Biochemical Pharmacology, 1964, Vol. 13, pp. 1421–1435. Pergamon Press Ltd., Printed in Great Britain.

THE DISTRIBUTION, EXCRETION AND METABOLISM OF BENZQUINAMIDE*

EDWARD H. WISEMAN, ERIC C. SCHREIBER† and REX PINSON, JR.

Medical Research Laboratories, Chas. Pfizer & Co., Inc., Groton, Conn., U.S.A.

(*Received* 30 *April* 1964; *accepted* 11 *June* 1964)

Abstract—Benzquinamide, a new benzoquinolizine psychotherapeutic agent, is rapidly absorbed after oral administration to man, dog, and rat. The drug is rapidly distributed throughout the tissues of experimental animals, but in spite of its high lipid: water partition ratio, it is not concentrated in adipose tissue. In the dog, benzquinamide is removed from plasma with a half-life of 30–40 min, chiefly by metabolism in the liver and, to a minor extent (2%–10%), by renal excretion. The principal metabolic reaction in the dog and man is N-dealkylation, the major urinary metabolite being N-deethylbenzquinamide (VI). Ten other metabolites, products of N-dealkylation, O-demethylation and, to a much lesser extent, deacetylation, have been identified. The metabolites are excreted, both in the urine and in the bile, from which the more polar metabolites are not reabsorbed and are excreted in the feces.

BENZQUINAMIDE (1) is a new psychotherapeutic agent which contains the benzoquinolizine ring system (Fig. 1) as the central structural unit.[1–3] It is useful in the management of certain mental states.[4–6] Benzquinamide is structurally related to tetrabenazine (V), the only other benzoquinolizine drug for which metabolic data have been reported.[7] This paper is an account of some studies of the physiological distribution and excretion of benzquinamide in man, dog, and rat.

EXPERIMENTAL‡

Metabolism and distribution experiments in animals were carried out in albino rats (Charles River) and mongrel dogs. The drug was administered orally or intraperitoneally to rats and orally or intravenously to dogs. For excretion studies the animals were maintained in metabolism cages with free access to food and water. Urine and feces were collected from the cages. For tissue distribution studies, the

* Quantril, Chas. Pfizer & Co., Inc.

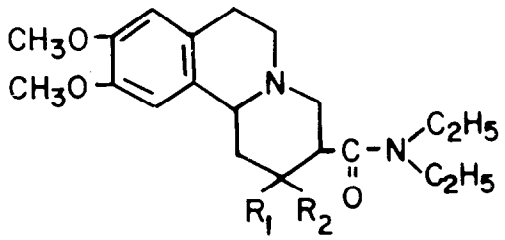
A preliminary account of these studies was presented at the Fall Meeting of the American Society for Pharmacology and Experimental Therapeutics, Nashville, Tenn., 1962.

† Present address: William S. Merrell Co., Cincinnati, Onio

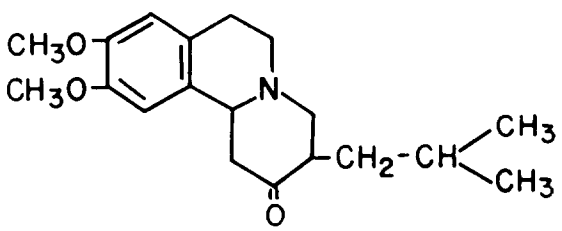
‡ The authors gratefully acknowledge the contributions of their colleagues in many phases of this study. Dr. C. S. Delahunt conducted the experiments in dogs with biliary fistulas and provided tissue samples from those animals; Dr. E. J. Gralla carried out the *in situ* dog liver perfusion experiments; Dr. P. F. Moore prepared the rats with biliary fistulas; Drs P. P. Ruseski and D. G. Iezzoni conducted experiments in human subjects. Miss D. Baumgartner, and Messrs. R. Seidell and E. Lang provided valuable technical assistance.

animals were sacrificed at various intervals by intraperitoneal injection of pentobarbital solution, and selected tissues were removed and immediately frozen.

Investigations in human subjects were conducted in normal male volunteers. Urine and plasma samples were stored in the frozen state until assayed.



		R_1	R_2
I	Benzquinamide	H	CH_3CO
II	Benzquinamide Alcohol	H	OH
III	Benzquinamide Ketone	=O	
IV	2-H^3-Benzquinamide	H^3	CH_3CO



V Tetrabenazine

FIG. 1. Structures of the benzoquinolizines.

Paper chromatography. Three descending systems, run on Whatman 4 paper treated with 40% formamide in methanol, were used. The mobile phases were: system 1, carbon tetrachloride:diethylamine (9:1), saturated with formamide; system 2, hexane:benzene:diethylamine (27:9:4), saturated with formamide; system 3, cyclohexane:diethylamine (9:1), saturated with formamide. Benzquinamide and its metabolites were detected on the chromatograms by their fluorescence under u.v. light. Approximate R_F's are shown in Table 1.

2-^{3}*H-Benzquinamide* (*IV*). This was prepared as follows. The ketone (III; 100 mg) was reduced† with tritium (total activity 10·0 c) and hydrogen (total uptake 7·0 ml)

† This reduction was carried out by New England Nuclear Corp., Boston, Mass., using the experimental conditions designed by Dr J. R. Tretter of these laboratories.

in the presence of platinum catalyst (5·0 mg) in methanol (1·5 ml). Three portions of methanol (10 ml) were evaporated from the residue to remove labile tritium. The residue was dissolved in ethanol (2 ml) and treated with ether (10 ml) saturated with hydrogen chloride. The hydrochloride so formed was reconverted to the free base and subjected to repeated column chromatography (No. 3 grade neutral alumina; methanol used as eluant) to give pure tritiated benzquinamide alcohol (II; 1·95 mc).

TABLE 1. PAPER CHROMATOGRAPHIC SYSTEMS

Compound	Relative R_F value		
	System 1*	System 2†	System 3‡
I	0·9	0·3	0·5
II	0·8	0·2	0·4
IV	0·5	0·1	0·1

*Carbon tetrachloride: diethylamine (9:1), saturated with formamide.

