Renal Contribution to Overall Metabolism of Drugs III: Metabolism of *p*-Aminobenzoic Acid

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Abstract □ The metabolic processes involved in the conversion of *p*-aminobenzoic acid to *p*-acetamidobenzoic acid, *p*-aminohippuric acid, and *p*-acetamidohippuric acid in the rabbit were reanalyzed. The contribution made by the kidney to the overall metabolism was estimated from analysis of clearance data.

Keyphrases □ *p*-Aminobenzoic acid—metabolism, disposition kinetics, reevaluation of contribution of kidney to clearance □ *p*-Acetamidobenzoic acid as *p*-aminobenzoic acid metabolite—renal metabolism and clearance, apparent clearance, role of kidney reevaluated □ *p*-Aminohippuric acid as *p*-aminobenzoic acid metabolite—renal metabolism and clearance, apparent clearance, role of kidney reevaluated □ *p*-Acetamidohippuric acid as *p*-aminobenzoic acid metabolite—renal metabolism and clearance, apparent clearance, role of kidney reevaluated □ Drug metabolism—role of kidney in *p*-aminobenzoic acid metabolism reevaluated from analysis of clearance data

p-Aminobenzoic acid occurs widely in nature and has been the subject of much interest since the discovery that it antagonizes the action of sulfonamides. It is also a component of folic acid. The compound is known to be metabolized by a number of pathways, the major ones being acetylation to *p*-acetamidobenzoic acid and conjugation with glycine to *p*-aminohippuric acid. There is also evidence that *p*-aminohippuric acid is further acetylated to form *p*-acetamidohippuric acid in some species (1) (Scheme I). One can also postulate conversion of *p*-acetamidobenzoic acid to *p*-acetamidohippuric acid.

It was observed that in patients with advanced liver disease, the capacity for acetylation of *p*-aminobenzoic acid and sulfadiazine was not correspondingly diminished. This situation was taken to infer that acetylation was not an exclusive function of the liver and that other tissues may have metabolizing activity (2). *In vitro* evidence of acetylation in tissues of the GI tract and kidney has been reported in man (3) and in animals (1, 4, 5). Frindt and Vial (6) studied the conjugation of *p*-aminohippuric acid and acetate by human kidney cortex and liver slices, and they found that the kidney showed an acetylating activity in the case of *p*-aminohippuric acid of 0.253 ± 0.023 μM/hr./g. of wet tissue as compared to 0.123 ± 0.015 μM/hr./g. for the liver. Acetylating and demethylating activities have both been shown in perfused kidney, brain, and uterine–placental–fetal preparations (7–9).

The aminobenzoic acids, especially the *o*- and *p*-isomers, occur widely in nature, but their fate in animals has been only randomly studied. Knoefel *et al.* (10) described the formation of the hippurate and glucuronide in rabbits. *p*-Aminobenzoic acid was administered by a constant intravenous infusion at the rate of 0.1 mM/kg./hr., and the fraction of the dose excreted as *p*-aminohippuric acid and the benzoyl glucuronide was 9 and 4%, respectively. *p*-Aminobenzoic acid is known to be acetylated *in vitro* in both liver and kidney tissues (11–13). *p*-Aminohippuric acid is also known to be acetylated in some animals including the rabbit (5, 17). It was decided that *p*-aminobenzoic acid would be an interesting compound for study in an attempt to ascertain the contribution of the kidney to metabolism. In initial studies it was found that the major metabolite was the acetyl conjugate, *p*-acetamidobenzoic acid.

EXPERIMENTAL

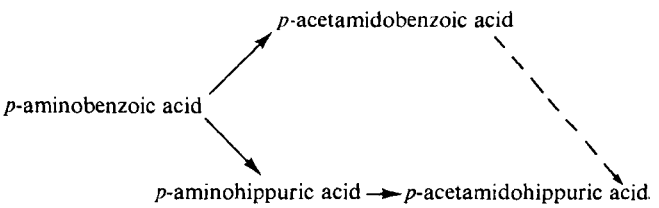
Intravenous Study—A 50-mg. sample of tritiated *p*-aminobenzoic acid, specific activity 14.3 μc./mg., was administered to a 3.7-kg. rabbit intravenously. Total urine was collected with an indwelling catheter (Foley) for 6 hr. A 20-μl. sample of the urine was spotted onto thin-layer plates and developed in a benzene–*p*-dioxane–acetic acid solvent system (90:75:8). Radioscan of the plates located the metabolites. Blood samples were obtained at 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min. after the injection.

