experiments in the dog[7] indicated that no important differences in blood levels could be attained following oral doses (2.5 mg./kg.) of micronized and nonmicronized naproxen. Neither was there any important difference between naproxen and its calcium or sodium salt. Oral doses of 100, 200, and 300 mg. in the human (Fig. 6) also produced peak plasma levels in 2 hr. Unless the encapsulated drug was passed from the stomach into the small intestine within 15 min. after ingestion, the rapid appearance of measurable blood levels in humans and in the dog suggests some gastric

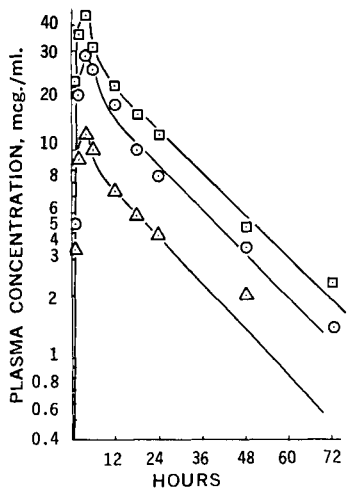

Figure 6—*Naproxen plasma concentrations in humans following oral administration of 100 mg. (△), 200 mg. (⊙), and 300 mg. (⊡) of naproxen.*

[6] Unpublished data.
[7] To be published.

Table II—Summary of Volumes of Distribution, V_d, and Plasma Half-Life, $t_{1/2}$, Values Obtained for all Species Studies

Species	Dose, mg./kg.	Sex[a]	$t_{1/2} \pm SD$, hr.	Number of Determinations	$V_d \pm SD$, l./kg.	Number of Determinations
Rat	3	M	5.1 ± 1.8	6	0.18 ± 0.06	3
Dog	2.5	F	35.0 ± 11.6	23	0.12 ± 0.8	6
Guinea pig	5	M and F	8.7 ± 2.1	3	0.12 ± 0.01	3
Rhesus monkey	5	F	1.9 ± 0.7	12	0.10 ± 0.04	10
Minipig	5	F	4.8 ± 0.8	6	0.12 ± 0.03	2
Human	1.1–1.4	M and F	13.9 ± 2.6	6	0.09 ± 0.03	6

[a] M = male; F = female.

absorption. However, no experiments specifically designed to isolate the major site of absorption were undertaken.

A measure of efficiency of drug absorption is the ratio of the area under the plasma drug concentration *versus* time curve for equal doses given orally and by intravenous injection [area (oral)/area (intravenous)]. A ratio less than 1 indicates either that the drug underwent biotransformation before it was distributed in the general circulation or that some of the drug was not absorbed. The ratio area (oral)/area (intravenous) of the 2.5-mg./kg. dose in the dog and the minipig is 1.0, indicating complete absorption, and no serious depletion of the drug before distribution equilibrium is reached. The rat, on the other hand, showed a ratio of 0.5. This low ratio was difficult to reconcile with the fact that urinary excretion rates after the intravenous and oral doses were almost identical, indicating efficient absorption of the orally administered radioactivity; no explanation could be found for this discrepancy. The value for the ratio in the human was 0.74. This ratio in the human experiments cannot have the significance that it might have if the oral and intravenous experiments had been done in the same subjects. The important facts are that oral doses do provide blood levels comparable to those obtained after intravenous dosing (Figs. 5 and 6) and that blood concentrations increase linearly on a 1:1 basis (Fig. 7) with the dose administered. The peak plasma concentrations after 100-, 200-, and 300-mg. oral doses are 12, 25, and 42 mcg./ml., respectively, which also suggest that a linear dose–blood level response is achieved.

Distribution—Twenty-four hours after oral administration of the radioactive dose in the rat, various tissues from one of the rats were obtained and analyzed. The results (Table I) show that very small residual radioactivity was detected in the tissues analyzed; approximately 0.01% was found in the spleen and heart, and 0.5% was found in the GI tract.

The apparent volumes of distribution, V_d, are approximately the same for all species studied (Table II). The V_d obtained from the human intravenous experiment is the smallest, indicating a greater fraction of the drug in the body is limited to the plasma compartment. By assuming a plasma volume of 4–5 l. and an extracellular fluid volume of 16–25% of total body weight (10), the results suggest that the human apparently distributes naproxen to a volume twice that of the plasma but only one-half that of the extracellular fluid. Hollister and Levy (11) reported apparent volumes of distribution in the range of 0.16 l./kg. in humans for a salicylate dose of 13 mg./kg. The values obtained following oral administration of naproxen at the three oral dose levels are virtually identical to that obtained for salicylate.

