demic, New York, N.Y., 1961, p. 179.
(10) P. J. Stewart, Ph.D. thesis, University of Queensland, St. Lucia, Australia, 1975.
(11) D. W. Marquardt, *J. Soc. Ind. Appl. Math.*, 11, 431 (1963).
(12) P. J. Davis, in "Survey of Numerical Analysis," J. Todd, Ed., McGraw-Hill, New York, N.Y., 1962, pp. 347, 363.
(13) L. Meites and H. C. Thomas, "Advanced Analytical Chemistry," McGraw-Hill, New York, N.Y., 1958, pp. 129, 131.
(14) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N.Y., 1953, pp. 306, 418.
(15) E. S. Amis, "Solvent Effects on Reaction Rates and Mechanisms," Academic, New York, N.Y., 1966, pp. 59, 69.
(16) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed., Wiley, New York, N.Y., 1961, pp. 123, 157.

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Effects of Aspirin on ^{14}C-Pirprofen Disposition in Rats

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Abstract □ The effects of aspirin on ^{14}C-pirprofen disposition in the rat were studied. An oral 60-mg/kg dose of aspirin significantly reduced plasma radioactivity during the 1–8-hr interval after an intravenous 5-mg/kg injection of ^{14}C-pirprofen. The aspirin-treated group had only 69% as much area under the radioactivity curve as the control group. The radioactive material in plasma consisted almost entirely of ^{14}C-pirprofen, as shown by GLC. The plasma clearance of ^{14}C-pirprofen was 7.4 ml/hr for the aspirin-treated group and 5.1 ml/hr for the control group, while the volumes of distribution were 0.32 and 0.20 liter/kg, respectively. The apparent elimination half-life was unchanged at 5.9 hr. ^{14}C-Pirprofen was approximately 98.6% bound to plasma proteins, and the binding decreased to an average of 97.2% in the presence of salicylate. Binding to blood cellular constituents was insignificant. Rats given ^{14}C-pirprofen by intravenous injection without aspirin secreted 36.0–42.8% of the dose radioactivity into bile during 4 hr while a comparable group given 60 mg of aspirin/kg secreted 46.4–70.8%. TLC and GLC demonstrated that the radioactivity in rat bile was 80–90% conjugated ^{14}C-pirprofen. The increased radioactive material secretion into bile was compensated in the intact rat by reabsorption, since the total radioactive material excreted in urine was not changed by aspirin administration.

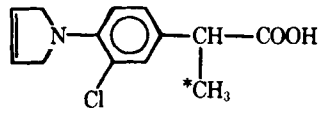
Keyphrases □ Aspirin—effect on pirprofen disposition, rats □ Pirprofen—disposition, effect of aspirin, rats □ Anti-inflammatory agents—pirprofen, disposition, effect of aspirin, rats

Pirprofen, 2-[3-chloro-4(3-pyrrolinyl)-phenyl] propionic acid, is a new anti-inflammatory drug of the arylalkanoic acid type (1).

Salicylate has been reported to reduce the plasma concentration of various arylalkanoic acid compounds including indomethacin (2), fenoprofen (3, 4), naproxen (5, 6), and diclofenac (7) in animals and humans. Pirprofen concentrations in human plasma as well as that bound to plasma proteins were reduced by salicylate[1], and the present study was undertaken to examine salicylate effects on the biological disposition of pirprofen in the rat.

EXPERIMENTAL

Radioactive Pirprofen—^{14}C-Pirprofen (I), with the radioactive atom in position three of the propionic acid moiety, was used[2]. The specific



I

activity was 13 μCi/mg. The labeled compound was examined by TLC using the procedures described later. The major constituent chromatographed like authentic pirprofin, and the only impurity was a substance comprising about 5% of the radioactivity.

Animal Procedures—Young adult male Wistar rats[3], ~200 g, were used.

^{14}C-Pirprofen dosage solutions were prepared in 0.2 *M* $NaHCO_3$ to provide 5 mg/kg and 3 μCi/animal in volumes of 0.3 ml for injection and 1.0 ml for intubation. Aspirin[4] was administered as a 60-mg/kg aqueous dose by intubation.