† Hexane:benzene:diethylamine (27:9:4), saturated with formamide.

‡ Cyclohexane:diethylamine (9:1), saturated with formamide

The alcohol (1·94 mc) in pyridine (0·3 ml) containing acetic anhydride (0·7 ml) was refluxed in an atmosphere of nitrogen for 2 hr. The solution was evaporated, the residue dissolved in sodium carbonate solution (0·4 N, 5 ml), and the solution extracted with benzene (4 × 5 ml). The benzene extracts were dried (Na_2SO_4) and evaporated. Nonisotopically labeled benzquinamide was added to this residue, and the whole was recrystallized three times from ethyl acetate:hexane solution. The recrystallized product melted at 134°–135° both alone and in admixture with pure benzquinamide. The u.v. and fluorescence spectra were identical with those of pure benzquinamide. The material was also chemically and radiochemically a single entity when examined in chromatographic systems 1, 2, and 3, all of which are capable of resolving benzquinamide, its parent alcohol, and the ketone (III). The yield of pure 2-^{3}H-benzquinamide (IV) was 39·6 mg, of specific activity 34·71 μc/mg.

Assay Procedures

Chemical. The assay procedure was based on the extraction of benzquinamide from the alkalinized biological sample into heptane, followed by re-extraction from the heptane into dilute hydrochloric acid and fluorometric assay of this solution. The assay is a modification of that previously used for tetrabenazine.[7] Analytical-grade reagents were used in all cases. In order to obtain adequate extraction, the heptane must contain 1·5% (v/v) of isoamyl alcohol. Benzquinamide could be extracted from urine, plasma, and tissue samples with a recovery of about 80%, and from feces with a recovery of about 50%. Recovery in the fecal assay was lower because an additional buffer extraction was required to eliminate interference from normal fecal constituents. Determinations were made on an Aminco-Bowman spectrophotofluorometer, which was standardized before each period of use with standard quinine sulfate in 0·1 N sulfuric acid solution. Benzquinamide was determined at an activation wavelength of 290 mμ and a fluorescence wavelength of 345 mμ. Drug concentrations

in unknown samples were determined graphically from the fluorescent readings, on a calibration curve constructed by carrying known amounts of benzquinamide through the complete assay. The assay proved to be useful in the range 1–20 μg/ml of sample. More concentrated solutions were diluted prior to assay. The assay was specific for benzquinamide and benzquinamide alcohol. Possible metabolites of benzquinamide arising from either N- or O-dealkylation were shown by appropriate experiments not to be extracted from urine, plasma, or tissue homogenates under the conditions of the assay and did not interfere even when present at concentrations as high as 100 μg/ml.

Urine samples (5 ml) were pipetted into 50-ml glass-stoppered centrifuge tubes containing 1 ml 0·2 N NaOH (final pH about 11). After the addition of 25 ml *n*-heptane, the mixture was shaken for 25 min and centrifuged. A 20-ml aliquot of the heptane was transferred to a second tube containing 10 ml 0·1 N HCl, shaken 15 min and centrifuged. The organic layer was removed by aspiration and the fluorescence reading of the aqueous layer determined with the spectrophotofluorometer.

Fecal samples were homogenized with three times their weight of 0·5 N HCl. The final homogenate was at about pH 4. A 3-ml sample of homogenate was pipetted into a 50-ml glass-stoppered centrifuge tube containing 1 ml 2 N NaOH and 15 ml *n*-heptane, the mixture shaken for 25 min and centrifuged. A 10-ml aliquot of the heptane phase was transferred to another tube containing 10 ml of pH 2 buffer (HCl-KCl), and the mixture shaken for 20 min and centrifuged. A 5-ml aliquot of the aqueous phase was transferred to another tube containing 0·5 ml 1 NaOH and 10 ml *n*-heptane, and shaken for 20 min and centrifuged. A 5-ml aliquot of the heptane phase was removed and shaken for 15 min with 5 ml 0·1 N HCl, centrifuged, and the heptane phase removed by aspiration. The fluorescence reading of the aqueous phase was then determined with the spectrophotofluorometer.

Plasma samples (1 ml) were alkalinized with 0·3 ml of 0·1 N NaOH (pH about 11), and 5 ml of *n*-heptane added. The mixture was shaken for 25 min and centrifuged. A 4-ml aliquot of the heptane phase was transferred to a second tube containing 4 ml of 0·1 HCl and shaken for 15 minutes and centrifuged. The organic phase was removed by aspiration, and the fluorescence reading of the aqueous phase was determined with the spectrophotofluorometer.

Tissue samples were homogenized in 0·01 N HCl (3 ml of acid/g of tissue), by a motor-driven Teflon pestle in a borosilicate glass grinding vessel.[8] A sample of the homogenate (1 ml) was made alkaline with 0·5 ml of 1 N NaOH, and 5 ml of *n*-heptane was added. The procedure for plasma was followed from this point.

Radiochemical. Samples were assayed in duplicate by liquid scintillation counting techniques in a Nuclear-Chicago liquid scintillation spectrometer (model 720). Samples were corrected for quenching and scintillator variation by internal standardization.

Urine samples were assayed directly by dissolving 0·2 ml of specimen in 15 ml of a scintillator solution composed of 30% ethanol and 70% toluene and containing 0·3% diphenyloxazole (PPO) and 0·01% *p*-bis-2-(5-phenyloxazolyl)benzene (POPOP).