Plasma protein binding may influence not only drug distribution but also metabolism and excretion (12, 13). Naproxen is extensively bound to the proteins in the plasma of all the species studied (14). At concentrations achieved in the human experiments, 99.9% of the drug in the plasma is protein bound. This property undoubtedly influences the ability of the plasma to sequester naproxen and limit the extent of distribution outside the blood.

The distribution of weak acids is also affected by their ability to permeate cell membranes and other lipoidal tissue (15, 16). The partitioning ability, in turn, is affected by a number of factors, including the pH of the aqueous phase and the pKa of the weak acid. The pKa of naproxen is approximately 4. In an aqueous medium with a pH of 7.4, virtually all of the drug present is in the anionic form. Such species are poorly soluble in lipids and, consequently, are not likely to show partition properties favorable to lipids. Thus, both extensive protein binding and the pH-partition theory of Brodie and Hogben (15) operate to restrict naproxen largely to the vascular compartment.

Metabolism—The presence in plasma of radioactivity other than that associated with naproxen was detected only in the rat and the rhesus monkey. In the rat, approximately 10% of the total radiolabeled material was found to be other than unchanged drug. More than 90% of the radioactivity was extractable into diethyl ether and identified as naproxen by TLC[8].

In the monkey, the difference between the radioactive and the GLC assay (Fig. 3) suggests an apparent accumulation of metabolic transformation products in plasma. The terminal segments of the two curves appear to be parallel, however, and this suggests that the clearance rate of the metabolic product(s) is approximately the same as that of naproxen.

Following intravenous administration of radioactive doses to dogs, the results from the two methods of analysis coincided closely at all time points, and virtually all of the radioactivity in the plasma was ether extractable. The human intravenous plasma concentration curves plotted from radioactivity and GLC determinations were

Table III—Mode of Excretion of Intravenously Administered ^{3}H-Labeled Naproxen

Species	Percent Administered Radioactivity Excreted—Urine		Feces
Rat	78	(1)[a]	2
Dog	29[b]	(7)	50[c]
Guinea pig	89	(4)	5
Rhesus monkey	78	(4)	1
	77	(3)	
Minipig	87	(7)	1
	85	(3)	
Human	94	(5)	1

[a] Numbers in parentheses indicate total number of days of collection. [b] Upon distillation of radioactive urine and fecal samples, only approximately 3% of the total ^{3}H present could be vaporized, presumably as tritiated water. [c] Radioactivity in considerable amounts was still being excreted in the feces when collection was stopped.

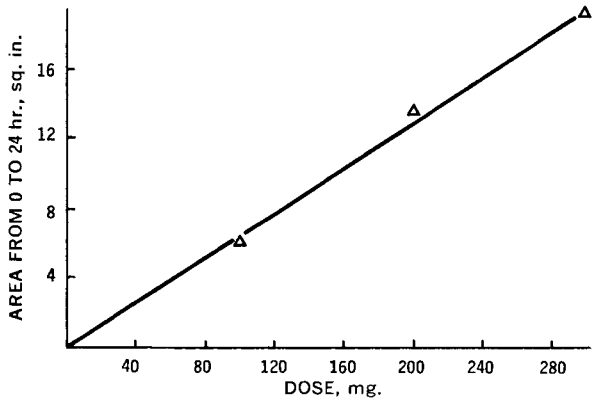

Figure 7—*Dose-area under the curve response for naproxen in human volunteers.*

[8] The association of the radioactivity with naproxen was based on mobility of the free acid using thin-layer silica gel chromatography and on retention time during GLC of the methyl ester. In both instances, the unknown substance behaved in a manner identical to standard naproxen and its methyl ester, respectively.