Blood samples were obtained by anesthetizing the rats with ether, opening the abdominal cavity, and exsanguinating *via* the abdominal aorta into a heparinized 10-ml syringe. Blood plasma was frozen pending analysis.

Bile duct cannulations were done under ether anesthesia for rats that were to receive intravenous ^{14}C-pirprofen, and these rats were confined in restraining cages[5] during bile collection. Rats receiving ^{14}C-pirprofen orally were anesthetized with urethan[4] rather than ether to preclude the necessity for restraint. A 25% solution of urethan in water was administered as a 1.25-g/kg ip dose.

Radioactivity Measurements—A solubilizer–phosphore solution was prepared by mixing one volume of a commercial solubilizer[6] with four volumes of toluene containing 0.5% 2,5-diphenyloxazole[7] and 0.01% *p*-bis(*o*-methylstyryl)benzene[8]. The solubilizer–phosphore solution (15 ml) was mixed with 10 μl of injection solutions, 50 μl of ultrafiltrates, or 0.1 ml of plasma, bile, or urine prior to duplicate sample counting in a liquid scintillation spectrometer[9]. Counting efficiency was measured by external standardization.

Whole blood (0.1 ml) was pipetted onto 2.5-cm circles of filter paper and dried before combustion[10]. The resulting carbon dioxide was absorbed in 4 ml of ethanolamine to which 9 ml of methanol and 6 ml of scintillator solution, containing 15.0 g of 2,5-diphenyloxazole and 1.0 g of *p*-bis(*o*-methylstyryl)benzene/liter of toluene, were added for counting.

TLC—Aliquots (20 μl) of bile or an equivalent amount of hydrolyzate

[1] To be published.
[2] The material was synthesized by Dr. Naba K. Chaudhuri and coworkers at Ciba-Geigy Corp., Ardsley, NY 10502.
[3] Charles River C.D. or Marland Farms.
[4] Merck and Co., West Point, PA 19486.
[5] Aerospace Industries, Garnerville, NY 10923.
[6] Bio-Solv BBS-3, Beckman Instruments, Fullerton, CA 92634.
[7] Eastman Kodak Co., Rochester, NY 14650.
[8] Packard Instrument Co., Downers Grove, IL 60515.
[9] Intertechnique SL-40, IN/US Service Corp., Fairfield, NJ 07006.
[10] Model 305 oxidizer, Packard Instrument Co., Downers Grove, IL 60515.

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Table I—Effect of Salicylate on ^{14}C-Pirprofen Radioactivity in Rat Plasma

Hours Postdose[a]	Concentration, average μg/ml[b] ± SD		
	Apparent ^{14}C-Pirprofen		
	Without Aspirin	With Aspirin	Salicylate
0.1	35.9 ± 1.6	31.0 ± 1.4	114 ± 8
0.25	31.1 ± 4.8	24.1 ± 4.0	127 ± 10
0.5	25.8 ± 2.0	19.9 ± 3.8	111 ± 6
0.75	24.2 ± 2.4	17.5 ± 1.2	121 ± 12
1.0	22.0 ± 1.4	13.3 ± 1.0	118 ± 8
2.0	18.6 ± 2.8	10.7 ± 2.2	113 ± 8
4.0	16.1 ± 1.2	10.3 ± 1.4	91 ± 26
6.0	11.3 ± 0.4	6.4 ± 1.0	66 ± 22
8.0	9.8 ± 1.6	5.7 ± 1.0	63 ± 16
11.0	4.1 ± 0.8	3.6 ± 0.2	11 ± 14
14.0	4.1 ± 1.2	3.0 ± 0.4	2 ± 0
17.0	2.3 ± 0.8	2.1 ± 0.8	—

[a] Rats were intubated with 60 mg of aspirin/kg in solution or with an equal volume of water and injected intravenously 1 hr later with 5 mg of ^{14}C-pirprofen/kg. [b] Four rats at each time point, except the aspirin-treated group at 4 hr which had three.

were spotted on silica gel plates[11] and developed in chloroform–methanol–formic acid (70:30:1) or benzene–ethanol–formic acid (90:10:1). The dried plates were examined under UV light and scanned for radioactivity[12].

