Effect of Aspirin on Fate of ^{14}C-Acetaminophen in Guinea Pigs

L. W. WHITEHOUSE [x], C. J. PAUL, and B. H. THOMAS

Abstract □ The interaction of ^{14}C-acetaminophen, 150 mg/kg (20 μCi/kg), and orally administered aspirin, 200 mg/kg, was studied in male guinea pigs. Aspirin-pretreated animals possessed higher ^{14}C blood levels than controls. Paper chromatography of 0–6-hr urines demonstrated that pretreated animals excreted significantly greater amounts of mercapturate than controls; however, it was only a minor metabolite, accounting for 1–3% of the counts in the urine. The major metabolite, the glucuronide, accounted for 90% of the counts, with free acetaminophen and its sulfate responsible for the remaining counts. Tissue distribution studies indicated that blood plasma and kidneys from aspirin-pretreated animals possessed statistically higher ^{14}C levels than did control tissues. Bile duct and ureter cannulation experiments indicated that aspirin inhibited the concentrating processes into the urine and bile.

Keyphrases □ Acetaminophen, radiolabeled—metabolism, effect of aspirin pretreatment, guinea pigs □ Aspirin—effect of pretreatment on ^{14}C-acetaminophen metabolism, guinea pigs

The restriction of phenacetin has caused acetaminophen, the principal metabolite of phenacetin, to come into widespread use. Since acetaminophen is now being used in combination with aspirin in some analgesic formulations, a study of the effect of aspirin upon acetaminophen was deemed desirable. A previous report (1) showed that, in rats, aspirin caused: *(a)* a reduction in the rate of acetaminophen absorption from the GI tract, *(b)* an enhanced blood level of radioactivity during the postabsorptive phase, *(c)* large changes in the proportions of acetaminophen and its metabolites excreted in the urine, *(d)* reduction in sulfate conjugation, and *(e)* an increase in glucuronide and mercapturate conjugation.

Amsel and Davison (2), studying the simultaneous metabolism of aspirin and acetaminophen in humans, found that aspirin had no effect on the formation of acetaminophen metabolites. This report confirmed earlier work in which a similar lack of effect was observed with lower doses (1.0 g) of sodium salicylate (3). In the present study the effect of aspirin upon the fate of acetaminophen in the guinea pig was investigated to determine if this species would serve as a better animal model than the rat for toxicological studies of this particular drug combination.

EXPERIMENTAL

Materials—^{14}C - Acetaminophen (*N* - acetyl - *p* - aminophenol-ring-UL-^{14}C)[1], with a specific activity of 44.64 μCi/mg, was custom-synthesized[2]. Aspirin[3], unlabeled acetaminophen[4], and gum tragacanth[5] were obtained commercially. Male Hartley strain guinea pigs, 250–350 g, were purchased locally[6].

[1] UL = uniformly labeled.
[2] Mallinckrodt, St. Louis, MO 63160
[3] B.D.H. Canada Ltd., Toronto, Canada.
[4] Matheson, Coleman and Bell, Norwood, Ohio.
[5] Fisher Scientific Co. Ltd., Toronto, Canada.
[6] High Oak Ranch, Goodwood, Ontario, Canada.