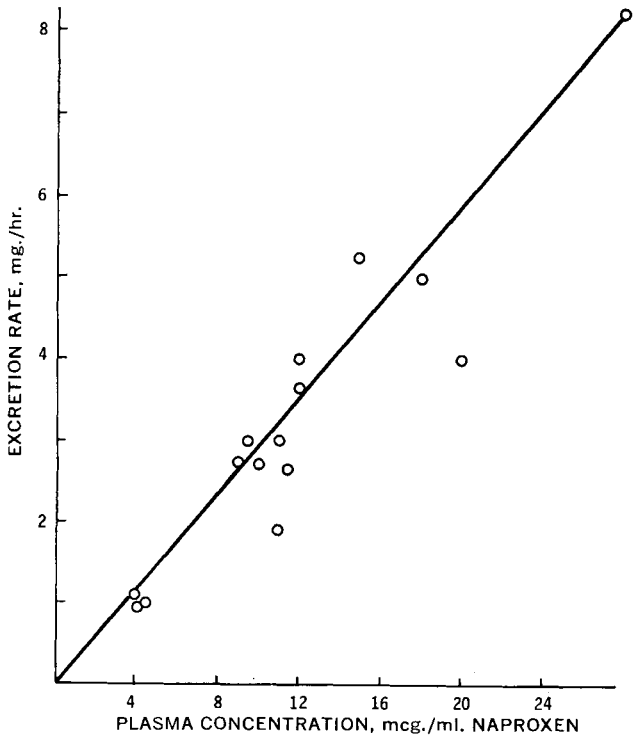

Figure 8—*Relationship of the total urinary excretion rate to plasma concentrations of unchanged naproxen in the three subjects who received radioactive intravenous doses. The milligrams per hour rate was calculated on the basis of the total radioactivity excreted in the urine and includes unchanged drug as well as its biotransformed products.*

identical (Fig. 4). Thus, all the plasma circulating radioactivity in the Day 1 human study was essentially unchanged naproxen.

Excretion—With the exception of the dog, all of the species studied excreted naproxen and its metabolic transformation products predominately in the urine (Table III). The preferred route of excretion in the dog was *via* the feces. Fifty percent of the injected radioactivity in the dog was excreted in the feces, suggesting extensive biliary involvement. Indomethacin (17) is excreted in a similar manner in the dog but apparently to a greater extent. Williams *et al.* (18) reported that solubility characteristics affected the degree of biliary involvement in the excretion of benzoic acid derivatives in the rat, also suggesting that the pKa of these weak acids may determine the mode of disposition.

In the three human subjects studied (Table III), an average of 94% of the injected radioactivity was excreted in the urine after the Day 1 and Day 15 experiments. Of the total radioactivity excreted in the urine, Thompsen (19) found only 5–6% unchanged naproxen. Some 28% was excreted as demethylated naproxen, while the remainder of the dose was in the form of conjugates of the drug, which consisted predominantly of the glucuronide ester. Fecal excretion of radioactivity ranged from 1 to 2% of the injected dose (Table III). Rates of urinary excretion of radioactivity coincided closely with rates of drug disappearance from the plasma. If the conversion products of naproxen are formed in the liver and transported *via* the plasma, clearance of these substances must be instantaneous. That is, the process that ultimately determines the rate of removal from the plasma is the conjugation of naproxen to a substance which is easily cleared by the kidneys. Hoffman and Nobe (20) made the same observation regarding salicylate excretion, but they elaborated by including the possibility that both the liver and the kidneys conjugated the salicylate.

Urinary excretion rates in all human subjects for both intravenous experiments were roughly proportional to the plasma concentrations (Fig. 8). Although the pH of the urine in Subject A on the Day 15 experiment ranged from 5.4 to 7.6, excretion rate fluctuations correlating to the urine pH's were not observed. Alkalinization of the urine enhanced salicylate clearance (21). Gutman *et al.* (21) believed the increased clearance was due to diminished tubular reabsorption of the salicylate anion when urine pH approached the pH of the extracellular fluids. Urine pH may have to reach higher values for naproxen (pKa 5) than for salicylic acid (pKa 3.5) before tubular reabsorption is affected. Presumably, a urinary pH above 7.5–8 would increase the fraction of naproxen excreted unchanged, as occurs with salicylates (19).

Effect of Pretreatment—According to the suggestion of Taylor *et al.* (22), a second intravenous radioactive dose was given following 14 days of continuous exposure to the drug. No change in the rates and routes of elimination was observed in the three human subjects under these conditions. The shapes of the plasma profiles remained the same; and although the average half-life values went from 12.3 to 15.5 hr., this could be considered normal experimental variation. Subject A, for example, excreted 93% of the injected radioactivity in the urine after the initial injection and 94% following the 14-day exposure period. The other subjects behaved similarly, and no signs of accommodation to the drug were observed. In this 2-week period of dosing, therefore, no evidence of induction of metabolizing enzymes was observed.

SUMMARY

Naproxen was found to be rapidly absorbed from the GI tract of all animals tested, with the dog being the most efficient and the rat the least. Blood levels achieved in the human following oral administration were only slightly lower than after rapid intravenous injection and were found to be proportional to the dose over a range of 100–300 mg. The plasma half-life of naproxen ranged from 2 hr. in the rhesus monkey to 35 hr. in the dog, with the value in the human being approximately 14 hr. The species nearest the human in this regard was the guinea pig with a half-life of 9 hr. The distribution of radioactivity in the various tissues and organs of the rat showed that no unusual amount was retained in the animal 24 hr. after administration, nor did there seem to be any selective uptake in any of the tissues analyzed.