Pirprofen Analysis—Pirprofen was determined quantitatively (8), using 3.0-ml aliquots of rat plasma containing 0–120 μg of pirprofen. The conjugated pirprofen in bile was hydrolyzed before analysis. Bile (0.2 ml) was mixed with 50 μl of 0.5 *N* KOH and stored 15 min at room temperature. The reaction mixture was neutralized with hydrochloric acid, and 1.0 ml of 0.4 *M*, pH 5.4 acetate buffer was added. Pirprofen was extracted by adding 2.0 ml of 20% methylene chloride in ether and rotating[13] for 20 min. The extraction was repeated, and the combined extracts were processed as described previously (8).

Protein Binding Measurement—Cellulose dialyzer tubing[14] was soaked 0.5 hr in water, and excess water was removed by blotting. A 20-cm length of tubing was formed into a U-shape, 2.0 ml of plasma was introduced, and the tubing was placed into a 2.8 × 10-cm polypropylene centrifuge tube containing eight or 10 0.5-cm diameter glass beads. A cork was used to close the tube and wedge the ends of the tubing with the bottom of the loop supported on the beads. Centrifugation at 1100×*g* for 30 min produced ~100 μl of ultrafiltrate, and 50-μl aliquots were taken for radioactivity counting.

Salicylate Analysis—Plasma (0.5 ml) was mixed with 1.0 ml of water, 0.3 ml of 6 *N* HCl, and 20 ml of methylene chloride. The tubes were rotated 30 min and centrifuged. The aqueous phase was aspirated off, and 10.0 ml of organic phase was mixed with 10.0 ml of 1 *N* NaOH. After rotating for 25 min and centrifuging, the aqueous phase fluorescence was measured[15] at an excitation wavelength of 310 nm and an emission wavelength of 400 nm.

RESULTS

The radioactivity concentrations found in the plasma of control and aspirin-treated rats are shown in Table I, expressed as the average and standard deviation for micrograms of apparent ^{14}C-pirprofen per milliliter. Lower apparent pirprofen concentrations were found for the aspirin-treated rats. Table I also contains the mean and standard deviation for the salicylate concentrations. Selected plasma samples from the same experiment also were analyzed for unbound radioactivity (Table II). For the aspirin-treated animals, binding was decreased slightly until the plasma salicylate concentrations declined to low values, at which point the binding had returned to the control value.

The radioactivity concentration in a few whole blood samples also was measured. No association of radioactive material with the cellular blood constituents was found.

A separate experiment was done to obtain sufficient plasma for quantitative pirprofen determination by GLC. As shown in Table III, very good agreement was found by radiometric and chromatographic analyses.

Table II—Effect of Aspirin on Binding of ^{14}C-Pirprofen by Rat Plasma Proteins *In Vivo*[a]

Time after ^{14}C-Pirprofen, 5 mg/kg iv	Plasma ^{14}C-Pirprofen, mean μg/ml ± SD	Plasma Salicylate, mean μg/ml ± SD	Binding, % ± SD
Controls—no aspirin			
5 min	35.9 ± 1.6	—	98.8 ± 0.4
6 hr	11.3 ± 0.4	—	98.6 ± 0.4
14 hr	4.1 ± 1.2	—	99.6 ± 0.2
Aspirin, 60 mg/kg po			
5 min	31.0 ± 1.4	114 ± 8	96.6 ± 1.4
6 hr	6.4 ± 1.0	66 ± 22	96.5 ± 1.2
14 hr	3.0 ± 0.4	2 ± 0	99.6 ± 0.2

[a] Four rats at each time point.

