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IN VIVO QUANTIFICATION OF RENAL GLUCURONIDE AND SULFATE CONJUGATION OF 1-NAPHTHOL AND *p*-NITROPHENOL IN THE RAT

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Abstract—The simultaneous *in vivo* renal sulfate and glucuronide conjugations of 1-naphthol (1-N) and *p*-nitrophenol (PNP) were determined in the rat. In mammals, 1-N and PNP are excreted almost entirely in the urine, mainly as the glucuronide and sulfate conjugates. In male Sprague–Dawley rats, greater than 98% of the infused [^{14}C]1-N (1.0 μmole·min^{-1}·kg^{-1}) or [^{14}C]PNP (2.0 μmoles·min^{-1}·kg^{-1}) recovered in urine was identified as the sulfate and glucuronide conjugates. Renal metabolism accounted for a minimum of 20% of the endogenously formed conjugates of either substrate excreted in the urine. The rat kidney formed the glucuronide and sulfate conjugates of PNP at equal rates, whereas the glucuronide:sulfate conjugate ratio for renally formed 1-N conjugates was 3:1. When the conjugates of either 1-N or PNP were infused systemically, *in vivo* hydrolysis contributed significantly to the amount of circulating parent phenol.

Glucuronide and sulfate conjugations are the major routes of metabolism of phenols in birds and mammals. Together these reactions function as important mechanisms for inactivation and elimination of numerous drugs, drug metabolites and other xenobiotics. Due to its large size and abundance of sulfotransferases and UDP-glucuronyltransferases, the liver is generally regarded as the dominant organ involved in the metabolism of xenobiotics by these enzymes. *In vitro* studies of maximal capacity of metabolite formation in various organs, when corrected for total weight of the organs, suggest that glucuronide and sulfate conjugations in the kidney generally represent only a small fraction of that occurring in the liver [1, 2]. However, most drugs and other xenobiotics are not present in the general circulation in concentrations which saturate their metabolism or excretion. Instead, first-order elimination is the general rule, suggesting that the rate at which a compound is delivered to a metabolizing organ may be a significant factor in determining the contribution of that organ to the metabolism of that compound. The liver receives only about 5% of cardiac output via the hepatic artery. The remaining hepatic flow consists of venous drainage from other organs, including the intestine which has been shown to have the capacity to form glucuronide and sulfate conjugates [2–6]. The kidneys of many animals including humans [7] have been shown to form glucuronide and sulfate conjugates of phenols. Renal blood flow is approximately 25% of cardiac output, suggesting that the renal contribution to the metabolism of phenols present in low concentration in the circulation may be greater than that suggested by data obtained from *in vitro* experiments.

Several techniques have been used to quantify the contribution of the kidneys to the *in vivo* metabolism of compounds present in the circulation. Clearance measurements, which have traditionally been used to quantify *in vivo* renal function, have been used by Riegelman and coworkers [8–11] to quantify renal metabolism. Their method involves measurement of the clearance of radiolabeled metabolite during steady-state infusion of radiolabeled metabolite (true clearance), and measurement of the clearance of unlabeled metabolite during steady-state infusion of radiolabeled metabolite and unlabeled precursor (apparent clearance). These workers concluded that, in the species studied (rabbits, rhesus monkeys, and humans), the kidneys contribute appreciably to glycine conjugation of several aromatic acids. Although their experiments have been criticized for their failure to measure simultaneously the true and apparent clearances of glycine conjugates during infusion of precursors [12], the renal contribution to the formation of urinary metabolite is proportional to the difference between the simultaneously determined apparent clearance and the true clearance. This valuable method is excellent for monitoring the renal formation of an end product of metabolism which is available in radioactive form. The technique has the following limitations: (1) the technique monitors the renal formation of metabolite but does not monitor the metabolism of precursor, nor does it detect unexpected shifts in routes of metabolism of precursor that may occur in disease unless the unexpected metabolite is infused in radiolabeled form; (2) to adapt this technique to measure the contribution of the kidneys to the detoxification of a compound when several metabolites are formed, the technique requires that all metabolites be infused

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simultaneously in radioactive form and that simultaneous apparent and true clearances be determined for each metabolite; and (3) the technique will not detect any renal metabolism of circulating radiolabeled precursor derived from *in vivo* hydrolysis of radiolabeled metabolites. Such hydrolysis has been shown to be significant [13].

Two other radioisotope techniques, neither of which is a clearance technique, have been used to quantify *in vivo* renal metabolism. The first of these, referred to as the specific activity ratio (SAR) technique, is a standard isotope dilution technique. It requires the steady-state infusion of radiolabeled metabolite, and the steady-state infusion of unlabeled precursor unless the precursor is present endogenously. The specific activities of metabolite in urine and plasma are measured. One minus the ratio of the specific activity of metabolite in urine to that in plasma indicates the fraction of urinary metabolite which was formed in the kidney and directly excreted. The SAR technique has been used to quantify the contribution of the kidney to the formation of uric acid in the chicken [14, 15] and rat [16]. Although the SAR technique is ideal for determining the renal contribution to the *in vivo* formation of an end product such as uric acid, it has the same limitations as those listed above for the dual clearance technique.