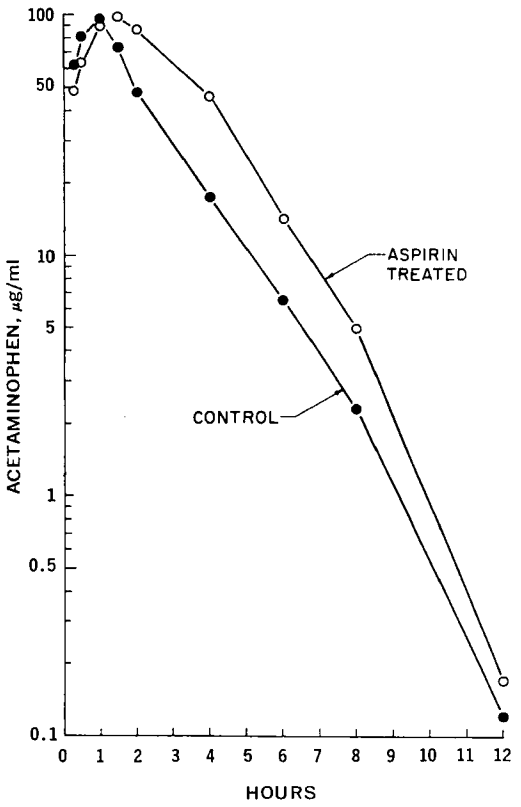

Figure 1—*Effect of aspirin (200 mg/kg) upon the blood profile of orally administered ^{14}C-acetaminophen (150 mg/kg). Blood concentrations of radioactivity are expressed as micrograms of acetaminophen per milliliter of blood. Values are means from five guinea pigs, with differences at 1.5, 2, and 4 hr being significant at* $p < 0.05$.

Methods—*Blood Profiles*—Guinea pigs were randomly divided into two groups of five animals each and deprived of food but not water for 16 hr. In the oral dosing study, all treatments were administered orally in volumes of 10 ml/kg as 0.25% gum tragacanth suspensions. One group of animals received aspirin (200 mg/kg) while the control group received 0.25% gum tragacanth. ^{14}C-Acetaminophen [150 mg/kg (20 μCi/kg)] was administered to both groups 30 min later.

Duplicate blood samples (10 μl) were collected from the toe at 0.25, 0.50, 1, 1.5, 2, 4, 6, 8, and 12 hr after dosing. The blood was digested and counted in a liquid scintillation counter as previously described (4). Urine was also collected at 6, 12, 24, and 48 hr after dosing and radioactivity was determined (4).

In the intraperitoneal dosing experiments, ^{14}C-acetaminophen [150 mg/kg (20 μCi/kg)] was administered in volumes of 7 ml/kg in a 10% ethanol solution, but the other routes of administration, dosing solutions, and procedures were otherwise identical to those used in the oral dosing study.

Urine Metabolites—Urines collected in the oral dosing experiments (0–6 hr) were chromatographed on medium flow rate chromatography paper[7]. Urines (10 μl) were applied as bands, and the chromatograms were developed using the alkaline paper chroma-

[7] Whatman No. 1.

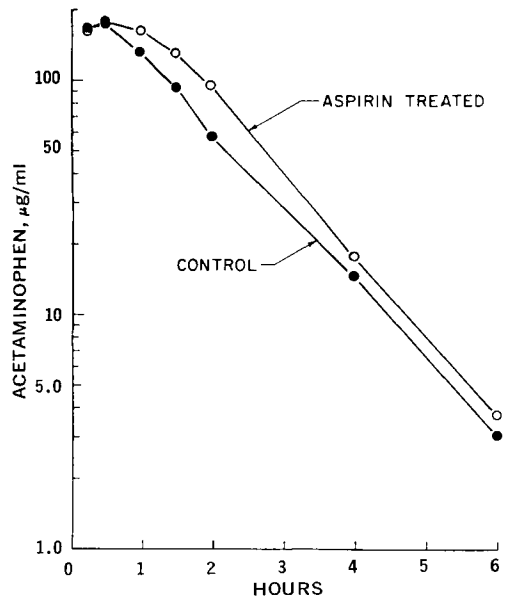

Figure 2—*Effect of aspirin (200 mg/kg) upon the blood profile of intraperitoneally administered ^{14}C-acetaminophen (150 mg/kg). Blood concentrations of radioactivity are expressed as micrograms of acetaminophen per milliliter of blood. Values are means from five guinea pigs, with differences at 1, 1.5, and 2 hr being significant at* $p < 0.05$.

tography system described by Shahidi (5, System C). The developed chromatograms were serially sectioned into 1-cm strips and radioactivity was determined by liquid scintillation counting.