Animal Preparation—Both ureters and the left and right marginal ear veins of a male New Zealand white rabbit were cannulated under barbiturate anesthesia. The infusion experiment was commenced after at least 16 hr. recuperation.

Infusion Studies—The format for the study of renal metabolism of *p*-aminobenzoic acid was similar to that for benzoic acid and salicylic acid reported earlier (14, 15). It was initially decided that the biotransformations of interest were the conjugations to *p*-acetamidobenzoic acid and *p*-aminohippuric acid. Therefore, the experiment was designed to serve this purpose. However, it was found that a significant fraction of infused *p*-aminohippuric acid was further acetylated to *p*-acetamidohippuric acid, and some deductions for this reaction have been made from the available data.

The first stage of the experiment involved an infusion of the metabolites, *p*-aminohippuric acid and *p*-acetamidobenzoic acid, to reach conditions of steady state. The renal clearance of each metabolite was determined. During the second stage of the infusion experiment, tritiated *p*-aminobenzoic acid was infused to five steady-state concentrations and plasma and apparent clearances for *p*-aminohippuric acid and *p*-acetamidobenzoic acid were again determined. The constancy of renal clearance was monitored by observing the ratios of the rate of excretion of cold metabolites, labeled metabolites, and unchanged drug at each infusion rate. Under conditions when renal clearance is a constant, the observed excretion ratio of cold to labeled compounds should be the same as the infusion ratio of cold and labeled compounds.

The *p*-aminohippuric acid and *p*-acetamidobenzoic acid infusion solution was prepared by dissolving 600 mg. of each compound in 88 ml. saline. This solution was infused at a rate of 500 mcg./min.



Scheme I—Metabolic pathways of p*-aminobenzoic acid*

Table I—Pharmacokinetic Parameters Obtained after Intravenous Administration of 50 mg. *p*-Aminobenzoic Acid to the Rabbit

Dose, mg.	A, mcg. ml.$^{-1}$	B, mcg. ml.$^{-1}$	C_p^0, mcg. ml.$^{-1}$	α, min.$^{-1}$	β, min.$^{-1}$	$(\dot{V}_{cl})_{pl}$, ml. min.$^{-1}$	V_p, ml.
50	24.5	2.1	26.6	0.048	0.0044	51	1880

Table II—Steady-State Plasma Concentrations and Rates of Excretion of *p*-Aminobenzoic Acid and Metabolites during Infusion in the Rabbit

Sequential Period of Infusion[a]	C_p, mcg. ml.$^{-1}$				$\Delta A/\Delta t$, mcg. min.$^{-1}$[b]			
	p-Aminobenzoic Acid	*p*-Acetamidobenzoic Acid	*p*-Aminohippuric Acid	*p*-Acetamidohippuric Acid	*p*-Aminobenzoic Acid	*p*-Acetamidobenzoic Acid	*p*-Aminohippuric Acid	*p*-Acetamidohippuric Acid
0	0	26	9.4	9.3	0	476	328	91
2	1.33	32.6	9.8	14.7	0	544	381	154
3	1.24	33.7	9.6	15.0	0	684	405	158
4	3.0	37.0	10.8	16.9	0	764	425	183
5	5.9	40.0	10.6	20.4	0	919	455	221

[a] The initial period of *p*-aminobenzoic acid infusion did not reach steady state and is not included in this table. [b] A constant infusion of 500 mcg./min. of both *p*-acetamidobenzoic acid + *p*-aminohippuric acid was maintained throughout the experiment.