Plasma samples (0·2 ml) were dissolved in 2 ml of Hyamine solution, to which was then added 15 ml of a toluene solution containing 0·6% PPO and 0·02% POPOP.[9]

Fecal and tissue samples were determined in either of two ways. Samples containing moderate amounts of radioactivity were homogenized in a little water, and an aliquot

of the homogenate was added to 10 volumes of formamide. The mixture was gently refluxed to obtain a clear solution, and an aliquot of this solution (0·1 to 0·5 ml) was added to a scintillator solution composed of 30% ethanol and 70% toluene, containing 0·3% PPO and 0·01% POPOP. For tissue containing low levels of activity, the sample was dried, finely ground, and a 50-mg sample, mixed with 50 mg potassium chlorate, was burned in an atmosphere of oxygen.[10] The gases were absorbed in 10 ml of methanol, and 5 ml of the solution was added to 10 ml of toluene containing 0·3% PPO and 0·01% POPOP for counting.

In vitro metabolism studies. The microsomal plus soluble fraction was prepared from the livers of adult male rats as described by Axelrod.[11] Preparations were stored at −15° for not longer than 1 week. For determination of rate of metabolism, about 4 μmoles of benzquinamide were incubated in 125-ml beakers at 37° in air in a mechanical shaker for varying times with the following mixture: microsomal plus soluble fraction from 4 g of liver, 212 μmoles of glucose-6-phosphate, 920 μmoles of nicotinamide, 500 μmoles of magnesium sulfate, 9·2 μmoles of NADP, and sufficient pH 7·4 phosphate buffer (0·15 M) to bring the final volume to 20 ml. One-ml samples of the mixture were analyzed at intervals for benzquinamide concentration.

RESULTS AND DISCUSSION

Physical properties of benzquinamide. These are shown in Table 2. The compound is a weak organic base which, as indicated by the oil–water partition data, is highly

TABLE 2. PHYSICAL PROPERTIES OF BENZQUINAMIDE*

Solubility:	Water	13·9 mg/ml
	Buffer (pH 7·4)	28·0 mg/ml
	Dilute acid	>200 mg/ml
p*K*a:	5·9 (50% dioxane/water	
Partition coefficient:	540 (chloroform/pH 7·4 buffer)	
	9 (heptane/pH 7·4 buffer)	

* All determinations were made in duplicate at 25°.

lipid soluble in the un-ionized form. It dissolves readily in dilute acid and retains substantial water solubility even at neutral pH. According to modern theories of drug absorption, this combination of dissociation constant and partition coefficient leads to the expectation that the compound would exist in physiological media largely in the undissociated form and that the high lipid solubility of this form would promote its ready transit across biological membranes.[12, 13] The extent of binding to human plasma protein was determined by equilibrium dialysis at 25° through Visking membranes against pH 7·4 buffer. At plasma concentrations of 5–80 μg/ml, 58 ± 2% of the drug was bound to the nondiffusible constituents of plasma, but it was not bound to the formed elements of blood.

Oral absorption and plasma half-life of benzquinamide. The time course of plasma drug concentrations following the oral and intravenous administration of benzquinamide (10 mg/kg) is shown in Fig. 2. The intravenous administration studies indicated that the drug has a plasma half-life of about 30 min and, since a straight-line

log plot was obtained, is removed from the plasma by a process which is first order with respect to benzquinamide.

Data from these observations were further analyzed by a mathematical model derived from chemical kinetics. Plasma drug concentrations following oral administration are defined by the rate of absorption from the gastrointestinal system and the

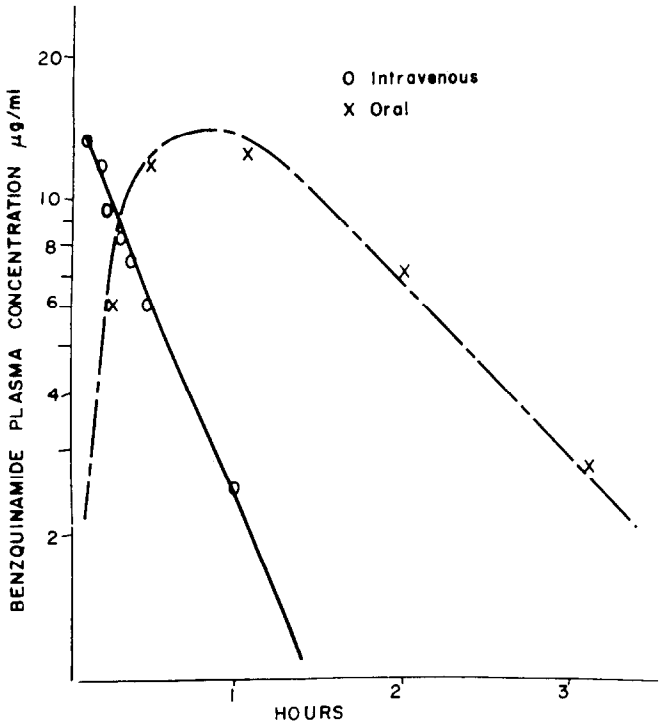


FIG. 2. Plasma concentrations of benzquinamide in the dog after dose of 10 mg/kg. Results are averaged data from two animals.

rate of removal of drug from plasma by the processes of excretion and/or metabolism. Within the assumption that both processes can be described by first-order kinetics, the plasma levels of a drug after oral administration can often be fitted to an equation of the type:

$$C = \frac{a_0 k_a}{V_d(k_d - k_a)} (e^{-k_a t} - e^{-k_d t}) \tag{1}$$

where C is the plasma drug concentration (μg/ml) produced at time t after an oral dose of a_0 (mg/kg) of drug, for which the volume of distribution is V_d (l/kg), and the rate constants for the processes of absorption and disappearance from plasma are k_a and k_d hr^{-1} respectively.[14-16] Values for k_d, the rate constant for the disappearance of drug from plasma, and V_d, the volume of distribution of the drug in equation (1), were approximated from the plot of the intravenous data shown in Fig. 2. The curve constructed from these values for V_d and k_d, together with an appropriate value for k_a, for a dose of 10 mg/kg is shown as a broken line in Fig. 2. The excellent fit

that was obtained between this curve and the experimental points (X) indicates that equation (1) is an adequate model for the time course of plasma concentrations after the oral administration of benzquinamide and can be used, in appropriate form, to calculate other quantities that are not directly measurable. For example, the value of k_a (absorption rate constant) which satisfies equation (1) indicates rapid absorption of the drug. This theoretical conclusion is corroborated by the experimental observation that in the dog, oral administration of benzquinamide resulted in peak plasma drug concentrations of comparable magnitude to those obtained after the same intravenous dose. Further evidence that benzquinamide was completely absorbed after oral administration was obtained from the fact that dogs receiving daily doses of 40 mg/kg over a period of several months excreted substantially less than 5% of the administered drug in the feces.