Tissue Distribution—While employing the dosage regimen used in the intraperitoneal blood profile study, levels of radioactivity were estimated in duplicate blood, plasma, kidney, and liver samples collected 90 min following ^{14}C-acetaminophen administration. A previously described method for preparing samples for liquid scintillation counting was used (4). In the case of bile, 10-μl samples were treated similarly to blood for estimation of radioactivity.

Levels of ^{14}C in the intestinal contents were determined by making the intestinal contents and washings to a constant volume of 10

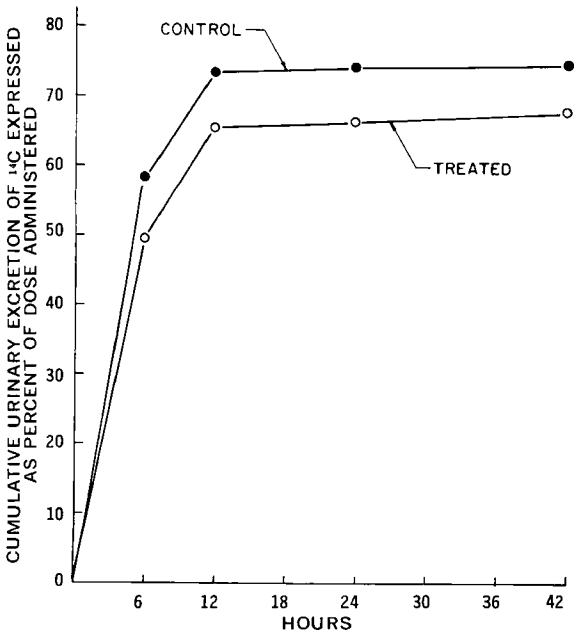

Figure 3—*Effect of aspirin (200 mg/kg) upon the cumulative urinary excretion of orally administered ^{14}C-acetaminophen (150 mg/kg). Values are means from five guinea pigs. A statistical difference of* $p < 0.05$ *was observed only at 12 hr.*

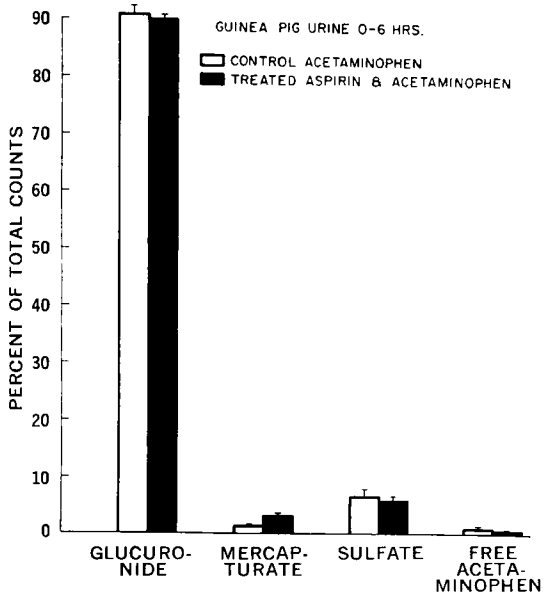

Figure 4—*Urinary excretion of acetaminophen and its metabolites in guinea pigs orally dosed with ^{14}C-acetaminophen (150 mg/kg). Values are means from five animals per group* ± SE.

ml with distilled water. Duplicate 0.5-ml aliquots were then dried in scintillation vials; the residue, digested with 1.0 ml of tissue digestant[8], was treated similarly to kidney and liver tissues.

Bile Duct and Ureter Cannulations—A median incision just below the xiphisternum was made in the abdomen of ether-anesthetized male guinea pigs. The common bile duct was exposed and surgical thread was positioned under the duct. A small incision was made in the upper part of the duct; polyethylene tubing[9] was inserted upward and tied into place. After stitching the abdominal incision, animals were placed in restraining cages and allowed to recover from the anesthesia.