A more recent radioisotope technique, referred to as the specific activity difference ratio (SADR) technique, was developed in this laboratory in an effort to avoid the limitations of the above techniques for measuring the *in vivo* renal contribution to the metabolism of a compound. The SADR technique requires only the infusion of precursor in radiolabeled form. Unlabeled metabolites are infused simultaneously if they are not present endogenously. The SADR technique can be used to quantify simultaneously the renal formation and direct excretion of any number of metabolites provided they are excreted in measurable amounts [13]. The process of renal formation and direct excretion of metabolite is referred to as excretory metabolism, and the urinary metabolite derived by this process is referred to as nephrogenic metabolite. The SADR technique has been used to quantify renal excretory metabolism of *p*-nitrophenol (PNP) in the chicken *in vivo* [13]. The authors concluded that the chicken kidney contributes significantly to the detoxification of PNP. The present study utilized the SADR technique to quantify the renal excretory conjugation of 1-naphthol (1-N) and PNP in the rat.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats weighing between 335 and 400 g (BioLab Corp., St. Paul, MN) were used. The rats were housed in air-conditioned quarters and had free access to food (Purina rat chow) and water. Animals were deprived of food, but not water, for 18 hr prior to the start of the experiment.

Chemicals. *p*-Nitro[2,6-^{14}C]phenol ([^{14}C]PNP, 34.8 mCi/mmole) and [methoxy-^{3}H]inulin-methoxy ([^{3}H]inulin, 114 mCi/g) were obtained from ICN Pharmaceuticals, Inc. (Irvine, CA). 1-[1-^{14}C]Naphthol ([^{14}C]1-N, 19.4 mCi/mmole) was obtained from the Amersham Corp. (Arlington Heights, IL). PNP, 1-N, 2-naphthol (2-N), *p*-nitrophenyl sulfate (PNPS), 1-naphthyl sulfate (1-NS), 1-naphthyl β-D-glucuronide (1-NG) and inulin were obtained from the Sigma Chemical Co. (St. Louis, MO). *p*-Nitrophenyl β-D-glucuronide (PNPG) was obtained from Boehringer Mannheim (Indianapolis, IN). All other chemicals and supplies were obtained from common commercial sources.

Experimental protocol. Rats were anesthetized with 5-ethyl-5-(1-methylpropyl)-malonyl-thio-urea (Inactin: 100–120 mg/kg, i.p.). A tracheotomy was performed, and a polyethylene cannula was placed in a jugular vein. The free end of the cannula was connected to an infusion pump (Harvard model 975) and a solution containing either 2.5% glucose and 0.45% NaCl (PNP experiments) or 0.9% NaCl (1-N experiments) was infused continuously at a constant rate between 0.02 and 0.08 ml/min, throughtout the experiment. One femoral or carotid artery was cannulated with polyethylene tubing (PE50, Clay Adams) to permit blood collection. An abdominal incision was made and, with the aid of a dissecting microscope, polyethylene cannulas (PE50, Clay Adams) were placed in each ureter. In all 1-N experiments and in one PNP experiment, a cannula (PE10, Clay Adams) was placed in the common bile duct for the continuous collection of bile. All cannulas were then exteriorized, and the abdominal incision was closed. Animal body temperature was maintained at 38.0 ± 1.0° by use of a temperature-controlled chamber or by use of a heating pad and temperature monitoring with a digital rectal thermometer (Curtin Matheson Scientific, Minneapolis, MN). The ^{14}C-labeled precursor and its unlabeled glucuronide and sulfate conjugates were infused simultaneously into the jugular vein. [^{14}C]PNP was infused at 2.0 μmoles·min^{-1}·kg^{-1}. PNPG and PNPS were each infused at 0.3 μmole·min^{-1}·kg^{-1}. [^{14}C]1-N and its conjugates were each infused at 1.0 μmole·min^{-1}·kg^{-1}. In experiments in which glomerular filtration rate was measured, [^{3}H]inulin was also infused into the jugular vein (0.3 μCi/min). Steady state was maintained during the interval of 75–180 min after beginning a continuous infusion, as judged by a constancy in blood concentrations and specific activities for precursor and each metabolite. Routinely, following a 90-min equilibration period, four to eight consecutive 15-min urine samples were collected from each ureter. Simultaneously, bile samples were collected in experiments in which the bile duct was also cannulated. Arterial blood samples (200–400 μl) were drawn at the midpoint of each collection period and transferred to heparin-coated microcentrifuge tubes. In experiments using PNP as the test substrate, the blood samples were immediately centrifuged and the plasma was collected and stored at 7°, along with the urine samples, until analysis the following day. In experiments using 1-N as the test substrate, 100 μl of whole blood was removed and mixed in a tube containing 10 μl of a 2-naphthol internal standard solution (20 μg/ml). The remainder of each blood sample was centrifuged immediately and plasma was

collected. All samples were then stored at 7° until they were analyzed.