The binding of ^{14}C-pirprofen to rat plasma proteins also was measured in the presence and absence of salicylate in an *in vitro* experiment. ^{14}C-Pirprofen (17 μg/ml) was added to pooled plasma from control rats, and sodium salicylate (100 μg/ml) was added to half of the plasma aliquots prior to a 30-min equilibration and binding measurements. All three control samples showed 98.6% binding. Samples containing salicylate had values of 97.6, 97.7, and 97.6%.

The urinary radioactivity excretion after 5 mg of ^{14}C-pirprofen/kg iv was measured after intubation with 60 mg of aspirin/kg or an equal volume of water. As shown in Table IV, the amount and time course for urinary radioactivity excretion were not altered by aspirin administration under these conditions.

The effect of aspirin on biliary secretion of radioactivity from ^{14}C-pirprofen was examined in bile duct-cannulated rats. Rats were given 60 mg of aspirin in aqueous solution/kg or an equal volume of water by intubation. The bile ducts were cannulated. One hour after the aspirin or water intubation, the animals were given 5 mg of ^{14}C-pirprofen/kg by tail vein injection or by intubation. The bile volume collected each hour was not different for the aspirin and water-treated rats. The radioactivity secretion into bile was much greater for the aspirin-treated rats than for the control rats (Fig. 1). Similar results were seen after oral rather than intravenous ^{14}C-pirprofen administration.

TLC of rat bile collected within 2 hr of oral ^{14}C-pirprofen administration showed one major constituent, which comprised 80–90% of the radioactivity. Four or five minor, unknown metabolites also were present. None of these constituents was identical to pirprofen. Base treatment

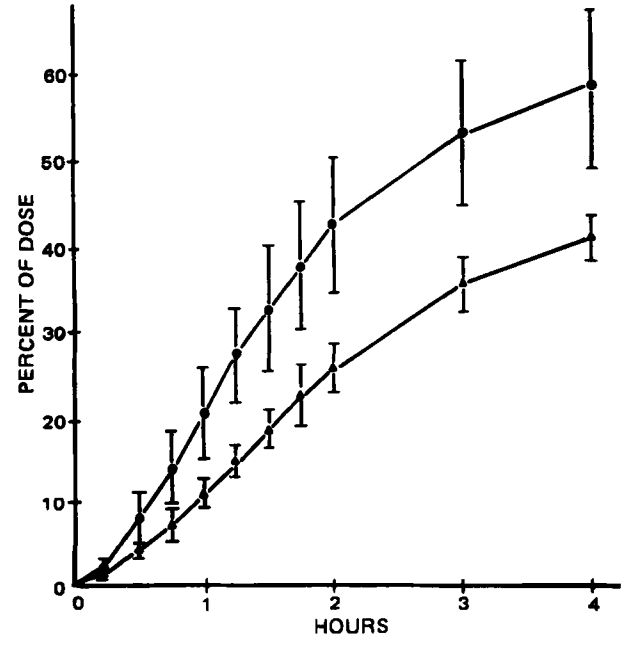

Figure 1—*Effect of 60 mg of aspirin/kg po on biliary radioactivity in rats after 5 mg of ^{14}C-pirprofen/kg iv, showing mean and standard deviation for five rats per treatment. Key: ●, aspirin-treated rats; and ▲, control rats.*

[11] E. Merck, 0.25-mm type 60 F-254 plates, EM Laboratories, Elmsford, NY 10523.
[12] Berthold radioscanner, Varian Instrument Division, Palo Alto, CA 94303.
[13] Rugged rotator model RD-250, Kraft Apparatus, South Richmond Hill, NY 11419.
[14] Catalog No. 3787-D22, 1.5-cm diameter, Arthur Thomas Co., Philadelphia, PA 19105.
[15] Model MPF-2A spectrofluorometer, Perkin-Elmer Corp., Norwalk, CT 06856.