Similar comparisons between oral and intravenous administration in humans were undertaken but, unfortunately, in these experiments the dose employed (100 mg) produced plasma concentrations too low (usually less than 2 μg/ml) to permit quantitative comparisons. The presence of the drug in plasma within 15–30 min after oral administration could be demonstrated in all cases, however.

Studies at lower doses (2·0 mg/kg i.v.) were also carried out in the dog, using benzquinamide labeled with tritium at the 2-position. The disappearance of plasma radioactivity in these experiments is shown in Fig. 3. Two phases in the decline of the

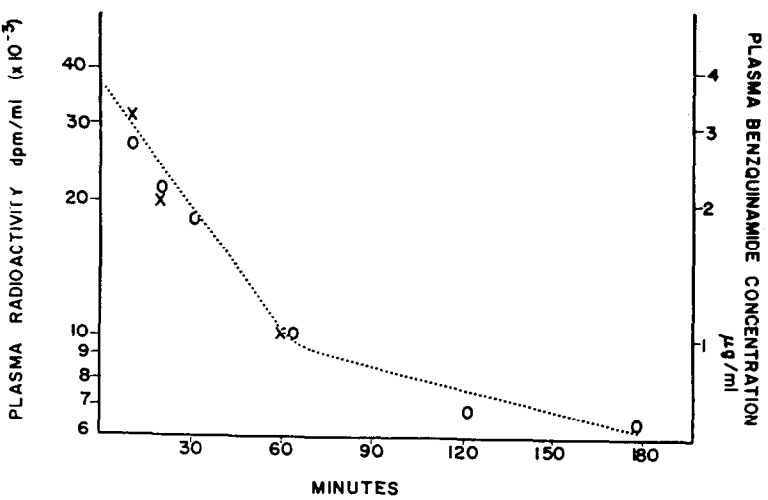


FIG. 3. Time course of benzquinamide plasma concentrations in the dog after the administration of 2-^{3}H-benzquinamide (2·0 mg/kg, specific activity 4·54 μc/mg, i.v.).
Assays performed by fluorometric measurement (X) and by radiochemical techniques (O).
Results are averaged data from two animals.

plasma concentration can be observed. During the initial phase, which had a half-life of about 30 min, blood concentrations obtained either by radioactivity measurements or by fluorometric assay were in good agreement. Since the fluorometric assay responds only to benzquinamide or to the alcohol derived from it by deacetylation and does not respond to other metabolites, it seems likely that the circulating radioactivity is

predominantly in the form of benzquinamide itself, and the disappearance of radioactivity from the blood represents metabolism, renal excretion, and distribution of the drug into tissue. During the second phase, which had a half-life of 3–4 hr, benzquinamide was not detectable in the plasma by fluorometric assay, and drug concentrations calculated from plasma radioactivity indicated levels below the limits of detection by the fluorometric assay. When it is considered that distribution studies in the dog show that many tissues contain appreciable concentrations of benzquinamide 90 min after administration, although at that time the drug had essentially disappeared from plasma, it seems reasonable to suggest that the chief contribution to plasma radioactivity during this second phase is from metabolites which appear in the blood as the drug is metabolized in tissue. However, some contribution to the plasma radioactivity may also result from the slow removal of unchanged benzquinamide from tissue.

Tissue distribution of benzquinamide. The volume of distribution of benzquinamide, calculated by extrapolation of the intravenous plasma concentration plot (Fig. 2) to zero time, was 700 ml/kg of body weight. This value, approximately equal to the volume of total body water, made it appear likely that the drug would be widely distributed throughout body tissues. This expectation was borne out by tissue distribution experiments in the dog and rat. Tissue distribution in the dog 45 and 90 min after oral or intravenous dosage is shown in Table 3. The time course of benzquinamide tissue concentrations in the rat after intraperitoneal administration is shown in Fig 4.

TABLE 3. BENZQUINAMIDE TISSUE DISTRIBUTION IN THE DOG

Sex	M	F	M
Time after admin. (min)	90	90	45
Route of admin.	p.o.	p.o.	i.v.
Dose (mg/kg)	80	80	10
Tissue	Benzquinamide tissue concentrations (μg/g)*		
Kidney	8 (0·04)	13 (0·04)	9 (0·21)
Liver	13 (0·03)	23 (0·05)	11 (0·91)
Brain	5 (0·02)	5 (0·02)	<0·5
Reproductive organs	24 (0·03)	3 (0·01)	
Eye	7 (0·01)	10 (0·01)	
Heart			<0·5

* Figures in parentheses are per cent of administered dose.

Concentrations significantly higher than that in blood were found only in the liver and kidney—organs of metabolism and excretion—and in those only during the first 45 min. Concentrations of the drug in fat were not significantly higher than those in blood indicating that, although the compound is highly lipophilic, fat does not constitute an important drug depot in the body. Drug concentrations in the brain closely paralleled those in plasma.

Excretion of benzquinamide. Less than 10% of the administered benzquinamide was excreted unchanged in the urine, in the three species studied. In man, significantly greater excretion was observed after intravenous and intramuscular administration, as compared with oral administration, and this difference was greatest in the early phase of the experiment (Fig. 5). Since parenterally administered material need not

pass through the liver before reaching the kidney, a relatively larger amount apparently escapes metabolism in the liver and is eliminated unchanged by renal excretion.