Analytical methods. Urine and bile volumes were determined by weight, assuming a specific gravity of 1.0. The urine and bile samples were then diluted with 0.50 ml of distilled water before any further analysis was performed. Aliquots (50 μl) of diluted bile, urine, infusion solution or undiluted plasma were pipetted into scintillation vials and 5.5 ml of Aquasol-2 (New England Nuclear, Boston, MA) was added to each vial. Plasma, urine, bile and infusion solutions were analyzed, by use of either a Packard model 3375 (PNP experiments) or a Beckman model 8000 (1-N experiments) liquid scintillation spectrophotometer, to determine ^{14}C and ^{3}H dpm. Relative counting efficiency was determined in each sample by internal standardization with a [^{14}C]PNP standard (PNP experiments) or by the H number method (1-N experiments). The H number is an external standardization technique for quench monitoring by measurement of the Compton edge of an external gamma source [17, 18].

1-Naphthol experiments. The specific activities of [^{14}C]1-N, [^{14}C]1-NG and [^{14}C]1-NS in plasma and urine and the specific activities of [^{14}C]1-N in blood and infusion solution were determined by high performance liquid chromatography (HPLC) followed by liquid scintillation analysis of the eluted peaks. This permitted calculation of the specific activity of each compound from a single sample. Ten microliters of an aqueous 2-N solution (20 μg/ml) was added to 50 μl plasma and 100 μl blood as an internal standard for 1-N. Each blood sample was extracted 4 times with 400 μl heptane. The heptane fractions were pooled and then evaporated to dryness under N_2. Plasma samples were extracted twice with 200 μl methanol. The two supernatant fractions of each sample were pooled and evaporated to dryness under N_2. Each sample was then redissolved in 250 μl of the appropriate HPLC eluant for subsequent injection. Concentrations of 1-N in blood and plasma were based on extraction ratios of 1-N/2-N of 1.3 for the heptane extract of blood and 1.0 for the methanol extract of plasma. The blood extract, but not the plasma extract, provided a sufficient amount of [^{14}C]1-N for calculation of precursor specific activity. In two SADR experiments, [^{14}C]1-N was extracted from the last blood sample immediately after collection. Extraction of duplicate samples stored up to 5 days resulted in the same concentrations and specific activities, indicating that there was no *in vitro* hydrolysis of metabolites under the conditions used in our experiments. The already diluted urine samples were further diluted with the HPLC eluant so that approximately 1.0 to 10 μg of each metabolite was contained in the 200 μl injected onto the column.

The HPLC separation of 1-N, 1-NS, 1-NG and 2-N was similar to that reported by Karakaya and Carter [19]. The chromatography system employed a Beckman model 110A solvent-delivery system. The eluant was a 50 mM NaH_2PO_4 solution, containing 55% methanol and 3.0 mM (urine samples) or 4.5 mM (plasma extracts) tetrabutylammonium hydrogen sulfate (TBA-HSO_4). TBA-HSO_4 was omitted for analysis of 1-N and 2-N from blood extracts and infusion solution. The flow rate was 2.0 ml/min. The column (Partisil 5 ODS-3 RAC, 100 mm × 9.4 mm i.d., Whatman, Inc., Clifton, NJ) was operated at ambient temperature with an inlet pressure of approximatly 1200 psi. Samples were introduced onto the column through a loop injector valve (Rheodyne model 7125 with a 200-μl loop). When 4.5 mM TBA-HSO_4 was present in the eluant, the following capacity ratios were obtained: 1-NG: $k' = 1.1$; 1-NS: $k' = 2.3$; 2-N: $k' = 3.8$; and 1-N: $k' = 4.5$. Each peak was fully contained in a 4.0-ml volume. All compounds were eluted in less than 17 min. The eluted metabolites (1-NG and 1-NS) were detected with a variable-wavelength u.v. photometer (Hitachi model 100-10) operated at 295 nm; the signal from the photometer was recorded and the peaks were integrated by a reporting integrator (Hewlett–Packard model 3390A). Both 1-N and the internal standard (2-N) were detected by electrochemical analysis. An electrical potential of +750 mV vs an Ag/AgCl reference electrode was applied to the column eluant flowing through an electrochemical cell (model TL-8A Kel-F, Bioanalytical Systems Inc., West Lafayette, IN) connected in series distally to the u.v. spectrophotometer. An amperometric controller (model LC-3, Bioanalytical Systems) was used to control the applied potential and monitor the changes in current during electrochemical oxidation of 1-N and 2-N. The current changes were displayed on a conventional strip-chart recorder set at 1.0 V full-scale.

The ^{14}C-labeled compounds eluted from the column were quantified by liquid scintillation spectrometry. The effluent was collected in 6- to 9-ml fractions with a fraction collector (ISCO model 328, Lincoln, NB) to ensure that each ^{14}C-labeled compound was completely contained in a single and separate 20-ml scintillation vial. Each fraction was then combined with 12 ml Aquasol-2 and counted. Counting efficiency for each sample was determined and data are expressed as dpm/nmole.