The half-lives of *p*-aminohippuric acid and *p*-acetamidobenzoic acid are expected to be in the same range of magnitude as hippuric acid, which was 10–21 min. in the three animals studied. It was assumed that 60 min. was sufficient for *p*-aminohippuric acid and *p*-acetamidobenzoic acid to reach steady state, after which time blood and urine samples were obtained. The *p*-aminobenzoic acid solution was prepared by dissolving 400 mg. tritiated *p*-aminobenzoic acid (specific activity 29.2 μc./mg.) in 80 ml. saline. An initial priming dose was given followed by infusion rates of 93, 131, 182.5, 358, and 502 mcg./min. for 2 hr. at each infusion rate. Half of each plasma sample was used for the Bratton–Marshall analysis, and the remaining half was used in the determination of radioactivity. All urine samples were analyzed by scintillation counting and by the Bratton–Marshall procedure.

Analytical Methods—*Analysis of* p-*Aminobenzoic Acid,* p-*Acetamidobenzoic Acid,* p-*Aminohippuric Acid, and* p-*Acetamidohippuric Acid in Plasma*—The Bratton–Marshall (16) method was used for the analysis of *p*-aminobenzoic acid and its metabolites. *p*-Aminobenzoic acid and *p*-acetamidobenzoic acid were separated from *p*-aminobenzoic acid and *p*-acetamidohippuric acid by a modified version of the partition fractionation method of Cohen and McGilvery (17). The extraction procedure for plasma was as follows. One milliliter of plasma was diluted to 2 ml. with distilled water. Then 1.0 ml. of 0.61 *M* trichloroacetic acid was added to precipitate plasma proteins. The mixture was centrifuged for 10 min., and 2.2 ml. of the supernate was removed for extraction. To the supernate was added 0.6 ml. 0.61 *N* sodium hydroxide and 2.2 ml. Sorenson citrate buffer, pH 4.0. The solution was extracted three times with 10-ml. portions of anhydrous reagent grade ether to extract *p*-aminobenzoic acid and *p*-acetamidobenzoic acid into the ether phase, leaving the hippurates and glucuronides in the aqueous phase.

There was some extraction of *p*-aminohippuric acid into the ether layer, which averaged 18 ± 2%. The *p*-aminohippuric acid data were, therefore, corrected for this extraction factor. The extraction of *p*-aminohippuric acid into the ether phase only affects the determination of *p*-aminobenzoic acid concentrations. Since *p*-aminobenzoic acid was tritiated, the absolute values of *p*-aminobenzoic acid could be checked by TLC and scintillation counting. The ether extract was evaporated under a stream of nitrogen in a 40–50° water bath. The residue was dissolved in 5-ml. distilled water, of which 2.5 ml. was removed for determination of *p*-aminobenzoic acid by the Bratton–Marshall method. To the remaining 2.5 ml. was added 0.5 ml. 6 *N* hydrochloric acid. The test tube was capped and heated at 100° for 30 min. to hydrolyze *p*-acetamidobenzoic acid to *p*-aminobenzoic acid. The Bratton–Marshall analysis was applied to the hydrolysate. The aqueous layer was divided into two fractions. One portion was analyzed for *p*-aminohippuric acid. The second fraction was hydrolyzed for determination of *p*-aminohippuric acid and *p*-acetamidohippuric acid. Acid hydrolysis of *p*-acetamidohippuric acid and *p*-acetamidobenzoic acid was found to be complete after 30 min. at 100°. *p*-Acetamidobenzoic acid and *p*-acetamidohippuric acid were thus determined by the difference method.