The balance of the drug was apparently disposed of by metabolic destruction, and total excretion studies were therefore carried out in animals with tritium-labeled benzquinamide (IV). It should be noted that the tritium occupies a stable position in

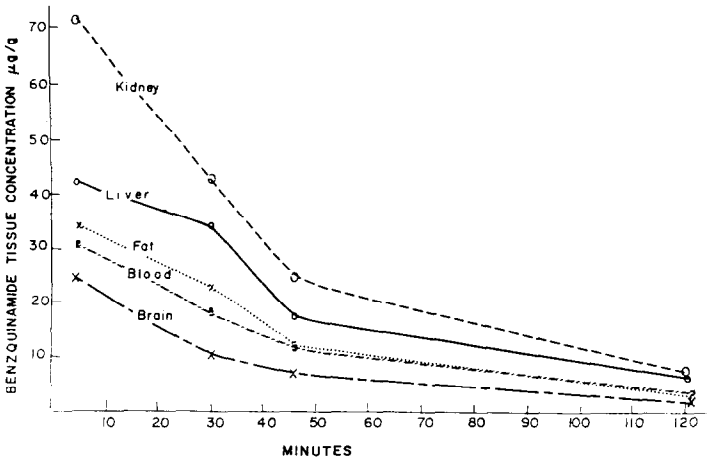


FIG. 4. Tissue concentrations of benzquinamide in the rat after intraperitoneal dose of 50 mg/kg. Each point represents the average of five animals.

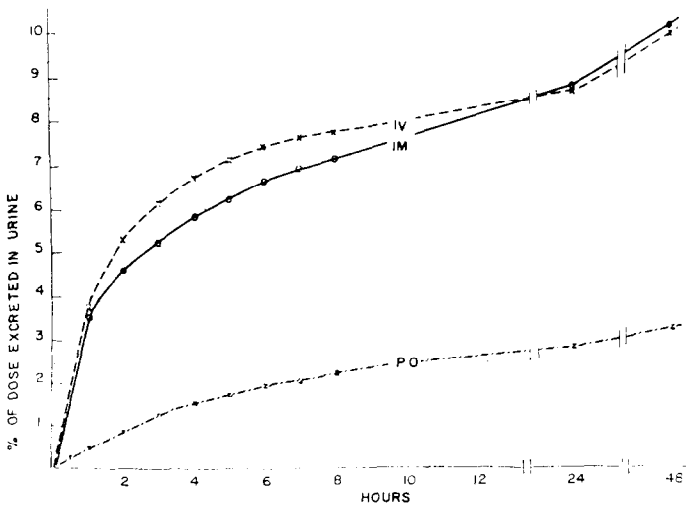


FIG. 5. Excretion of benzquinamide by human male subjects receiving 100 mg by various routes. Results are averaged data from four subjects.

the benzquinamide molecule and is not exchangeable with hydrophilic solvents under most conditions and certainly not under physiological conditions. Moreover, tritium in this position remains stable in all the known metabolites and can be removed as water only by such transformations as deacetylation, followed by oxidation to the ketone; by other reactions that would activate the 2-position by the introduction of

conjugated unsaturation; or by more extensive degradation of the ring system. The elimination of radioactivity in the urine and feces of animals receiving 2-^{3}H benzquinamide is shown in Fig. 6 (rat) and Fig. 7 (dog). In both species, about 70% of the administered activity was recoverable in the excreta.

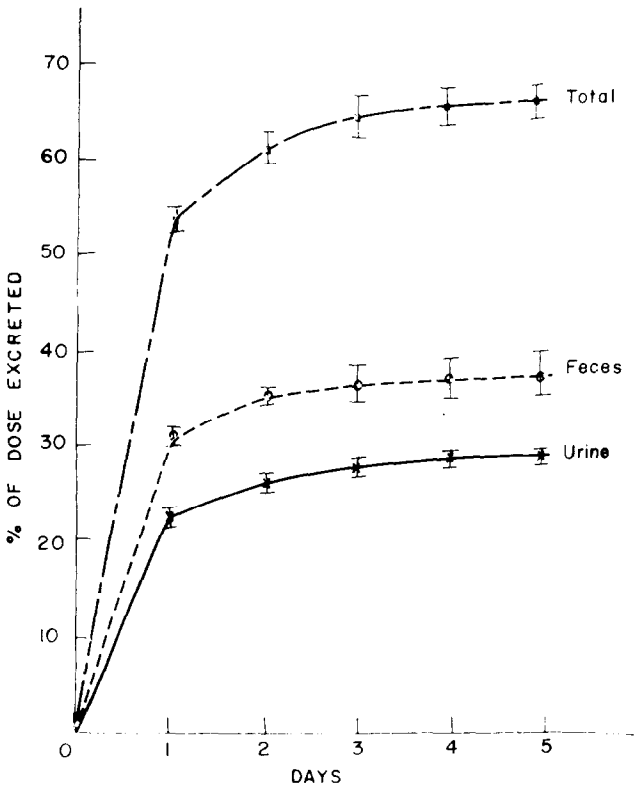


FIG. 6. Excretion of radioactivity by the rat after administration of 2-^{3}H-benzquinamide (8·0 mg/kg i.p.). Bars on graph show excretion range for two animals.

A large proportion of the administered material that is not detectable in the urine as unchanged drug can be accounted for as closely related metabolites (see below). However, the lack of a complete material balance and the appearance of tritiated water in the urine are evidence that benzquinamide must also undergo more profound metabolic changes. Assumed that the isotopic composition of water is constant throughout the body, and that respiratory loss of water is accounted for,[17–19] it was calculated that in the rat, approximately 2%–5% of the dose of 2-^{3}H-benzquinamide was converted to tritiated water.