PNP experiments. *p*-Nitrophenol, PNPS and PNPG were quantified in plasma and urine by HPLC using the methods of Diamond and Quebbemann [20]. Identification of [^{14}C]PNP, [^{14}C]PNPS and [^{14}C]PNPG in plasma and urine was accomplished by thin-layer chromatography. Samples of deproteinized plasma (prepared as above for HPLC), urine and nonradioactive standards were applied to 5 × 20 cm silica gel plates (Whatman LDQ5). The plates were developed by ascending chromatography in a solvent system consisting of 1-butanol–acetic acid–water (8:1:1, by vol.). Standard compounds were detected by fluorescent quenching and radioactivity was detected with the aid of a Packard model 7201 radiochromatogram scanner. The radioactive compounds were identified by comparison of R_F values with nonradioactive standards. The mean R_F values (±S.D.) for PNP, PNPS, and PNPG in this system were 0.93 ± 0.01, 0.76 ± 0.03, and 0.33 ± 0.03 respectively. Quantification of the ^{14}C-labeled compounds on the developed thin-layer plates was accomplished by individually scraping the radioactive areas of each chromatogram into liquid scintillation vials (8-ml vol.) containing 0.5 ml of distilled water. The vials were gently shaken for 120 min to elute the ^{14}C-labeled compounds from the silica gel.

Aquasol-2 (5 ml) was added to each vial before counting in a liquid scintillation spectrometer. Relative counting efficiency was determined by internal standardization and was found to be equal in samples containing eluted [^{14}C]PNP, [^{14}C]PNPS and [^{14}C]PNPG. Recoveries of [^{14}C]PNP, [^{14}C]PNPS and [^{14}C]PNPG from plasma and urine samples by this procedure were 98, 97 and 95%, respectively.

Calculations. The SADR indicates the fraction of urinary metabolite which was formed in the kidney and directly excreted: $SADR = (SAM_u - SAM_p)/(SAP_p - SAM_p)$, where SAM_u, SAM_p and SAP_p represent the specific activities of metabolite in urine, metabolite in plasma, and precursor in plasma, respectively. By chemically determining the rate at which that metabolite is excreted in the urine ($\dot{M}_{u:total}$), one can calculate the rate of excretion of nephrogenic metabolite ($\dot{M}_N$).

$$\dot{M}_N = (\dot{M}_{u:total}) \times SADR$$

The urinary excretion rate of total M formed *in vivo* ($\dot{M}_{u:in\ vivo}$) is calculated as follows:

$$\dot{M}_{u:in\ vivo} = \dot{M}^*_{u:total}/SAP_p$$

where $\dot{M}^*_{u:total}$ represents the urinary excretion rate of radiolabeled M in dpm/min. Non-nephrogenic M formed *in vivo* is calculated from the difference between $\dot{M}_{u:in\ vivo}$ and $\dot{M}_N$. The SADR technique has been described in detail elsewhere [13].

RESULTS

The complete results of a single SADR experiment are presented in Table 1. [^{14}C]1-N (2320 dpm/nmole), 1-NG and 1-NS were simultaneously infused, each at 1.0 μmole·min^{-1}·kg^{-1}, into the systemic circulation by way of the jugular vein. This infusion resulted in a steady-state plasma concentration of 1-N of 1.10 ± 0.04 (mean ± S.D.) nmoles/ml and a urinary percent recovery of infused ^{14}C-label of 77 ± 5 (mean ± S.D.). Greater than 99% of the ^{14}C-labeled material excreted in the urine was identified as either [^{14}C]1-NG (66%) or [^{14}C]1-NS (33%). As shown in Table 1, the specific activities of [^{14}C]1-N, [^{14}C]1-NG and [^{14}C]1-NS were relatively constant in the arterial circulation and urine throughout the experiment. The concentration of each compound in the arterial circulation (data not shown) and urinary excretion rate of each metabolite (Table 1) varied approximately 10% during this period, indicating that steady state was reached in blood and kidney. The specific activities of [^{14}C]1-NG and [^{14}C]1-NS in the urine were greater than the respective specific activities in plasma, indicating that the kidneys formed [^{14}C]1-NG and [^{14}C]1-NS and that some, if not all, of the renally synthesized conjugates were excreted directly into the urine. The mean SADR for [^{14}C]1-NG indicates that 12.0 and 11.8% of the 1-NG excreted by the left and right kidneys respectively, were nephrogenic. The total excretion rates of 1-NG by the left and right kidneys were 695 and 717 nmoles·min^{-1}·kg^{-1}, respectively. Therefore, nephrogenic 1-NG was formed at rates of 84.4 and 84.0 nmoles·min^{-1}·kg^{-1} by the left and right kidneys, respectively. By similar analysis, the SADR for [^{14}C]1-NS indicates that nephrogenic 1-NS was formed at rates of 21.2 and 22.6 nmoles·min^{-1}·kg^{-1} by the left and right kidneys, respectively. The sum of the excretion rates of nephrogenic 1-NG and 1-NS, when expressed as a fraction of the sum of the urinary excretion rates of all 1-NG and 1-NS synthesized *in vivo*, is designated as the nephrogenic fraction. The mean nephrogenic fractions were 0.23 and 0.22 for the left and right kidneys, respectively, suggesting that nephrogenic 1-NG and 1-NS amounted to approximately 23% of the 1-N conjugates formed *in vivo* and excreted in the urine.

