The Metabolism of a Basic Amide Analgesic, SQ 10,269. (30023)

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SQ 10,269, 2-Ethoxy-N-methyl-N-[2-(methylphenethylamino)-ethyl]-2,2-diphenylacetamide, hydrochloride, has been shown to be a potent analgesic agent in animals, and clinical studies have proven this compound effective in the relief of many types of pain, including pain associated with chronic and recurrent diseases(1). This report is concerned with the pattern of excretion of radioactivity following administration of C^{14} labeled SQ 10,269.

Materials and methods. Radioactive SQ 10,269. C^{14}-labeled SQ 10,269 was prepared by one of us (J.K.). The structure of this compound and the position of the radioactive label are shown in Fig. 1.

Whole animal experiments. Adult, male albino rats of the Wistar strain and mongrel dogs were employed in all experiments. Animals were placed in standard metabolic cages so designed that urine and feces could be collected uncontaminated by each other. Rats were fed *ad libitum* on standard Purina Laboratory Chow, ground and moistened with water to prevent contamination of urine and feces. Dogs were fed once daily on Pard, a moist canned dog food.

Formation of 1° and 2° amines. The possible presence of primary and/or secondary amines resulting from incubation of rat liver slices in the presence of SQ 10,269 was determined by extracting suitable aliquots of the incubation medium with chloroform. The chloroform extracts were taken to dryness, dissolved in 0.1 ml water, and treated with 1-fluoro-2,4-dinitrobenzene(2). This reagent forms products with 1° and 2° amines which have an absorption maximum in cyclohexane at 350 mμ.

Measurement of radioactivity. All measurements of radioactivity were made with a Packard Tri-Carb liquid scintillation spectrometer. Urine, feces, and carcass were assayed separately by use of a modified

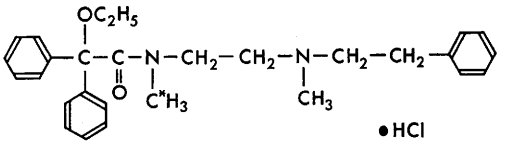

FIG. 1. Structural formula of SQ 10,269-C^{14}, 2-ethoxy-N-methyl-C^{14}-N-[2-methyl-phenethylamino)-ethyl]-2,2-diphenylacetamide, hydrochloride. The position of the radioactive carbon has been indicated by an asterisk.

Schoniger combustion technique(3). All determinations were corrected to 100% efficiency by use of internal standards. Respiratory $C^{14}O_2$ was measured by placing a rat in a glass metabolism cage immediately after its i.p. injection with 2.5 mg of SQ 10,269-C^{14} containing 0.35 μc. Oxygen was admitted into the cage in direct proportion to the amount of CO_2 liberated. CO_2 was absorbed in N NaOH during a 19-hour experiment. The absorbed CO_2 was then converted to $BaCO_3$ by addition of $BaCl_2$, washed with water and acetone, dried, finely pulverized, and assayed for radioactivity in a toluene scintillation solution as a suspension stabilized with Cab-O-Sil.† Suitable corrections were made for quenching and self-absorption.

Results and discussion. Table I contains data on the amount of radioactivity excreted in rat urine and feces during a 4-day period following oral administration of SQ 10,269-C^{14}. It is evident that in 24 hours more than 70% of the dose had been eliminated; of this amount approximately ⅔ was found in the feces. The quantitative distribution of radioactivity between urine and feces was essentially constant during the entire 96-hour period. Of significance is the fact that greater than 90% of the dose could be accounted for in the excreta within 96 hours. This rate of excretion was confirmed by the low values for radioactivity remaining in the carcass after the 72- and 96-hour periods. Less than 2% of the total radioactivity administered could be accounted for as respiratory $C^{14}O_2$.

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TABLE I. Rate and Route of Excretion of SQ 10,269 Orally Administered to Rats.

Hr after admin	% of administered dose: Urine	Feces	Total excretion	Carcass	Total recovered
0-24 (8)	25.0 ± 5.2	48.4 ± 12.4	73.4	9.8 ± 2.3 (2)	83.2
24-48 (6)	4.3 ± 1.5	10.7 ± 4.2	15.0	3.3 ± .3 (2)	91.0
48-72 (4)	.9 ± .6	1.8 ± 1.1	2.7	2.5 ± .6 (2)	93.2
72-96 (2)	.4 ± .09	.6 ± .0	1.0	1.3 ± .2 (2)	93.0
Total	30.6	61.5	92.1		

Rats weighing 90-110 g received orally 2.5 mg SQ 10,269-C^{14} containing 0.35 μc.
Values are expressed as mean per cent of administered dose, and are given with standard deviation.
Figures in parentheses represent number of animals.

The small amounts of radioactivity in respired air corroborated the conclusion that the major routes of elimination of the drug were urine and feces.

Intraperitoneal injection of rats with 2.5 mg of labeled drug resulted in a pattern of excretion similar to that observed for orally dosed animals (Fig. 2). This observation was compatible with the view that the drug was probably well absorbed and that the radioactivity in the feces may not have been due primarily to unabsorbed compound. Proof of this must be sought in the identity of the excretion products in urine and feces.