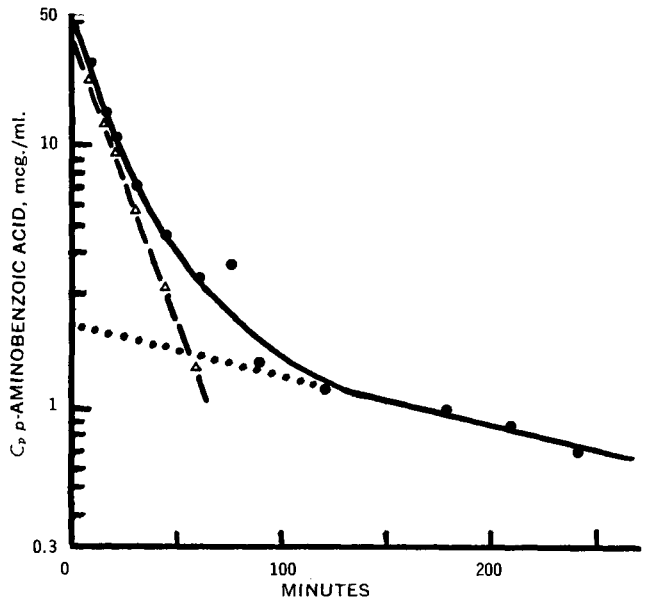


Figure 1—*Plasma concentration–time curve obtained after intravenous administration of 50 mg.* p-*aminobenzoic acid to the rabbit.*

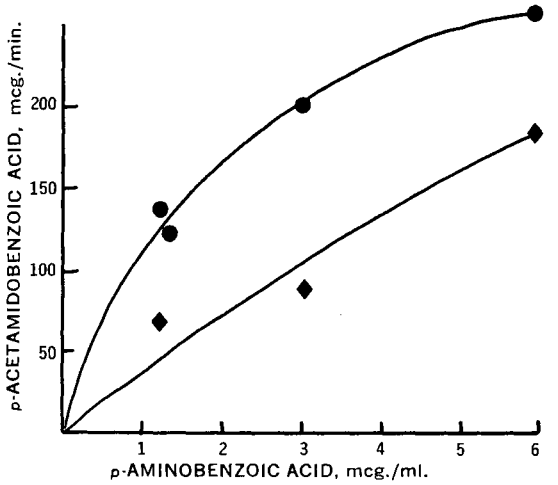


Figure 2—*Rate of formation of* p-*acetamidobenzoic acid by the liver and kidney at various plasma concentrations of* p-*aminobenzoic acid in the rabbit. Key:* ●, *liver; and* ◆, *kidney.*

Table III—$(\dot{V}_{cl})_{pl}$[a] and $(\dot{V}_{cl})_{app.}$ of Metabolites of *p*-Aminobenzoic Acid

Compound	$(\dot{V}_{cl})_{pl}$[a]	$(\dot{V}_{cl})_{app.}$ at Levels 2	3	4	5
p-Acetamidobenzoic acid	18.3	16.7	20.2	20.6	22.9
p-Aminohippuric acid	35	38.8	42.1	39.3	42.9
p-Acetamidohippuric acid	9.8	10.4	10.5	10.8	10.8

[a] These values were calculated under the initial infusion conditions when no *p*-aminobenzoic acid was infused.

Table IV—K_m and $V_{max.}$ Values Obtained from the Woolf Plot

Metabolite	Metabolizing Organ	K_m, mcg./ml.	$V_{max.}$, mcg. min.$^{-1}$	r
p-Acetamidobenzoic acid	Liver	2.0	345	0.75
	Kidney	6.0	333	0.99
p-Aminohippuric acid	Liver	—	—	—[a]
	Kidney	2.1	112	0.90

[a] Too scattered to fit.

Analysis of p-*Aminobenzoic Acid and Metabolites in Urine*—The procedure for urine analysis was similar to that described for plasma. Samples were diluted to an estimated concentration of 10–40 mcg./ml. before extraction. A summary of the Bratton–Marshall procedure for primary aryl amines follows. The aqueous sample was acidified with 0.5 ml. 6 *N* hydrochloric acid, and 0.4 ml. of a 0.1% sodium nitrate solution was added and allowed to stand 3 min. Then 0.4 ml. of a 0.5% solution of ammonium sulfamate was added, mixed, and allowed to stand 2 min. Then 1.2 ml. of *N*-naphthylethylenediamine hydrochloride, 0.1%, was added. After standing for 5 min. or more, the solutions were read at 545 nm. on a spectrophotometer[1].

