Absorption and Elimination Profile of Isoproterenol III

The Metabolic Fate of *dl*-Isoproterenol-7-^{3}H in the Dog

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Intravenous injection of isoproterenol-7-^{3}H in dogs, in doses ranging from 0.4 to 1.6 mcg./kg. body weight, immediately induced tachycardia which declined with a half-life of about 1 min. The resulting levels of radioactivity in blood declined with a significantly longer half-life of 3–4 min. Unchanged isoproterenol accounted for less than 1 percent of the total radioactivity in plasma after intravenous medication. After oral administration of 15 mg. of isoproterenol-7-^{3}H hydrochloride to dogs, a biphasic time course was observed for both heart rate and radioactivity in blood with maxima occurring at 0.5 and 1–2 hr. after medication. The metabolic fate of isoproterenol was determined by the route of medication, the intravenous and intraportal route giving rise mainly to 3-*O*-methylisoproterenol, while oral medication was converted mainly to a sulfuric acid ester which was formed either by the intestinal flora or during passage through the intestinal wall. The rapid decrease in tachycardia after intravenous medication is consistent with the rapid conversion of the drug to inactive metabolites while the more prolonged tachycardia induced by oral medication results from slow absorption.

THE EFFICACY of the sympathomimetic amine, isoproterenol hydrochloride,[1] [1-(3,4-dihydroxyphenyl)-2-isopropylaminoethanol] in the treatment of bronchial asthma and other bronchopulmonary diseases as well as heart block, when administered by various routes of medication, prompted recent studies of the absorption profile of the drug in dogs (1, 2). The drug was found to be poorly absorbed from the stomach but well absorbed from the small intestine, proximal colon, rectum, and from the mucous membrane of the trachea as measured indirectly by the resulting tachycardia. The rapid disappearance of chronotropic activity observed after intravenous administration in contrast to the prolonged activity following oral medication and the relatively greater efficiency in producing tachycardia by infusion of the drug into the femoral vein in contrast to the gastroepiploic vein, prompted the biochemical studies of the metabolic fate of isoproterenol after various routes of administration. The tachycardia is believed to reflect only the concentration of free isoproterenol in plasma and it was hoped that this could be confirmed by direct measurement.

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[1] Trademarked as Isuprel, by Winthrop Labs., New York, N. Y.

EXPERIMENTAL

Materials—*dl*-Isoproterenol-7-^{3}H hydrochloride with a specific activity of 287 μc./mg. base was used for the intravenous injection studies. This was diluted with nonradioactive carrier to prepare medication with specific activities ranging from 4 to 66 μc./mg. base for the oral and intravenous infusion studies. The drug was dissolved in 5% dextrose solution containing sodium bisulfite (1:100,000) and ethylenediaminetetraacetic acid disodium salt (1:10,000). All doses are expressed in terms of isoproterenol base.

Animal Preparations—The heart rate was monitored by means of an electrocardiograph (Viso-Cardiette, Sanborn) using the Lead I attachments. Food was withheld 17–18 hr. prior to medication. Dogs of either sex, weighing between 8 and 17 kg., were anesthetized with sodium pentobarbital (30 mg./kg., i.v.). Both femoral veins were exposed, one for drug injection and the other for drawing blood *via* an implanted catheter. Urine samples were obtained periodically by rinsing the bladder with two 20-ml. portions of distilled water through an indwelling catheter. Ethylenediaminetetraacetic acid (100 p.p.m.) and α-thioglycerol (10 p.p.m.) were added to urine and blood samples as preservatives. All samples were kept frozen until analyzed. Where collection of bile was desired, the cystic duct was ligated and a polyethylene catheter was implanted in the common bile duct.

Conscious dogs of either sex, which weighed from 12 to 18 kg., were placed in sling-stand frames designed to keep the animal still but with a minimum of restraint. Blood was withdrawn periodically from the saphenous and radial veins. Urine was obtained periodically as described above.

Analytical Methods—Duplicate samples of blood (0.5 ml.) were dried and prepared for counting by a modification of the oxygen-flask combustion technique (3). Samples of urine were dissolved in a water-miscible scintillator for counting (4). Plasma samples (0.1 ml.) were digested with 1 *N* NaOH at 60° for 1 hr., neutralized with 0.5 ml. of oleic

acid, diluted with 1 ml. of water, and added to 15 ml. of the water-miscible scintillator. All samples were counted for 10 min. in a Packard Tricarb model 3324 liquid scintillation spectrometer operated at 2° and were corrected for quenching by an internal standard technique. The sensitivity of the assays was determined by the specific activity of the drug used in the particular experiment. Concentrations indicated as not significant in the text were based on net count rates less than 7 c.p.m.; corresponding to a standard error in the net count of ±20%.