Following recovery (at least 1 hr), animals were treated with acetaminophen and aspirin or gum tragacanth; the intraperitoneal dosage regimen used in the blood profile experiment was employed. Blood and bile were collected every 15 min for 180 min, and the concentration of radioactivity was determined by liquid scintillation counting using 10-μl samples.

In another group of animals, the left ureters were similarly exposed and cannulated while the right ureters were ligated close to the kidneys. Following recovery from anesthesia, animals were treated according to the intraperitoneal dosage regimen. Blood and urines were collected every 15 min, and the levels of ^{14}C in blood (10 μl) and urine (total) were determined as previously described (4).

RESULTS

Blood concentrations of radioactivity in treated and control animals dosed by the oral route are shown in Fig. 1. Animals pretreated with aspirin showed a 30-min delay in peaking of blood levels of radioactivity; only at 1.5, 2, and 4 hr did pretreated animals show significantly higher ^{14}C blood levels than did controls. A closer examination of the blood profiles indicated that aspirin did not change the half-life of ^{14}C-acetaminophen ($T_{1/2}$ = 1.40 hr, control; $T_{1/2}$ = 1.39 hr, aspirin pretreatment), but the total areas under the blood profile curves were significantly greater in pretreated animals (350.4 μg/ml × hr) than in controls (235.8 μg/ml × hr).

Initially, these results were attributed to inhibition of acetaminophen absorption from the GI tract by aspirin. An intraperitoneal study, in which acetaminophen was administered intraperitoneally while aspirin pretreatment was given orally, resulted in a blood profile (Fig. 2) similar to that obtained in the oral study (Fig. 1). This suggested that the delay in the blood profile was not caused

[8] Soluene, Packard Instrument Co., Downers Grove, Ill.
[9] PE-50, Clay-Adams, New York, N.Y.

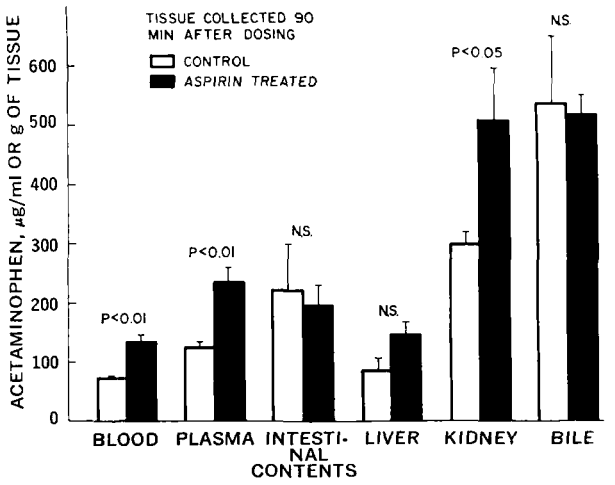

Figure 5—*Tissue distribution of radioactivity in control and aspirin-pretreated guinea pigs. Concentration is expressed as micrograms of acetaminophen per milliliter or gram of tissue. Values are means of five animals per group* ± SE.

by an inhibition of absorption due to a chemical interaction between the two chemicals in the GI tract.

The cumulative urinary excretion in orally dosed animals is presented in Fig. 3. Only at 12 hr did aspirin-pretreated animals excrete a significantly lower percentage of the administered dose than did controls. At the other time periods studied, the same trend was observed, although statistical significance at the 0.05 probability level could not be demonstrated.

Paper chromatography of the 0–6-hr urine samples collected from the oral dosing experiments separated the radioactivity into four distinct peaks, identified by R_f values from a previous study as the glucuronide, mercapturate, sulfate, and free acetaminophen (1). As shown in Fig. 4, the major metabolite in the urines of both treated and control guinea pigs was the glucuronide, which represented at least 90% of the total radioactivity excreted. Pretreatment with aspirin caused only a slight, but significant, increase in the percentage of mercapturate excreted in the urine.