Analysis of Tritiated p-*Aminobenzoic Acid and Metabolites in Plasma and Urine*—The protein in 0.2–0.5 ml. plasma was precipitated by the addition of 50–100 μl. of 0.61 *M* trichloroacetic acid. After centrifuging, a known aliquot was spotted on silica gel plates and developed in a benzene–*p*-dioxane–acetic acid (90:75:8) system. The compounds were visualized by UV fluorescence, located by radioscan, and manually scraped off the plates. For scintillation counting, the scrapings were placed in 30-ml. vials and 10 ml. of cocktail was added. The vial was capped and shaken for 15–20 min. on a shaker. A liquid scintillation counter[2] was used under the following conditions: window, 50–1000; and gain, 50%. The efficiency of counting was determined by the internal standard method. Plasma samples which had not been subjected to the

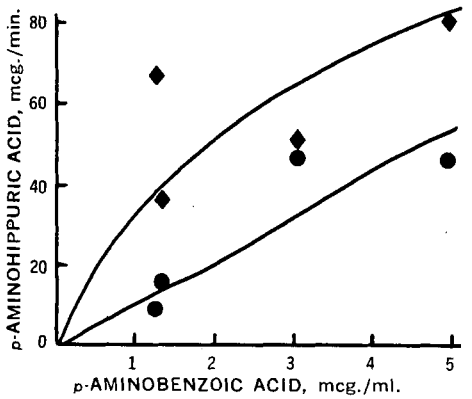


Figure 3—*Rate of formation of* p-*aminohippuric acid by the liver and kidney at various plasma concentrations of* p-*aminobenzoic acid in the rabbit. Key:* ●, *liver; and* ◆, *kidney.*

[1] Beckman DB
[2] Packard Tri-Carb, model 3003.

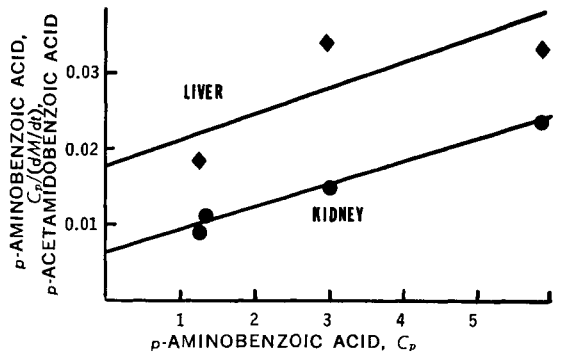


Figure 4—*Woolf plot for the kinetics of biosynthesis of* p-*acetamidobenzoic acid during infusion of* p-*aminobenzoic acid in the rabbit. Key:* ◆, *liver; and* ●, *kidney.*

TLC process were counted to determine total radioactivity. The elution of compounds from silica gel scrapings averaged 60 ± 8% for plasma samples.

The procedure for urine was similar to that described for plasma. The protein precipitation step was omitted. Two-microliter samples of urine were spotted directly onto silica gel plates. The elution of compounds from silica gel scrapings was more complete in urine; 90–100% of the radioactivity could be recovered.

RESULTS AND DISCUSSION

Radioscan of urine spotted on silica gel plates showed that the component with the highest activity, estimated to be 40–80% of total activity, had an R_f of 0.33, which corresponded to the R_f of *p*-acetamidobenzoic acid. Other radioactive components had R_f values of 0.08 and 0.03 and corresponded to those of *p*-aminohippuric acid and *p*-acetamidohippuric acid, respectively; they were therefore dealt with as one substance. A very negligible fraction of the dose was excreted as *p*-aminobenzoic acid, which had an R_f of 0.47. A small quantity of glucuronides is possibly included in the *p*-aminohippuric acid fraction. However, no attempt was made to identify them since the metabolites of interest were *p*-acetamidobenzoic acid, *p*-aminohippuric acid, and *p*-acetamidohippuric acid.

The plasma concentration–time curve is shown in Fig. 1, and the calculated parameters were tabulated in Table I. The half-lives of α and β were 14.5 and 157 min., respectively. The half-life of elimination was 26 min., and the V_p of 1800 ml. is comparable to that of benzoic acid.