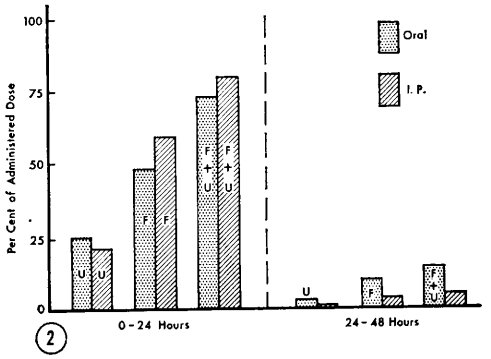
In dog, as in rat, the feces accounted for the largest portion of administered radioactivity (Table II). In contrast to the rat, however, there was proportionally less radioactivity in urine as compared with feces. This may have been due either to less efficient absorption from the gut or to preferential excretion by way of the feces.

TABLE II. Rate and Route of Excretion of SQ 10,269 Orally Administered to Dogs.

Hr after admin	% of administered dose: Dog No. 1 Urine	Dog No. 1 Feces	Dog No. 2* Urine	Dog No. 2* Feces	Dog No. 3 Urine	Dog No. 3 Feces
24	11.8	69.3			.1	.2
			5.4	39.5		
48	3.0	4.8			16.8	26.4
72	1.4	.9			3.3	23.4
			3.6	18.7		
96	1.0	.9			1.5	8.5
Total	17.2	75.9	9.0	58.2	21.7	58.5

Dog No. 1 received (in a gelatin capsule) 200 mg SQ 10,269-C^{14} containing 27 μc. Dogs No. 2 and 3 received 200 mg SQ 10,269-C^{14} containing 14 μc. Dogs were fasted for 20 hours prior to dosing. Values expressed as per cent of administered dose.
* Urine and feces could only be obtained at 48 and 96 hr; therefore, each value for dog No. 2 represents the total of 2 successive 24-hour periods.

Incubation of rat liver slices in the presence of unlabeled drug resulted in the production of material that reacted with 1-fluoro-2,4-dinitrobenzene (Fig. 3), which indicated



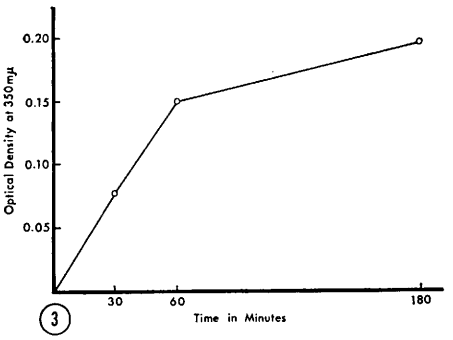


FIG. 2. Comparison of excretion of radioactivity following oral or intraperitoneal administration to rats of 2.5 mg of SQ 10,269-C^{14}.

FIG. 3. Formation of 1-fluoro-2,4-dinitrobenzene derivatives by slices of rat liver incubated in presence of SQ 10,269. Slices weighing approximately 450 mg were incubated at 37°C in 10 ml Krebs-Ringer phosphate buffer (pH 7.4), containing 3 mg/ml SQ 10,269. 2 ml aliquots were removed and assayed for dinitrobenzene reacting substances.

the formation of either primary and/or secondary amines. An unidentified metabolite was produced by the incubation of rat liver slices at 37°C in the presence of Krebs-Ringer phosphate buffer (pH 7.4) containing 0.05 μc of SQ 10,269-C^{14}. At the end of the incubation 1 ml of the medium was removed, made alkaline to pH 10 with N NaOH and extracted 3 times with 5 volumes each of chloroform. The extracts were combined, evaporated to dryness and the residue was taken up in 0.5 ml of chloroform; 100 μl was chromatographed. The solvent used was 5% formamide in butylformate. The chromatogram was developed by the descending technique for 3½ hours on Whatman #1 filter paper previously impregnated with formamide. Two radioactive areas were found: unchanged SQ 10,269 (R_f 0.77) and the metabolite (R_f 0.25). This radioactive zone did not correspond to the zones for any of the analogues or potential metabolites available for testing. In similar studies, kidney, muscle (diaphragm) and brain showed no activity in metabolizing SQ 10,269 as judged by the formation of dinitrophenyl derivatives.

Paper chromatography with the formamide-butylformate system of urine from animals dosed with the labeled drug revealed only one significant radioactive area; it was located at the origin of the chromatogram. Chloroform extracts of alkaline urine revealed trace amounts (<2%) of SQ 10,269 and another slower moving radioactive area which were only barely detectable on chromatography of whole urine. Thus, although it was demonstrated that SQ 10,269-C^{14} dissolved in urine, methanol or chloroform, traveled with essentially the same R_f, there was no significant migration of radioactive material in the urine of the dosed animals. Paper electrophoresis of experimental urine did reveal the presence of 3 distinct radioactive areas; none of these areas corresponded either to SQ 10,269 or to a series of analogues or possible metabolites available for testing (Fig. 4). The pure compounds were checked in urine since the migrations in water differed.