Approximately 0.5 mg. each of authentic samples of isoproterenol (I), 3-*O*-methylisoproterenol (OMI), normetanephrine (NM), and norepinephrine (NE) were added to aliquots (1–2 ml.) of urine or plasma which contained 100,000–200,000 d.p.m. of tritium in the form of radiochemically labeled isoproterenol and its metabolites, after which the samples were adjusted to pH 8. Metabolites were then separated by ion-exchange chromatography on 1 × 3-cm. columns of Bio-Rex 70 resin (approx. 10 g., 200–325 mesh, Biorad Laboratories, Richmond, Calif.) developed with ammonium phosphate buffer (0.05 *M*, pH 8.0) containing 0.001 *M* thioglycerol as an antioxidant. The elution volumes of the compounds added to the sample were determined by continuous monitoring of the absorption of UV light (254 mμ) by the effluent. The effluent stream was also split to provide aliquots for monitoring the elution of radioactivity. The presence of conjugates was established by the shift in the elution pattern of metabolites brought about by enzymatic hydrolysis of samples prior to chromatography.

Paper electrophoresis was conducted using phosphate buffer (0.05 *M*, pH 8.0) and a voltage gradient of 900 v./56 cm. Authentic compounds, added as carriers, were located by spraying with iodoplatinate reagent. Mobilities, as cm., migrated toward the cathode in 1 hr., were: I, 11; OMI and NE, 12; NM, 13. Electrically neutral material migrated 3.5 cm. as a result of electroosmosis. The distribution of radioactivity was determined by cutting the electrophoregrams into 1-cm. strips which were soaked for 2 hr. in counting vials with 2 ml. of water, then mixed with 15 ml. of dioxane scintillator (4).

Conjugates of metabolites with glucuronic and sulfuric acids were hydrolyzed by adding 0.1 ml. of glucuronidase[2] mixture A to 2.0 ml. of plasma or urine (adjusted to pH 5.5 with acetate buffer) and incubating for 18 hr. at 37° in a closed vessel containing a nitrogen atmosphere and a drop of chloroform. This assay indicated that such an incubation mixture contained 77 arbitrary units of glucuronidase/ml. and 560 arbitrary units of sulfatase/ml. One arbitrary unit is defined as the quantity of enzyme required in 1 ml. of the reaction mixture to hydrolyze 1 μmole of substrate per hr. at 37°, pH 5.5, substrate concentration 0.0025 *M* in the presence of bovine serum albumin, 0.25 mg./ml. Substrates used in the assay were potassium *p*-nitrophenyl sulfate and *p*-nitrophenylglucosiduronic acid.

RESULTS

Chromatographic Analyses—The typical pattern for elution of metabolites from the weak cation exchanger is shown in Fig. 1. Neutral, acidic, and conjugated metabolites are not retained by the resin and the elution volume for these metabolites (approximately 8 ml.) represents the hold-up volume of the column. The authentic compounds, OMI, I, NM, and NE, were eluted in that order as indicated by the lower curve. The upper curves present the distribution of radioactivity in the column effluent from samples of a urine before and after hydrolysis with glucuronidase mixture A, a potent source of the enzymes β-glucuronidase and sulfatase. The radioactivity eluted in a particular peak before hydrolysis represents the free compound and that eluted after hydrolysis represents the total (free + conjugated) amount of the metabolite.

It is apparent from the shift of the pattern after glucuronidase treatment that the major metabolite in the particular urine sample illustrated is conjugated isoproterenol. From the difference in the radioactivity contained in each peak before and after enzymatic hydrolysis, the composition of this particular urine (as % of radioactivity in urine) can be calculated as I (free 0%; conjugate, 67.5%), OMI (free 6.7%; conjugate, 7.3%), NM (free 0.8%; conjugate, 0.0%), neutral and acidic materials (17.5%), NE (0%). NE was not detected as a metabolite in any urine or plasma sample. The presence of 0.8% NM in the sample illustrated is of borderline significance. Failure of the numbers to total 100% may result from rounding off errors or from elution of small amounts of radioactivity in areas not represented by discrete peaks. The results of many such analyses are presented later in the text and figures, usually calculated as a percentage of the administered dose. Where the concentrations of metabolites or conjugates are expressed as mcg./l., the figures refer to concentrations of molecularly equivalent solutions of isoproterenol.

Blood Levels After Intravenous Administration—Rapid intravenous injection of 0.4, 0.8, and 1.6 mcg./kg. of isoproterenol-^{3}H increased the heart rate of anesthetized dogs by 70, 105, and 120 b.p.m. within 30 sec. The tachycardia regressed with a

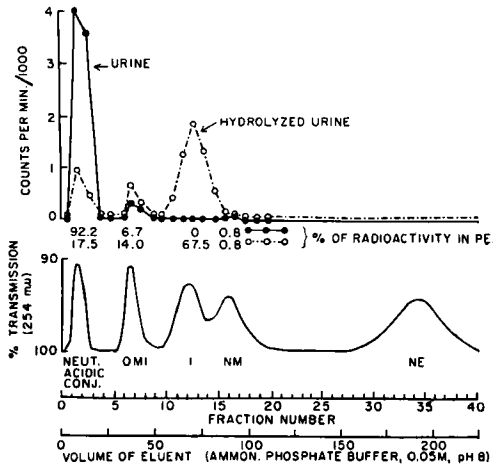

Fig. 1—Fractionation of metabolites on weak cation-exchange resin.