The results of three experiments in which the renal conjugation of 1-N was quantified are summarized in Table 2. The urinary percent recovery of infused ^{14}C-label was 74.6 ± 4.8 (mean ± S.E.). In all experiments, the bile duct was cannulated and bile was collected continuously throughout the experiment. The bile was found to contain 10.9 ± 1.8% (mean ± S.E.) of the infused ^{14}C-label and thus provides an alternate route of excretion for 1-naphthol. In the three 1-N experiments summarized in Table 2, 98.5 ± 0.4% (mean ± S.E.) of the ^{14}C-label in urine was identified as either [^{14}C]1-NG or [^{14}C]1-NS. No [^{14}C]1-N was detected in urine. Analysis was sufficiently sensitive to detect [^{14}C]1-N if it exceeded 0.5% of excreted ^{14}C-label. The mean nephrogenic fraction for conjugates of 1-N was 0.20. Of this, approximately 75% was nephrogenic 1-NG and 25% was nephrogenic 1-NS.

Also presented in Table 2 are the results of four experiments in which the renal conjugation of PNP was quantified. The recovery of infused ^{14}C-label in urine was 75.2 ± 2.5% (mean ± S.E.). In one PNP experiment the bile duct was cannulated and bile was collected continuously during the experiment. The bile was found to contain approximately 0.9% of the infused ^{14}C-label, while the recovery in urine was 72.6%, suggesting that bile was not a significant route of excretion of PNP in our experiments. In each of four PNP experiments summarized in Table 2, greater than 99% of the ^{14}C-label excreted in the urine was identified as either [^{14}C]PNPS or [^{14}C]PNPG. No [^{14}C]PNP was detected in the urine. The mean nephrogenic fraction was 0.23. Of this approximately 50% was nephrogenic PNPS and 50% was nephrogenic PNPG.

In all the 1-N experiments and in several PNP experiments the specific activity of circulating precursor was less than the specific activity of precursor in the infusion solution. As no endogenous 1-N or PNP was detected in rat blood, this indicates that the infused ^{14}C-labeled precursor (1-N or PNP) was diluted with unlabeled precursor derived from the hydrolysis of infused sulfate and/or glucuronide conjugates. The specific activity dilution was not an analytical artifact, since in animals where [^{14}C]1-N or [^{14}C]PNP was infused without the simultaneous infusion of its unlabeled conjugates, the specific activity of precursor in plasma, blood and infusion solution was the same. When [^{14}C]PNPS was isolated from urine and infused intravenously, [^{14}C]PNP was detected in plasma. The hydrolysis of infused sulfate and/or glucuronide conjugates could yield potentially significant amounts of precursor for subsequent *in vivo* conjugation. This precursor would be present

Table 1. Renal sulfate and glucuronide conjugation of 1-N in the rat: Results of a single SADR experiment*

	Specific activity (dpm/nmole)						SADR		1-NS excretion (nmoles·min^{-1}·kg^{-1})				1-NG excretion (nmoles·min^{-1}·kg^{-1})				
	Circulation				Urine				Total	*In vivo*			Total	*In vivo*			
Sample	[^{14}C]1-N	[^{14}C]1-NS	[^{14}C]1-NG	Sample	[^{14}C]1-NS	[^{14}C]1-NG	[^{14}C]1-NS	[^{14}C]1-NG	Infused and *in vivo*	Nephrogenic	Non-nephrogenic	Total	Infused and *in vivo*	Nephrogenic	Non-nephrogenic	Total	Nephrogenic fraction
P-1	2087	558	794	1-1	595	918	0.024	0.095	518	12.4	135	147	707	66.6	238	305	0.18
				R-1	624	923	0.043	0.099	547	23.7	140	164	719	71.3	246	317	0.20
P-2	2088	539	748	L-2	614	925	0.048	0.132	503	24.3	123	147	660	87.3	206	293	0.25
				R-2	611	927	0.047	0.134	595	27.5	146	174	754	101	234	335	0.25
P-3	2253	539	807	L-3	619	948	0.046	0.198	476	21.9	109	131	621	60.7	200	261	0.21
				R-3	602	947	0.036	0.097	559	20.1	129	149	748	72.5	243	316	0.20
P-4	2024	552	756	L-4	613	954	0.042	0.156	624	26.0	163	189	790	123	249	372	0.27
				R-4	604	935	0.035	0.141	538	18.9	142	161	645	91.1	207	298	0.24
Mean	2113	547	776	L	610	936	0.040	0.120	530	21.2	133	154	695	84.4	223	308	0.23
± S.D.	98	10	29		11	17	0.011	0.029	65	6.1	23	25	73	28.1	24	47	0.04
Mean				R	610	933	0.040	0.118	560	22.6	139	162	717	84.0	233	317	0.22
± S.D.					10	11	0.006	0.023	25	3.9	7	10	50	14.5	18	15	0.03