Volumes of distribution were found to be uniformly about 0.1 l./kg. in all species. In the human the estimated volume of distribution of naproxen was approximately the same as the value reported for salicylates. Plasma protein binding and pH partitioning behavior of naproxen were suggested as the most likely explanations for a relatively large fraction of the drug being held in the vascular compartment. Minor amounts of radioactivity other than that associated with tritiated naproxen were found in the plasma of the rat and the rhesus monkey. However, following intravenous administration in humans, all of the radioactivity present in the plasma was unchanged drug. With the exception of the dog, all of the species excreted naproxen and its metabolic transformation products predominantly in the urine. In the dog the preferred route was the feces. In the human experiments, 94% of the administered radioactivity appeared in the urine after 5 days, while 1–2% appeared in the feces. Following 14 days of continuous exposure to the drug, the three human test subjects displayed no evidence of induction of metabolizing enzymes.

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ACKNOWLEDGMENTS AND ADDRESSES

Received August 27, 1971, from the *Syntex Research Center, Stanford Industrial Park, Palo Alto, CA 94304*

Accepted for publication January 11, 1972.

The authors gratefully acknowledge the invaluable, expert technical assistance and cooperation of Miss H. Louise Gammell, Miss Karla Kraft, Mr. Ralph Magoun, Mr. Donald Parsons, and Mr. Janos Szakacs.

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Kinetics of Equilibration of Bisulfite and Dexamethasone-21-phosphate in Aqueous Solution

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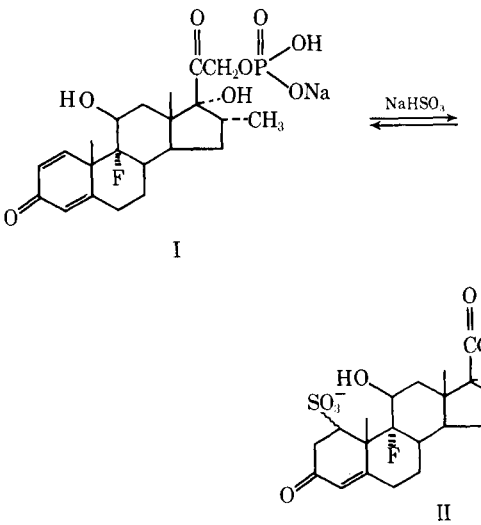
Abstract □ Dexamethasone phosphate in aqueous solution is susceptible to reversible bisulfite addition to form an A-ring-substituted sulfonic acid salt, sodium 16α-methyl-9α-fluorohydrocortisone-1-sulfonate-21-phosphate. Addition of bisulfite and dissociation of adduct proceed at comparable rates in neutral solution. The addition is a second-order reaction in which sulfite ion and monoanionic dexamethasone phosphate are the major participants; that is, a long-range effect of the steroid phosphate group on the A ring is observed. The adduct dissociation is a second-order general-base-catalyzed reaction in which dianionic adduct is more reactive than trianionic adduct. A large isotope effect was observed for dissociations of protonated and C_2-deuterated adducts. Strong dependence of equilibrium steroid composition on temperature was correlated with the temperature dependence of the ionization constant of water.

Keyphrases □ Dexamethasone phosphate and bisulfite—equilibration kinetics in aqueous solution □ Sulfite addition—aqueous solutions of dexamethasone phosphate, kinetics □ Kinetics—sulfite addition to dexamethasone phosphate in aqueous solution □ NMR spectroscopy—structure identification

Sodium dexamethasone-21-phosphate (I) enters a slow reversible reaction with sodium bisulfite, forming sodium 16α-methyl-9α-fluorohydrocortisone-1-sulfonate-21-phosphate (II) (Scheme I). This reaction is typical of conjugate sulfite addition exhibited by α,β-unsaturated ketones, *e.g.*, chalcone (1) and prednisolone phosphate (2). Since sodium bisulfite is commonly employed as an antioxidant in dexamethasone phosphate injection formulations (3), a detailed study of the kinetics and equilibria of this system was undertaken.

The structure of the adduct (II) was determined by NMR spectroscopy. The NMR parameters of the adduct are summarized in the *Experimental* section. It was evident that attack on the A ring of the dexamethasone had occurred since the signals corresponding to the vinylic protons at C_1 and C_2 were absent. The transformation involved more than simple reduction to the hydrocortisone analog, since both the C_4 and 19-methyl resonances were displaced downfield from their characteristic positions in this series. The latter observation strongly suggested that a substituent had been introduced in the A ring, most probably at C_1 in view of the large perturbation of the 19-methyl signal. This inference was supported by the presence of a $-CH_2CH-$ pattern novel to both the dexamethasone and hydrocortisone systems. It was, therefore, reasonable to attribute these signals to protons on C_1 and C_2. The chem-



Scheme I