Owing to the slow turnover of body water, tritium in this form could be expected to remain in the animals for considerable periods. Radioactivity was still present in the tissues 30 days after drug administration. The radioactivity was present in all tissues of the body but, because of low concentrations, has not been identified. Some possibilities, however, may be eliminated. The tissue radioactivity apparently was not due entirely to tritiated water. Repeated lyophilization of tissue homogenates by the

addition and removal of water eventually produced a sublimate which contained no radioactivity, although measurable levels were still present in the dry residue. Moreover, the specific activity of the tissue homogenates considerably exceeded the specific activity of the water removed, and the rate of tritium excretion at the time tissue concentrations were determined was far slower than would be anticipated from the half-life of water in the rat.*,[18]

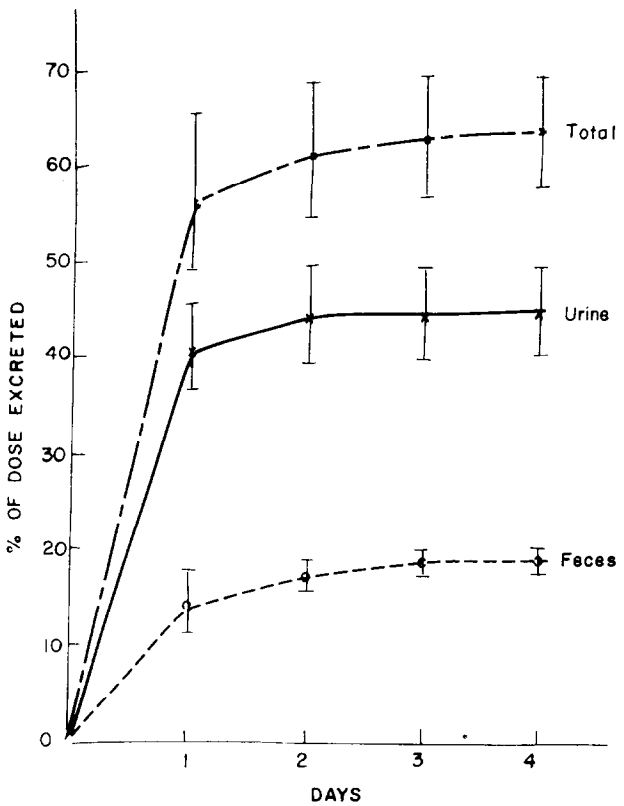


FIG. 7. Excretion of radioactivity by the dog after administration of 2-^{3}H-benzquinamide (2·0 mg/kg i.v.). Bars on graph show excretion range for two animals.

Chromatographic examination of a number of tissue extracts, prepared in different ways, failed to show the presence of benzquinamide or any of its recognized metabolites. The only radioactive species detectable in the urine during the final phase of the experiment was tritiated water. A possible explanation of these findings would be the presence of a hitherto unrecognized metabolite of benzquinamide which is tightly bound at some tissue site from which it is slowly released by metabolism. However, it is believed that the data may also be accommodated by the hypothesis that the tissue radioactivity is due to the presence of normal body constituents that have been labeled as the result of biosynthesis which in some way utilized the tritium atom of benzquinamide.

* The reported half-life of water in the rat (3·3 days) was confirmed in our laboratories.

It was pointed out earlier that only reactions involving the 2-position of benzquinamide could lead to liberation of the tritium label. It is reasonable to assume that the sequence of reactions is deacetylation of benzquinamide to the alcohol, followed by oxidation by an alcohol dehydrogenase with NADP serving as the initial hydrogen acceptor. By analogy with studies on deuterated ethanol,[20] this must lead to the formation of tritiated reduced NADP, which would then be available for participation in subsequent biochemical reductions. It has been shown recently that the fatty acids synthesized in the presence of tritiated reduced NADP carry a tritium label.[21] It appears reasonable to postulate, therefore, that the low levels of tissue radioactivity remaining in animals treated with 2-^{3}H-benzquinamide are the result of the biochemical ubiquity of reduced NADP which has been labeled by participation in the oxidation of benzquinamide. Because of the small amount of benzquinamide that follows this metabolic route, the specific activity of any biochemically-labeled product would be extremely low. For example, cholesterol isolated by conventional methods from the liver of a rat killed five days after administration of 2-^{3}H-benzquinamide did show an increase in count rate over background.* The increase however, was statistically insignificant (90% confidence limits). The residual tissue radioactivity, calculated as benzquinamide, represented a tissue concentration in the range of 0·1–0·5 μg/g.

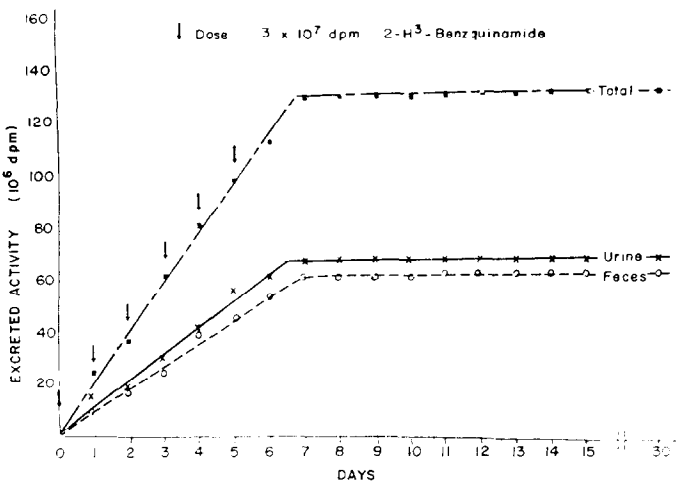


FIG. 8. Excretion of radioactivity by rat receiving daily doses of 2-^{3}H-benzquinamide (8·0 mg/kg/day i.p.).