* [^{14}C]1-N, 1-NG and 1-NS were each infused at 1.0 μmole·min^{-1}·kg^{-1} into the systemic circulation by way of the jugular vein. After an initial infusion period of 90 min, four consecutive 15-min urine samples and midpoint blood samples were collected, and the specific activities were determined for each sample. The SADR was the fraction of the total metabolite excreted (*in vivo* and infused) which was formed in the kidney and directly excreted. Nephrogenic conjugate was conjugate synthesized within the kidney and excreted directly into the urine without being absorbed into the circulation. Non-nephrogenic conjugate was conjugate synthesized *in vivo* which entered the circulation before being excreted in the urine. Non-nephrogenic conjugate was calculated from the difference between the total *in vivo* conjugate and nephrogenic conjugate. Total *in vivo* conjugate was calculated by the following formula: $\dot{M}_{u:in\ vivo} = \dot{M}^{*}_{u:total}/SAP_p$. The nephrogenic fraction is the sum of nephrogenic 1-NG and 1-NS taken as a fraction of the excretion rate of all 1-NG and 1-NS synthesized *in vivo*. Rat No. 15, 338 g.

Table 2. *In vivo* renal sulfate and glucuronide conjugation of 1-naphthol in three rats and *p*-nitrophenol in four rats*

Precursor infused (nmoles·min^{-1}·kg^{-1})	Sulfate (nmoles·min^{-1}·kg^{-1} per kidney)			Glucuronide (nmoles·min^{-1}·kg^{-1} per kidney)			Nephrogenic fraction
	Nephrogenic	Non-nephrogenic	Total	Nephrogenic	Non-nephrogenic	Total	
1-N (1177)	35 ± 12	149 ± 16	184 ± 23	62 ± 8	268 ± 34	330 ± 40	0.19 ± 0.02
1-N (985)	14 ± 2	96 ± 11	110 ± 13	56 ± 7	226 ± 26	282 ± 30	0.18 ± 0.01
1-N (1033)	21 ± 5	135 ± 16	156 ± 18	84 ± 21	228 ± 20	312 ± 32	0.23 ± 0.04
Mean ± S.E.	23 ± 6	127 ± 16	150 ± 22	67 ± 8	241 ± 14	308 ± 14	0.20 ± 0.01
PNP (2118)	71 ± 48	306 ± 42	377 ± 26	64 ± 26	229 ± 54	292 ± 45	0.20 ± 0.08
PNP (2020)	89 ± 86	403 ± 52	492 ± 72	119 ± 72	276 ± 75	395 ± 81	0.22 ± 0.14
PNP (2102)	97 ± 23	311 ± 65	407 ± 37	82 ± 37	350 ± 80	432 ± 59	0.22 ± 0.05
PNP (1600)	126 ± 79	345 ± 144	471 ± 46	120 ± 46	322 ± 160	442 ± 200	0.27 ± 0.07
Mean ± S.E.	96 ± 11	341 ± 22	437 ± 27	96 ± 14	294 ± 26	390 ± 34	0.23 ± 0.01

* The measured infusion rate of radiolabeled precursor for each experiment is shown in parentheses. Four to six determinations of urine and plasma specific activities were made in each experiment. Each value represents the mean ± S.D. for each rat. Descriptions of nephrogenic, non-nephrogenic and total *in vivo* urinary metabolites are found in the legend of Table 1.

in the circulation in addition to the infused radiolabeled precursor. To determine whether precursor derived from *in vivo* breakdown of conjugate was available for conjugation, the rate of hydrolysis of infused conjugates was measured by isotope dilution. The rate of *in vivo* conjugate hydrolysis was calculated using the ratio of the specific activity of the circulating precursor to the specific activity of precursor in the infusion solution. Under steady-state conditions, this ratio is inversely proportional to the amount of circulating precursor which was derived from unlabeled conjugates. Thus, in the experiment summarized in Table 1, the steady-state ratio of the specific activity of [^{14}C]1-N in the arterial circulation to that in the infusion was 0.91, suggesting that 9% of the circulating 1-N was derived from hydrolysis of unlabeled conjugates. This hydrolysis, combined with the 1-N infusion of 1.0 μmole·min^{-1}·kg^{-1}, resulted in the availability of 1134 nmoles·min^{-1}·kg^{-1} 1-N (1023/0.91) for *in vivo* conjugation. Based on this calculated availability of 1-N and the measured urinary recovery of infused ^{14}C-label (76.6%), it is possible to predict the urinary excretion rate of 1-N conjugates synthesized *in vivo*. In the experiment outlined in Table 1, this calculation predicts the rate of urinary excretion of *in vivo* synthesized conjugates as 869 nmoles·min^{-1}·kg^{-1} (1134 × 0.766). The actual urinary excretion rate, as measured by chemical analysis, was 939 nmoles·min^{-1}·kg^{-1}. Table 3 lists the results of similar calculations for seven rat experiments in which either [^{14}C]1-N or [^{14}C]PNP was the infused precursor. The mean difference between the predicted rate and the measured rate of excretion of *in vivo* synthesized conjugates was 4 ± 1% (mean ± S.E.). When precursor derived from conjugate hydrolysis was not considered, the resulting predicted rate of excretion of *in vivo* synthesized conjugates was 12 ± 2% (mean ± S.E.) less than the measured rate.