Table III—Radioactivity and Pirprofen in Rat Plasma after 5 mg of ^{14}C-Pirprofen/kg iv

Hours Postdose	Concentration, μg/ml[a] Liquid Scintillation Counting	GLC Analysis
0.25	23.6	28.6
0.25	25.6	22.2
1	19.4	19.4
1	16.4	14.8
3	10.6	9.6
3	8.2	7.3
6	8.7	6.0
6	5.9	4.6
12	2.4	2.0
12	1.1	1.2
18	0.8	0.6
18	0.2	0.9
24	0.4	<0.1

[a] Plasma from three rats was pooled and analyzed in duplicate by the two methods.

of bile resulted in a change in the TLC pattern. The major radioactive constituent was converted to a material that behaved like pirprofen on TLC in two different solvent mixtures. Quantitative analysis by GLC confirmed that the hydrolyzed material was pirprofen.

DISCUSSION

Oral administration of aspirin (60 mg/kg) to rats 1 hr before intravenous ^{14}C-pirprofen administration (5 mg/kg) resulted in markedly reduced radioactive material in plasma, and GLC analysis showed the radioactive material to be unchanged ^{14}C-pirprofen. The effect was significant ($p = 0.01$) during the 1–8-hr interval and decreased as salicylate concentrations declined to average values of 11 μg/ml or less (Table I). Comparison of area under the curve (*AUC*) values, calculated from time zero to infinity, showed the aspirin group to have 69% as much area as the control group.

Accordingly, pirprofen is another example of an arylalkanoic acid for which concentrations in plasma are reduced by salicylate. Yesair *et al.* (2) found that salicylate altered indomethacin disposition in the rat, resulting in reduced concentrations in plasma, decreased excretion in urine, and increased elimination *via* the bile and feces. For pirprofen, aspirin had no effect on the amount of radioactivity excreted in urine. Four rats excreted 74.9 ± 4.5% of the radioactivity from a dose of 5 mg of ^{14}C-pirprofen/kg iv in urine during 48 hr when given 60 mg of aspirin/kg po. This finding can be compared to 77.4 ± 2.3% for four rats given ^{14}C-pirprofen without aspirin (Table IV).

A similar situation was described by Rubin *et al.* (3) for fenoprofen in humans. The fenoprofen concentration in plasma and the *AUC* were reduced by concomitant aspirin administration without a change in the total amount of material excreted in urine. This finding could result from increased clearance by the liver if the rate with which metabolites are released into blood by the liver is greatly exceeded by renal metabolite clearance. The metabolites will be present in plasma only in low concentrations and will contribute little to the *AUC* for radioactive material, while the bile serves as a depot due to increased recycling.

Aspirin administration with ^{14}C-pirprofen greatly affected biliary radioactivity in rats. Five rats given intravenous ^{14}C-pirprofen without aspirin secreted 40.5 ± 2.7% of the dose radioactivity into bile during 4 hr, while a comparable aspirin-treated group secreted 58.6 ± 9.6% (Fig. 1). The bile production rate was similar for the aspirin-treated and the control animals, precluding increased choleresis as a factor. Similar results were obtained on administration of ^{14}C-pirprofen by intubation rather than by intravenous injection.

The major radioactive constituent (80–90%) in bile of both the aspirin-treated and the control animals was a material that was converted on alkaline hydrolysis to a product indistinguishable from pirprofen by TLC and GLC. In the intact rat, reabsorption compensated for the increased secretion into bile caused by aspirin, as indicated by unaltered urinary excretion.

Calculation of plasma clearance according to the equation:

$$\text{clearance} = \frac{\text{dose}}{AUC} \qquad \text{(Eq. 1)}$$

Table IV—Effect of Aspirin on Urinary Excretion of Radioactivity by Rats after an Intravenous Dose of ^{14}C-Pirprofen

Postdose[a], hr	Average ± *SD* for Cumulated Percent of Dose[b] Control Rats	Aspirin-Treated Rats
1	6.0 ± 1.7	4.9 ± 4.4
2	11.7 ± 4.7	13.0 ± 1.8
3	19.3 ± 5.8	18.2 ± 3.1
4	21.4 ± 6.2	25.5 ± 4.3
6	34.5 ± 4.8	37.8 ± 7.0
8	42.0 ± 7.2	44.6 ± 9.4
10	51.7 ± 8.4	51.3 ± 7.0
24	74.3 ± 3.9	70.2 ± 7.1
48	77.4 ± 2.3	74.9 ± 4.5