The presence of these low, but detectable, levels of radioactivity in tissue 5 and 30 days after a single dose of 2-^{3}H-benzquinamide made it desirable to determine if these levels were measurably increased by repeated administration of the drug. Accordingly, a rat was treated with six consecutive daily doses of 2-^{3}H-benzquinamide ($3{\cdot}0 \times 10^7$ dpm/kg/day) and excretion was monitored for 30 days. Excretion of activity by this animal is shown in Fig. 8. During the six days of treatment the fraction of the daily

* The liver was saponified with 10% potassium hydroxide in 50% aqueous ethanol and the solution extracted with hexane. The dried hexane extract was chromatographed over deactivated alumina[22], and the eluant fraction containing cholesterol (confirmed by paper chromatography [23]) was assayed by liquid scintillation counting.

dose excreted per day was uniformly greater than that excreted after a single dose. In this experiment, an average of 77% of the daily dose was excreted per day as opposed to an average of about 60% for the single-dose experiments. Twenty-four hours after the last dose, 74% of the total of six doses had been excreted, and at the end of 30 days approximately 90% had been excreted. Thus, in the subacute experiment, the proportion of the total dose excreted was substantially greater than that observed after the acute single-dose experiments. The amount of radioactivity remaining unaccounted for at the end of the experiment was about the same after the six-dose experiment as it was after single-dose administration. Examination of residual radioactivity in skeletal muscle, taken as a representative tissue, from the subacute experiment showed levels of 1,200 dpm/g of wet tissue or 0·2 μg/g calculated as benzquinamide. The results of this experiment indicate that chronic administration does not result in cumulative increase in residual tissue radioactivity. Since it will be recalled that, in the latter phases of the 30-day experiment, the entire urinary excretion of radioactivity was in the form of tritiated water, it seems logical to conclude that the residual tissue radioactivity is in the form of tritium-labeled body constituents that are eventually metabolized, releasing tritium in the form of water, which is then excreted.

Metabolic products of benzquinamide also appear to be removed by biliary excretion. In dogs receiving benzquinamide chronically (40 mg/kg/day), fluorometric assay revealed substantially less than 5% of the drug in the feces. Subsequent experiments in dogs prepared with biliary fistulas showed that small amounts of unchanged drug (0%–2% in 6 hr) were cleared by this route. In a rat similarly prepared, 15% of the radioactivity from administered 2-^{3}H-benzquinamide could be recovered in the bile in 24 hr. Normal rats excreted about 35% of administered radioactivity in the feces over 3 days. Chromatography, utilizing unlabeled carrier benzquinamide, failed to show any radioactivity associated with the unchanged drug. Presumably, any unchanged benzquinamide appearing in the bile was largely reabsorbed, but metabolites, in all probability of greater polarity than the drug, were incompletely reabsorbed and were excreted in the feces.

Site of metabolism of benzquinamide

In vitro *studies.* Incubation of benzquinamide with human and dog plasma for periods as long as 24 hr demonstrated that plasma esterases were incapable of deacetylating the drug. Examination by paper chromatography of the incubated solution showed only the presence of unchanged benzquinamide. Homogenates prepared from brain and kidney tissue proved similarly incapable of degrading benzquinamide. However, incubation of benzquinamide with rat liver microsomal preparations, with appropriate added cofactors, caused rapid destruction (ca. 50% in 40 min) of material responsive to the fluorometric assay. If the incubation was stopped after 35–40 min and the residue examined in chromatographic systems capable of resolving benzquinamide and the corresponding alcohol (systems 1, 2, and 3), little or no benzquinamide alcohol (II) was detectable. Only unchanged benzquinamide and more polar metabolites could be found.

In situ *liver perfusion studies.* Dogs were anesthetized and the liver, together with the portal vein, exposed. With other veins occluded, a solution of benzquinamide was injected rapidly into the portal vein, and samples of blood were drawn at intervals from the inferior vena cava. At the conclusion of the experiment the dogs were sacrificed, and the livers were removed and frozen prior to assay. The liver was capable

of handling high plasma levels of benzquinamide, rapidly transforming the drug to materials unresponsive to the fluorometric assay. After injection of benzquinamide at a concentration of 190 μg/ml into the portal vein, drug concentrations in the inferior vena cava, between 1 and 10 min after injection, were 0–6 μg/ml (2 animals). Assays for benzquinamide in liver tissue at completion of the experiment were below the limit of detectability of the fluorometric assay (<1 μg/g).