The half-life for β, estimated from the intravenous injection of *p*-aminobenzoic acid in the initial rabbit studied, was used for the estimation of rates of infusion in the second animal. However, it appears that the rate of metabolism of *p*-aminobenzoic acid proceeds at a higher rate in the animal used for the infusion experiment, resulting in lower plasma concentrations than expected. Also, except for the lowest infusion rate of *p*-aminobenzoic acid of 93 mcg./min., all other rates of infusion were carried to steady state. Data for the first rate of infusion were, therefore, not con-

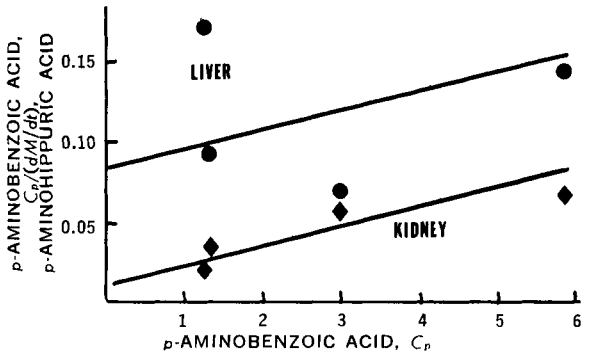


Figure 5—*Woolf plot for the kinetics of biosynthesis of* p-*aminohippuric acid during infusion of* p-*aminobenzoic acid in the rabbit. Key:* ●, *liver; and* ◆, *kidney.*

Table V—Contribution of Liver and Kidney to Total Body Metabolism of *p*-Acetamidobenzoic Acid and *p*-Aminohippuric Acid in the Rabbit

C_p *p*-Aminobenzoic Acid, mcg. ml.$^{-1}$	Percent *p*-Acetamidobenzoic Acid Formed by		Percent of Dose Excreted as *p*-Acetamidobenzoic Acid	Percent *p*-Aminohippuric Acid Formed by		Percent of Dose Excreted as *p*-Aminohippuric Acid
	Kidney	Liver		Kidney	Liver	
1.33	0	100	39.7	73.6	26.4	28.5
1.24	32.4	67.6	87.2	90.9	9.1	29.8
3.00	30.2	69.8	61.6	49.6	50.4	19.1
5.90	42.2	57.8	67.5	67.0	33.0	17.9

sidered further. During the infusion of *p*-acetamidobenzoic acid and *p*-aminohippuric acid at 500 mcg. of each per minute, it was found that the output of *p*-acetamidobenzoic acid was approximately equal to the rate of input; *i.e.*, the infused *p*-acetamidobenzoic acid could be quantitatively recovered from urine. However, only 66% of the infused *p*-aminohippuric acid could be recovered intact (Table II). Material balance was achieved when the Bratton–Marshall procedure was carried through acid-hydrolyzed urine samples. A significant increase in spectrophotometric absorbance was also obtained with plasma samples after hydrolysis. TLC indicated that the acid-hydrolyzable fraction was probably *p*-acetamidohippuric acid. This would explain the increase in absorbance, because *p*-acetamidohippuric acid yields *p*-aminohippuric acid after acid hydrolysis.

The contribution of the kidney to overall metabolism was assessed by analyzing renal clearance and apparent clearance data as discussed previously (14).