[a] Rats were intubated with 60 mg of aspirin/kg in 10 ml of aqueous solution or with an equal volume of water and injected intravenously 1 hr later with 5 mg of ^{14}C-pirprofen/kg. [b] Each group contained four rats.

gave values of 5.1 ml/hr for control rats and 7.4 ml/hr for aspirin-treated rats. The apparent volumes of distribution were 0.20 liter/kg for the control group and 0.32 liter/kg for the aspirin group. As a result of the compensatory changes in plasma clearance and apparent volume of distribution, no change in half-life resulted from aspirin administration; half-life values of 5.9 hr were found for both the aspirin and control groups.

Rubin *et al.* (4) reported that the fenoprofen half-life in humans was decreased by aspirin, but the change only became apparent after repeated fenoprofen and aspirin administration. A short study reported in the same publication showed no change in either the half-life or the *AUC* for indomethacin in humans, although the indomethacin concentrations were reduced by aspirin in the early part of the plasma curve.

Binding of ^{14}C-pirprofen to plasma proteins in the rat was extensive and reduced slightly by salicylate. Binding of 98.6% was reduced by salicylate to an average value of 97.2% in the *in vivo* and *in vitro* experiments. This modest decrease in binding represents a marked increase in the unbound drug availability. Binding to the blood cellular constituents did not occur in either the presence or absence of salicylate.

Pirprofen, indomethacin (9), naproxen (5, 6), and fenoprofen (3) are all highly bound to plasma proteins, and all except fenoprofen are displaced by salicylate. Chaplin (5) suggested that the mechanism by which aspirin reduced plasma naproxen concentrations was by increasing the unbound naproxen availability for transformation or excretion. Evidently, this factor is not the sole causative mechanism for decreasing the plasma concentrations of all arylalkanoic acids, because Rubin *et al.* (3) found that fenoprofen was not displaced. An additional factor such as altered hepatic extraction or increased splanchnic blood flow may be involved.

In conclusion, concomitant administration of aspirin and ^{14}C-pirprofen to rats reduced plasma concentrations and reduced *AUC* values of radioactive material. No change in half-life was found, due to increases in both the plasma clearance and the volume of distribution. Binding to plasma proteins decreased slightly. Urinary radioactivity excretion was unchanged by aspirin administration. Secretion of pirprofen metabolite(s) into bile was increased but was compensated by reabsorption.

REFERENCES

(1) R. W. J. Carney, J. J. Chart, R. Goldstein, N. Howie, and J. Wojtkunski, *Experientia*, **29**, 938 (1973).

(2) D. W. Yesair, L. Remington, M. Callahan, and C. J. Kensler, *Biochem. Pharmacol.*, **19**, 1591 (1970).

(3) A. Rubin, P. Warrick, R. L. Wolen, S. M. Chernish, A. S. Ridolfo, and C. M. Gruber, Jr., *J. Pharmacol. Exp. Ther.*, **183**, 449 (1972).

(4) A. Rubin, B. E. Rodda, P. Warrick, C. M. Gruber, Jr., and A. S. Ridolfo, *Arthritis Rheum.*, **16**, 635 (1973).

(5) M. D. Chaplin, *Biochem. Pharmacol.*, **22**, 1589 (1973).

(6) E. J. Segre, M. Chaplin, E. Forchielli, R. Runkel, and H. Sevelius, *Clin. Pharmacol. Ther.*, **15**, 374 (1974).

(7) F. O. Muller, H. K. L. Hundt, and D. G. Muller, *Int. J. Clin. Pharmacol.*, **15**, 397 (1977).

(8) R. C. Luders, M. B. Maggio-Cavaliere, H. Egger, O. B. Gum, O. Resnick, M. F. Bartlett, M. J. Gaito, A. Soo, and C. Li, *Clin. Pharmacol. Ther.*, **21**, 721 (1977).

(9) R. W. Mason, and E. G. McQueen, *Pharmacology (Basle)*, **12**, 12 (1974).