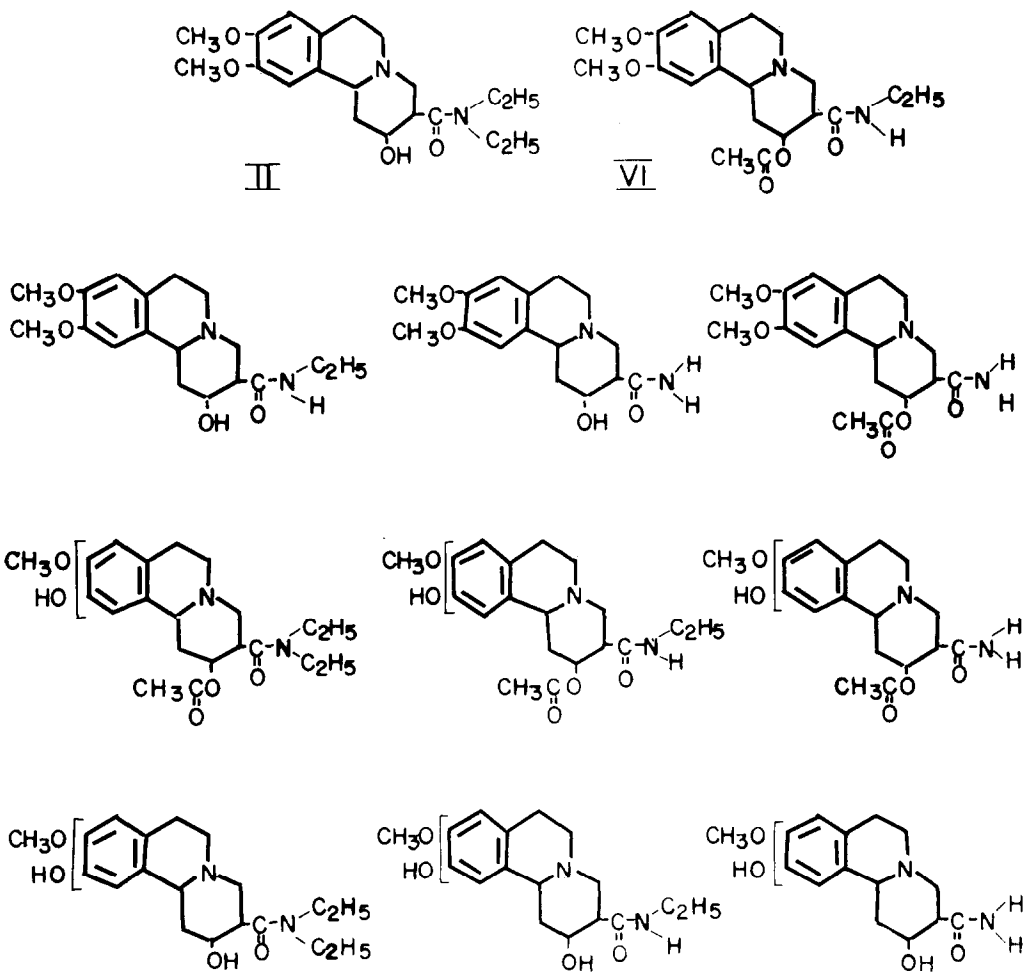


FIG. 9. The urinary metabolites of benzquinamide.

Urinary metabolites of benzquinamide. As noted in the preceding sections, benzquinamide is extensively metabolized, and only a small fraction of the administered drug is excreted unchanged in the urine. Isolation and chemical structure studies have led to the identification, in the urine of both dogs and human subjects treated with benzquinamide, of the eleven metabolites shown in Fig. 9.[24] The structures of these metabolites indicate the existence of at least three metabolic pathways. Of these, N-dealkylation appears to predominate, N-deethyl benzquinamide (VI), the most

abundant metabolite, exceeding in urine by three- to six-fold the amount of unchanged drug. This compound has no significant central nervous system activity of the type shown by benzquinamide and is without effect on conditioned avoidance behavior of rats in doses several times those at which benzquinamide is active*. O-Demethylation occurs also, but which of the two methoxy groups is dealkylated has not been established. Although metabolites with free 2-hydroxyl groups corresponding to all the identified 2-acetyl metabolites have been detected, deacetylation is apparently a relatively minor pathway. In human subjects receiving benzquinamide, benzquinamide alcohol (II) was barely detectable in the urine. In contrast, subjects receiving benzquinamide alcohol excreted 5%–10% unchanged in the urine over a 24 hr period.

The experimental evidence presented characterizes benzquinamide as a drug which is rapidly absorbed and distributed throughout body tissues but, because of facile renal excretion and metabolism, has only a short plasma half-life. This rapid removal of benzquinamide from the body accords well with the observed time course of its pharmacological action[3] and with the fact that dosage three or four times a day is usually required in the clinical situation.[25] It thus appears reasonable to suppose that unlike reserpine, for example, the continued presence of benzquinamide in the body is necessary for its action and that its relatively short sojourn in the organism leads to a relatively short duration of effect.

* A. Weissman, personal communication.

REFERENCES

1. K. F. Finger, A. Weissman and J. R. Tretter, *Pharmacologist* **3**, 75 (1961).
2. J. R. Tretter, J. G. Lombardino, K. F. Finger and A. Weissman, *Abstracts*, pp. 4–0, 138th Meeting of American Chemical Society, Chicago, Sept. 1961.
3. A. Weissman and K. F. Finger, *Biochem. Pharmacol.* **11**, 871 (1962).
4. M. E. Smith, *Amer. J. Psychiat.* **118**, 937 (1962).
5. P. E. Feldman, *Psychosomatics* **3**, 148 (1962).
6. M. W. Shulkin, R. Dykyj, T. Bodi, M. Lim, P. E. Siegler, Y. Mapp and J. H. Nodine, *Curr. ther. Res.* **4**, 285 (1962).
7. G. P. Quinn, P. A. Shore and B. B. Brodie, *J. Pharmacol. exp. Ther.* **127**, 103 (1959).
8. H. Brendler, *Science* **114**, 61 (1951).
9. P. S. Chen, *Proc. Soc. exp. Biol. (N.Y.)* **98**, 546 (1958).
10. F. Kalberer and J. Rutschmann, *Helv. chim. Acta* **44**, 1956 (1961).
11. J. Axelrod, *J. Pharmacol. exp. Ther.* **117**, 322 (1956).
12. B. B. Brodie and C. A. M. Hogben, *J. Pharm. Pharmacol.* **9**, 345 (1957).
13. L. S. Schanker, *J. Med. pharm. Chem.* **2**, 343 (1960).
14. T. Teorell, *Arch. int. Pharmacodyn.* **57**, 205 (1937).
15. H. G. Bray and K. White, *Kinetics and Thermodynamics in Biochemistry*, pp. 166. Academic Press, New York (1957).
16. R. G. Wiegand and J. D. Taylor, *Biochem. Pharmacol.* **3**, 256 (1960).
17. A. Krogh and H. H. Ussing, *Skand. Arch. Physiol.* **75**, 90 (1936).
18. R. C. Thompson, *J. biol. Chem.* **200**, 731 (1953).
19. W. S. Spector, *Handbook of Biological Data*, p. 339 Saunders, Philadelphia (1956).
20. H. F. Fisher, E. E. Conn, B. Vennesland and F. H. Westheimer, *J. biol. Chem.* **202**, 687 (1953).
21. D. W. Foster and B. Bloom, *J. biol. Chem.* **238**, 888 (1963).
22. P. B. Schneider, R. B. Clayton and K. Bloch, *J. biol. Chem.* **224**, 175 (1957).
23. S. K. Figdor, E. C. Schreiber, R. B. Stebbins and R. Pinson, Jr., *J. med. Chem.* **7**, 508 (1964).
24. B. K. Koe and R. Pinson, Jr., *J. med. Chem. In press* (1964).
25. M. E. Smith, *Dis. nerv. Syst.* **24**, 116 (1963).