Table III includes a summary of clearance data obtained from the infusion study. The plasma clearance of *p*-acetamidobenzoic acid $(\dot{V}_{cl})_{pl}$, which in this case is its renal clearance, $(\dot{V}_{cl})_{ex}$, since it is not metabolized further, was calculated to be 18.5 ml./min. or 4.1 ml./min./kg. The higher *p*-aminohippuric acid clearance was due in part to metabolic clearance, while the plasma clearance of *p*-acetamidobenzoic acid was essentially equivalent to its renal clearance. The data listed in Table III were, of course, calculated from values in Table II. While 95% of infused *p*-acetamidobenzoic acid was recovered in the urine, only 66% of the *p*-aminohippuric acid was recovered. It is likely, therefore, that the *p*-aminohippuric acid is the major source for the *p*-acetamidohippuric acid found in the blood and recovered in the urine. This, of course, means that $(\dot{V}_{cl})_{pl}$ for *p*-aminohippuric acid represents both metabolic and renal clearance. It should be once again noted that plasma clearances for *p*-acetamidobenzoic acid and *p*-aminohippuric acid are lower than the expected clearance values for these compounds. The low clearance, as in the case of the benzoic acid and salicylic acid experiments, could be due to ischemia and a reduced blood flow to the kidneys from back-pressure building up in the catheters. However, the apparent clearance of both *p*-aminohippuric acid and *p*-acetamidobenzoic acid increased during infusion of *p*-aminobenzoic acid, indicating renal conversion of *p*-aminobenzoic acid to *p*-aminohippuric acid and *p*-acetamidobenzoic acid. The apparent clearance of *p*-acetamidohippuric acid also increased to a small extent. Unfortunately, these low clearance values were seen in all studies to date and steps are being taken to eliminate the experimental deficiency. It undoubtedly affects the absolute values of metabolism and clearance. It is conjectured, however, that the percent of dose metabolized by the kidney would have been higher if renal blood flow had not been impaired.

Table VI—Comparison of Infusion Ratios of Nonlabeled and Labeled Compounds to Their Excretion Ratios

Level of Infusion	Cold Input[a] / Hot Input	Cold Output[b] / Hot Output
2	5.6	5.3
3	4.1	4.7
4	2.1	2.5
5	1.5	1.8

[a] Calculated from infusion rates. [b] Calculated from experimental excretion data.

An estimation of the rates of metabolism for each metabolite and the contribution of the kidney as compared to total body metabolism was made. While the maximum rates of metabolism had not been reached for the systems, the plots of dM/dt against C_p *p*-aminobenzoic acid showed that the kinetics resembled Michaelis–Menten kinetics (Figs. 2 and 3). A similar plot for *p*-acetamidohippuric acid formation was not carried out. The substrate for *p*-acetamidohippuric acid formation can be derived from two sources, *p*-aminohippuric acid and *p*-acetamidobenzoic acid, and unless the contribution of each substrate is known, a plot of (dM/dt) *p*-aminohippuric acid against substrate concentration would be difficult to interpret.

The data were treated according to Woolf's linearizing procedure for enzymatic reactions (Figs. 4 and 5). The K_m and $V_{max.}$ values were obtained from the slope and intercept of the regression lines (Table IV). The kidney showed a relatively high $V_{max.}$ for both *p*-acetamidobenzoic acid and *p*-aminohippuric acid, although initially a larger percentage of the metabolites was formed in the liver (Table V). At low plasma concentrations of *p*-aminobenzoic acid, the fraction of the dose excreted as *p*-acetamidobenzoic acid, *p*-aminohippuric acid, and *p*-acetamidohippuric acid totaled almost 100%. As plasma *p*-aminobenzoic acid concentration increased, however, the fraction excreted as the acetyl and glycine conjugates dropped, and only 71% was excreted as these conjugates at a *p*-aminobenzoic acid concentration of 5.9 mcg./ml. It is probable that glucuronic acid conjugates are also formed, but no attempt was made to ascertain the amount present.

Renal clearance in this experiment was presumed to be constant for all compounds under study. This has some basis of validity since the infused ratio of labeled to nonlabeled compounds was comparable to the excretion ratio of these two chemical species at all rates of infusion (Table VI). This is being investigated further using the double-label isotope technique.

In summarizing, it can be said that *p*-aminobenzoic acid administered intravenously to the rabbit is excreted mainly as the acetyl conjugate, *p*-acetamidobenzoic acid. Smaller amounts of *p*-aminohippuric acid and *p*-acetamidohippuric acid are formed. The *p*-aminohippuric acid that is infused or formed by metabolism can be further acetylated to *p*-acetamidohippuric acid. It is possible that *p*-acetamidobenzoic acid is further metabolized to *p*-acetamidohippuric acid, but the magnitude of the reaction was not sufficiently large to be detectable. Note that in Table V, at Levels 2 and 3, 90 and 84% of the dose were excreted as *p*-acetamidobenzoic acid, while the total of *p*-acetamidohippuric acid and *p*-aminohippuric acid combined did not exceed 8%.