The data obtained from the SADR experiments were used to calculate the simultaneous apparent and true renal excretory clearances of 1-N and PNP. The apparent renal excretory clearance was determined by the conventional clearance calculation in which the urinary excretion rate of precursor (estimated from the minimum detectable concentration of 1-N or PNP in urine since no 1-N or PNP was detected in urine) is divided by the plasma or blood concentration of the precursor. The true renal excretory clearance was determined by calculating the sum of the excretion rates of all nephrogenic conjugates of test substrate, obtained from the SADR measurements, plus the urinary excretion rate of unchanged precursor (essentially zero in these experiments) and dividing by the precursor concentration in plasma or blood. We have designated this clearance as true renal excretory clearance rather than true renal clearance, since a portion of precursor cleared from the circulation by renal metabolism may be transferred to the renal venous circulation. The calculated clearance values are presented in Table 4. In three experiments, in which the mean plasma concentration of PNP was 30.9 μM, the true renal plasma excretory clearance of PNP was more than 64 times the apparent excretory clearance for PNP and was greater than the simultaneously measured glomerular filtration rate. In experiments in which 1-N was the infused precursor, the mean plasma concentration of 1-N was 1.2 μM, and the true renal plasma excretory clearance was 74 ml·min^{-1}·kg^{-1} per kidney, a rate almost 15 times the simultaneously measured glomerular filtration rate of 4.7 ± 0.7 ml·min^{-1}·kg^{-1} per kidney (mean ± S.E.). In seven separate rats, undergoing similar surgical procedures as outlined above, the [^{3}H]inulin/^{14}CPAH clearance ratio was 0.28 ± 0.03 (mean ± S.E.), suggesting that the effective renal blood flow (ERBF) in the three SADR experiments using ^{14}C-1-N was 33.6 ml·min^{-1}·kg^{-1} per kidney. The true renal excretory clearance for 1-N, based on blood concentrations, was 21.3 ± 5.8 ml blood·min^{-1}·kg^{-1} per kidney or 63% of the estimated ERBF.

DISCUSSION

The present study was designed to determine the contribution of the kidney to the *in vivo* metabolism of two phenols in the rat. We have reported previously that the human kidney is capable of converting both of these phenols, 1-naphthol and *p*-nitrophenol, to the sulfate and glucuronide conjugates [7]. These are the same metabolites formed by several organs of the rat *in vitro* [21–24]. The data obtained in the present studies support the following principal conclusions: (1) the SADR technique provides a useful experimental approach to estimating the contribution of renal excretory metabolism to drug metabolism *in vivo* in mammals; (2) the renal contribution to the *in vivo* metabolism of 1-N and PNP, quantified at single infusion rates, is significant. In rats, a minimum of 20% of the urinary sulfate and glucuronide conjugates of intravenously infused 1-N or PNP is synthesized in the kidney; (3) excretory conjugation is the principal mechanism for the renal elimination of 1-N and PNP from the circulation; and (4) hydrolysis of glucuronide and/or sulfate conjugates of 1-N and PNP occurs *in vivo*.

The SADR technique is ideally suited for use in quantifying the formation of multiple metabolites from a single precursor by an excretory organ. The technique requires steady-state concentrations of precursor and metabolite(s) in the circulation. It also requires that accurate measurements of precursor and metabolite specific activities in blood and urine be made. The technique is not compromised by interconversion of precursor and metabolite. In fact, the SADR technique permits calculation of the fraction of circulating precursor that is derived from the systemic hydrolysis of infused conjugates. This fraction is equal to [1-($SAP_{circulation}$/$SAP_{infusion}$)]. *In vivo* hydrolysis of infused 1-N conjugates was found to occur in all three SADR experiments and to account for 10.3 ± 1.3% (mean ± S.E.) of the circulating 1-N in the blood. Hydrolysis of PNP conjugates was more variable (6.5 ± 4.0%; mean ± S.E.), occurring only in two of four rats to a measurable extent.

The present study quantified the renal contribution to the elimination of 1-N and PNP in the rat *in vivo*. During a continuous infusion of [^{14}C]1-N (1.0 μmole·min^{-1}·kg^{-1}) or [^{14}C]PNP (2.0 μmoles·min^{-1}·kg^{-1}) and its unlabeled sulfate and glucuronide conjugates, a steady state was reached, indicating

Table 3. Measured and predicted excretion of *in vivo* synthesized conjugates of 1-naphthol and *p*-nitrophenol*

Precursor infused	Precursor specific activity ratio (circulation/infusion)	Substrate available for conjugation (nmoles · min^{-1} · kg^{-1})			Urinary recovery of infused ^{14}C (%)	Urinary excretion of *in vivo* synthesized conjugates (nmoles · min^{-1} · kg^{-1})				
		Infused	Hydrolyzed	Total		Measured	Predicted from infused plus hydrolysis	Difference (%)	Predicted from infusion only	Difference (%)
	Blood/Infusion									
1-N	0.91	1177	122	1299	81.8	1029	1063	+3	963	−6
1-N	0.87	985	145	1130	65.4	783	739	−6	644	−18
1-N	0.91	1023	101	1134	76.6	939	869	−7	784	−17
	Plasma/Infusion									
PNP	0.87	2118	275	2393	67.9	1680	1625	−3	1438	−14
PNP	0.88	2020	242	2262	77.4	1825	1751	−4	1563	−14
PNP	1.00	2102	0	2102	78.5	1774	1650	−7	1650	−7
PNP	0.99	1600	17	1677	78.8	1339	1321	−1	1261	−6
							Mean ± S.E.	−4 ± 1		−12 ± 2