The patterns of radioactivity produced by the electrophoretic separation of the constituents of rat and dog urine were similar; but

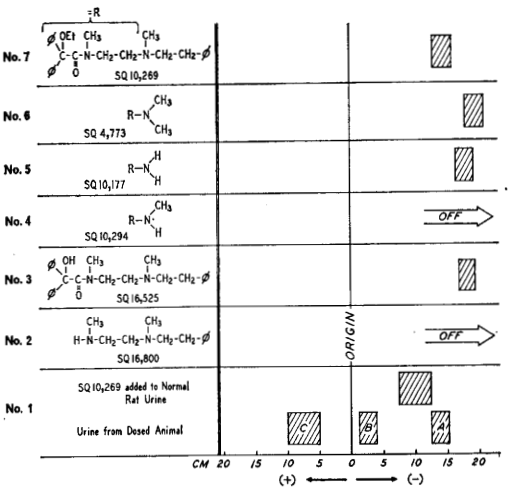


FIG. 4. Paper electrophoresis of rat urine following administration of 2.5 mg of SQ 10,269-C^{14}. Included are electrophoretograms of analogues and of possible metabolites. Shaded areas represent zones of radioactivity in the case of No. 1 and No. 7, and zones of either ninhydrin-reacting or ultraviolet-absorbing material in all other experiments. Buffer: pyridine-acetate, pH 4.0. Voltage: 14 v per cm. Time: 5 hr.

the distribution of radioactivity obtained for the rat differed from that obtained for the dog (Rat: A = 18%; B = 44%; C = 38%. Dog: A = 20%; B = 5%; C = 75%). Both areas B and C represent compounds more acidic than SQ 10,269, possibly phenolic metabolites.

Treatment of rat urine with β-glucuronidase liberated chloroform extractable material. Subsequent paper electrophoresis of the treated urine revealed that approximately 40% of area C had been hydrolyzed by β-glucuronidase, indicating the presence of a glucuronide.

Although area B was not identified, this may have represented a phenolic derivative. The sum of the radioactivities in areas B and C was the same for both rat and dog urine and this suggests a more efficient conversion of a phenol to a glucuronide by the dog.

Summary. After administration of SQ 10,269 to rats, more than 70% was eliminated in 24 hours. Approximately ⅔ of the dose was excreted in feces. By the fourth day following administration of the drug only 1.3% remained in the carcass and about 90% of the dose had been excreted in urine and

feces. After administration to dogs, the major portion of the labeled drug was excreted within 3 days but more radioactivity was present in feces than in urine. The electrophoretic patterns of radioactivity obtained for rat and for dog urine were similar but more glucuronide appeared in the urine of dogs.

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Effect of Triton Ingestion on Fat Absorption.* (30024)

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In an earlier study there appeared to be some delay in the absorption of fat when Triton† was administered orally along with the fat(1). Variations in fat absorption as measured by the appearance of radioactivity in the blood prompted a further study of this and associated phenomena. Janicki *et al*(2) have reported the rate of absorption of I-131 labeled oleic acid was not influenced by circulating Triton in the guinea pig.

If Triton should inhibit the lipolytic activity of pancreatic lipase, then its presence in the intestinal tract would be expected to decrease fat absorption. A study was undertaken to learn the extent of any effect of Triton on lipolytic activity and also to determine whether the presence of bile salts might alter this effect.

The possibility of a gradual loss of Triton from the intestinal tract when fed with the fat is suggested by a greater early decrease in the rate of appearance of fat in the blood, 39% as compared with only 16% later on(1). However, Cornforth *et al*(3) failed to detect any Triton in the blood of mice fed large amounts of Triton for one month.

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† Triton WR-1339 (oxyethylated tertiary octyl phenol formaldehyde polymer), Winthrop Laboratories, is a detergent which has been used as an aid in studying fat metabolism.

The results obtained in this study support the contention that Triton, given orally, is not absorbed and that it interferes with fat absorption by markedly inhibiting lipolysis of ingested fat.

Experimental. Initially 5 male albino rats, weighing about 250 g, were allowed 10% cerelose solution and water for 2 days. Then they were given orally by tube 1.5 ml of 10% Triton solution (WR-1339) and 0.3 ml of corn oil per sq dm of body surface. A similar amount of Triton solution was administered one hour later. Initially and 1, 2, 3 and 4 hours later, 0.2 ml blood samples were collected from the rat's tail for esterified fatty acid (EFA) determinations by the method of Stern and Shapiro(4). These determinations under similar conditions were repeated on the same rats after intervals of at least 2 weeks with only fat and then with Triton alone.

To determine the effect of Triton on the lipolytic activity of pancreatic lipase, the following procedure was employed. An incubation mixture was prepared with 1 ml of 0.05 M tris buffer (pH 8.5) containing 100 mg of bovine albumin (Armour), 0.4 ml supplement of water or various dilutions of Triton (0.0 to 0.125%) and 0.1 ml Lipomul-IV.‡ Aliquots of the incubation mixture were taken immediately after addition of 1.0

‡ Lipomul-IV, Upjohn Co., Kalamazoo, Mich., is a fat emulsion for intravenous infusion.