A tissue distribution study of radioactivity (Fig. 5) showed that blood, plasma, and kidney levels of radioactivity were statistically higher in aspirin-pretreated animals than in controls. Livers showed the same trend, although the difference was not significant. The level of radioactivity in the intestinal contents and bile of aspirin-pretreated and control animals did not differ statistically, although the trend was for the control group to possess higher levels of ^{14}C than the pretreated group, suggesting that aspirin was inhibiting the biliary excretion of acetaminophen. A comparison of the cumulative biliary ^{14}C excreted in 180 min with the total area under the blood profile curve for the same period gave ratios for aspirin-pretreated animals that did not statistically differ from control ratios. However, a statistical difference was demonstrated in the bile to blood ratio of radioactivity at 60 min. Three control guinea pigs possessed a 7.07 ± 0.82- (mean ± *SE*) fold higher concentration of ^{14}C in the bile than in the blood, while three treated animals gave a ratio of 3.47 ± 0.40.

Because biliary excretion over the 3-hr period studied accounted for only 3–10% of the dose administered while urinary excretion over 6 hr accounted for as much as 50–60% (Fig. 3), the effect of aspirin on the urinary excretion of acetaminophen in ureter-cannulated animals was examined. Blood and urine were collected every 15 min for a total of 180 min. Cumulative radioactivity excreted in 180 min in the urine over the total area under the blood profile curve for the same period gave a mean ratio of 1.22 ± 0.08 for four control animals, which was significantly greater than 0.85 ± 0.07, the mean ratio obtained from six aspirin-pretreated animals.

DISCUSSION

The combination of orally administered aspirin and acetaminophen appeared to reduce the rate of acetaminophen absorption during the absorptive phase (Fig. 1). Despite the slower rate of absorption, peak blood levels of radioactivity were comparable in the control and aspirin-pretreated animals. Similarly, comparable peak blood levels of radioactivity were observed in the intraperitoneal study for the treated and control groups (Fig. 2).

Data from the urinary metabolite study (Fig. 4) indicated that aspirin had no effect upon the sulfation or glucuronidation of acetaminophen in guinea pigs, an observation previously reported for humans (2). A significant increase in the percentage of mercapturate excreted in the urines of aspirin-pretreated animals was observed. Since this metabolite only accounted for 1–3% of the ^{14}C in the urine samples, the difference in metabolism between treated and control animals was not considered a likely contributor to the observed effect of aspirin on the blood profile of radioactivity.

The most significant effect of aspirin was the short-term effect on biliary and urinary excretion of radioactivity. Bile to blood ratios were significantly lower in aspirin-pretreated animals, suggesting that plasma clearance of acetaminophen by the liver was being inhibited by the pretreatment. Since differences in the major metabolites in the urine could not be demonstrated (Fig. 4), reduction in clearance could not be attributed to the inhibition of hepatic metabolism as previously reported for the rat (1). The 180-min cumulative urinary excretion of ^{14}C, adjusted for differences in blood levels of radioactivity, indicated that excretion by the kidney was also being suppressed by aspirin pretreatment, an effect previously reported for the clearance of *p*-aminohippuric acid from human plasma (6).

Since aspirin, acetaminophen, and its major metabolites are acidic drugs excreted in part by the kidney tubules *via* an active excretory process (7, 8), the most likely explanation for the observed interaction is a competition of both drugs for a common anionic excretory mechanism. Because aspirin is a stronger acid, it is preferentially excreted, resulting in higher blood levels of acetaminophen.

In humans, aspirin does not appear to inhibit the glucuronide or sulfate conjugation of acetaminophen (2), but urinary excretion of acidic drugs is reduced (6). Since aspirin-pretreated guinea pigs appeared to respond similarly to humans and the proportions of glucuronide and sulfate resembled those in humans (9), this species probably would serve as a better animal model than the rat for further toxicological studies.

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