* The measured rates of infusion of radiolabeled precursor are given. 1-NG and 1-NS were each infused at 1.0 μmole · min^{-1} · kg^{-1} for 1-N experiments. In PNP experiments, PNPG and PNPS were each infused at 0.3 μmole · min^{-1} · kg^{-1}. Rate of precursor availability due to *in vivo* hydrolysis of infused metabolite(s) is equal to the product of one minus the precursor specific activity ratio and the precursor infusion rate. Urinary excretion rate of conjugates was predicted in two ways: (1) multiplying the infusion rate of precursor by the fractional urinary recovery of infused ^{14}C-label, or (2) adding the rates of precursor infusion and conjugate hydrolysis and multiplying this sum by the fractional urinary recovery of infused ^{14}C-label. Data represent means for each experiment.

Table 4. Apparent and true renal excretory clearances of 1-naphthol and *p*-nitrophenol*

Precursor infused	Precursor concentration in plasma or blood (μM)	Renal excretory clearance		Clearance ratio ($Cl_{precursor}/Cl_{inulin}$)
		Apparent	True	
1-N	1.2 ± 0.1 (plasma)	<0.1	74.4 ± 13.1†	14.8 ± 2.7
1-N	4.4 ± 0.2 (blood)	<0.1	21.3 ± 5.8‡	
PNP	30.9 ± 4.4 (plasma)	<0.1	6.4 ± 2.1†	1.6 ± 0.4

* Data represent mean ± S.E. of three experiments for 1-N and four experiments for PNP. Clearance ratio calculation is based on the true excretory clearance of precursor.

† Units are ml plasma·min^{-1}·kg^{-1} per kidney.

‡ Units are ml blood·min^{-1}·kg^{-1} per kidney.

that first-order elimination existed for each of the infused compounds. During steady state, approximately 75% of the infused ^{14}C-label of either substrate was excreted in the urine. More than 98% of the ^{14}C-label appearing in urine was identified as either the sulfate or glucuronide conjugate of the test compounds. No unchanged [^{14}C]1-N or [^{14}C]PNP was detected in the urine. Excretion of nephrogenic conjugates accounted for approximately one-fifth of the conjugates of the test substrate which were formed *in vivo* and excreted in the urine. Thus, a significant portion of the urinary metabolites was derived from renal excretory conjugation.

Two factors should be considered when evaluating the nephrogenic fraction. First, lack of complete urinary recovery of infused ^{14}C-label. Since the nephrogenic fraction relates to total urinary conjugate, the portion that is cleared by metabolism and eliminated into another compartment (e.g. bile) will decrease the actual nephrogenic contribution to the total *in vivo* conjugation. In our experiments, approximately 25% of the infused ^{14}C-label was not excreted into the urine. If the entire 25% of infused ^{14}C-label was metabolized and excreted by routes other than the urine, the nephrogenic fraction would decrease from 0.20 to 0.15. In contrast, if the precursor simply moved into a more slowly equilibrating compartment (e.g. fat) without metabolism, the nephrogenic fraction would remain 0.20. Second, the nephrogenic fraction may also underestimate the contribution of the kidney to total body metabolism of a test compound if metabolite formed in the kidney enters the renal venous blood instead of the urine.

The importance of excretory conjugation in the renal clearance of 1-N and PNP can be seen by comparing the true and apparent renal excretory clearances of 1-N and PNP in Table 4. The renal clearance of unchanged 1-N or PNP (apparent excretory clearance) was essentially zero. However, the true excretory clearance for PNP, which includes renal excretion of precursor by excretory conjugation, was 6.4 ml plasma·min^{-1}·kg^{-1} per kidney. This is 1.6 times the glomerular filtration rate. The efficiency of 1-N elimination by the kidney was even greater than that for PNP. The true excretory clearance of 1-N from plasma was 14.8 times greater than the simultaneously measured clearance of [^{3}H]inulin and approximately 5 times greater than the estimated renal plasma flow. This indicates than 1-N associated with the blood cell fraction, which accounted for 85 ± 1% (mean ± S.E.) of 1-N present in the blood, was available for metabolism by the kidney. The high accumulation of 1-N in the blood cells is in agreement with a previous report by Bock and Winne [21]. The true renal clearance of 1-N based on blood concentrations of 1-N was 21.3 ± 5.8 ml blood·min^{-1}·kg^{-1} per kidney. Therefore, approximately two-thirds (63%) of the renal blood flow was cleared of 1-N by excretory conjugation, thus making the extensive renal metabolism of 1-N in blood analogous to the metabolism of propranolol by the liver [25]. In view of our previous demonstration that sulfate and glucuronide conjugation of PNP, 1-N and phenol occurs in the isolated perfused human kidney [7], it is reasonable to speculate than renal excretory conjugation may be important for the metabolism and excretion of phenolic compounds in humans.

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