In the animal studied, 30–40% of the acetylation to *p*-acetamidobenzoic acid occurred in the kidney. Seventy percent of the *p*-aminohippuric acid was formed in the kidney, and only 10–50% was formed in the liver. However, these results are very dependent upon a delineation of the sources of *p*-acetamidohippuric acid. It may be possible to clarify this in the future by administration of tritiated *p*-aminobenzoic acid at varying levels and a concomitant constant infusion of *p*-acetamido-^{14}C-benzoic acid. An analysis of the two radioisotopes could determine the contribution of *p*-aminohippuric acid and *p*-acetamidobenzoic acid to the formation of *p*-acetamidohippuric acid.

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Characterization of Adjuvant Mineral Oils

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Abstract □ Analysis of two lots of mineral oil indicated the overall composition to be quite similar with two major hydrocarbon ranges: a low range from C_{14} to C_{20} with a maximum at C_{16} and a high range from C_{20} to C_{30} with a maximum at C_{25}. The low range components represented 40.8 and 49.3 wt.% of Lots Q-9 and Q-10, respectively. Urea fractionation of the paraffin–naphthene fraction from silica gel chromatography yielded 28.2 and 28.8 wt.% for Lots Q-9 and Q-10, respectively. The nonadductable material consisted of highly branched and cyclic structures containing up to four-ring systems. The total butyl phthalate content in Lots Q-9 and Q-10 was 13.2 and 7.0 p.p.m., respectively, with the ratio of monobutyl phthalate to dibutyl phthalate being 9:1. No evidence was found to indicate the presence of polynuclear aromatic hydrocarbons.

Keyphrases □ Mineral oils, adjuvant—separation, UV and mass spectrometry characterization □ Phthalate esters—identification in adjuvant mineral oils □ Adjuvant mineral oils—separation, UV and mass spectrometry characterization □ GC—separation of mineral oil components □ UV spectrophotometry—characterization of mineral oils □ Mass spectroscopy—characterization of mineral oils

Mineral oil[1] for adjuvant use is a light oil consisting of a blend of oleum-treated charge stocks (1) and, along with the emulsifier mannide monooleate[2], constitutes Freund's incomplete adjuvant preparation (2). This adjuvant has found widespread experimental use over the years for enhancing antibody responses to viral and bacterial antigens and for hyposensitization of certain allergic individuals (3). Recently, the safety of this adjuvant for use in humans has been questioned due to the long-term persistency (4, 5) of the mineral oil in the tissue at the injection site and because of various pathological changes which have been observed in experimental animals and man (3, 6–8). In view of these findings as well as those of others relating to either the carcinogenic or cocarcinogenic properties of mineral oils in general (9–11), an analytical study was initiated.

EXPERIMENTAL

Materials—Two commercial lots of mineral oil (Lots Q-9 and Q-10) were obtained from the manufacturer and consisted of three basic charge stocks representing 61, 31, and 8% of the total oil[3]. Column chromatography was conducted employing silica gel[4] (mesh 60–200) and alumina[5] (chromatographic grade F20). All solvents were reagent grade and were distilled before use.

Column Chromatography—Mineral oil, Lot Q-9, was chromatographed over silica gel as the first step in the overall fractionation procedure (Scheme I). For this separation, 300 g. of mineral oil was dissolved in 500 ml. petroleum ether (30–60°) and introduced over 1140 g. of silica gel. The column was eluted sequentially with petroleum ether, benzene, and absolute methanol. Alumina chromatography of the derived benzene fraction was carried out by

[1] Drakeol 6VR mineral oil manufactured by the Pennsylvania Refining Co., Butler, Pa.
[2] Arlacel A, Atlas Chemical Industries, Wilmington, Del.
[3] Personal communication, Mr. Charles Steenbergen, Pennsylvania Refining Co., Butler, Pa.
[4] Davison Chemical, Baltimore, Md.
[5] Alcoa Chemicals, Bauxite, Ark.