[2] Glusulase, Endo Products Inc., Richmond Hill, N. Y.

half-life ranging from 0.9 to 1.3 min. during the first 3 min. after medication (Fig. 2) and returned to the premedication rate within 5–7 min. On the other hand, the concentration of radioactivity in whole blood declined with a much longer half-life which ranged from 3.0 to 4.1 min. The heart rate is believed to reflect only the concentration of unchanged isoproterenol, whereas the tritium concentration represents the sum of all radioactive compounds in the blood.

In order to obtain a plasma level of radioactivity sufficiently high to enable estimation of the circulating metabolites, a dose of 131 mcg. isoproterenol-7-^{3}H/kg. (specific activity 287 μc./mg. base) in 1 ml. of aqueous solution was given by rapid injection into the femoral vein of one anesthetized dog. The tritium declined in two phases, a rapid phase with a half-life of about 5 min. and after 30 min. a second slower decline with a half-life of about 4 hr.

One minute after injection, ion-exchange chromatography indicated that the major metabolite in the plasma was OMI which accounted for 63% of the radioactivity. Isoproterenol amounted to less than 1% of the total radioactivity and, in fact, could not be detected. The NM content was also less than 1%. Neutral, acidic, and conjugated metabolites, which were not further resolved, made up 35% of the total. Electrophoretic analysis of the 3-min. plasma gave similar results, 65% OMI and 35% electrically neutral material; isoproterenol and acidic materials were not detectable.

Urinary Excretion—The rate of excretion of radioactivity in urine was similar for the four dogs given intravenous medication. After 6 hr. the amounts excreted were 84, 82, 70, and 83% following doses of 0.4, 0.8, 1.6, and 131 mcg./kg., respectively. The urine from the dog given the highest dose was examined in detail by ion-exchange chromatography and these results are summarized in Fig. 3, where the cumulative excretion of both total radioactivity and individual metabolites are shown. Free isoproterenol was found only in the early urine, 0.4% of the dose in the first 20 min., and another 0.2% in the next 10 min. Conjugated I was detected throughout the 6-hr. period although a major portion had been excreted within the first hour. NM and its conjugates accounted for only 3% of the dose. The metabolite excreted in largest amount at all times was OMI which, in the free form, accounted for 43% of the administered dose over the 6-hr. period. Another 10% was excreted in conjugated form. Neutral and acidic metabolites accounted for about 20% of the dose. The exact nature of this fraction has not yet been determined, but preliminary results involving fractionation on an anion-exchange resin indicate that less than 2% of the dose can be accounted for as 3-methoxy-4-hydroxymandelic acid.

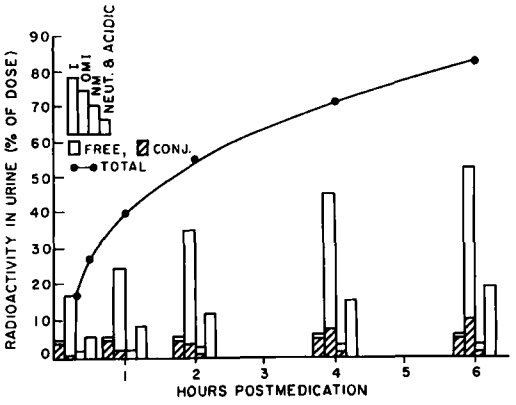

Fig. 3—Cumulative excretion of metabolites in the urine of dog No. 12 following rapid injection of 0.13 mg./kg. of isoproterenol-^{3}H into the femoral vein.

Blood Levels After Oral Administration—A dose of isoproterenol-7-^{3}H hydrochloride equivalent to about 0.8 mg./kg. was dissolved in 2 ml. of water and administered orally to four conscious dogs. The dose was delivered from a syringe at the back of the tongue and was followed immediately by a rinse with 5 ml. of water. The time course of the resulting tritium concentration in whole blood (Fig. 4) shows a distinct double peak in three of the four dogs. This double peak was also reflected in the heart rate.

The mean maximal increase in heart rate (173%) was obtained in 10 min. following medication, and the duration of action was approximately 3 hr. In comparison with the intravenous half-life of 1–2 min., this longer duration of heart-rate activity

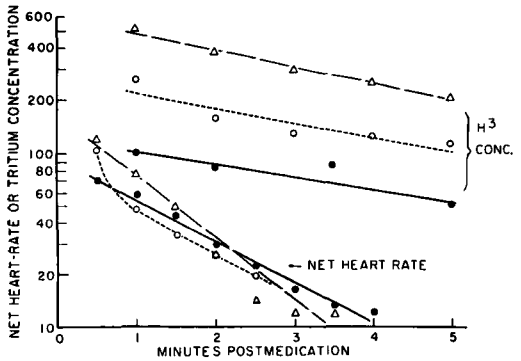

Fig. 2—Net heart rate (b.p.m. above premedication level) and tritium concentration (as 100 × the equivalent level of isoproterenol-^{3}H in mcg./l.) in whole blood following rapid intravenous injection of isoproterenol-^{3}H in dogs. Key (dose in mcg./kg.): ●, 0.4; ○ 0.8; △, 1.6.

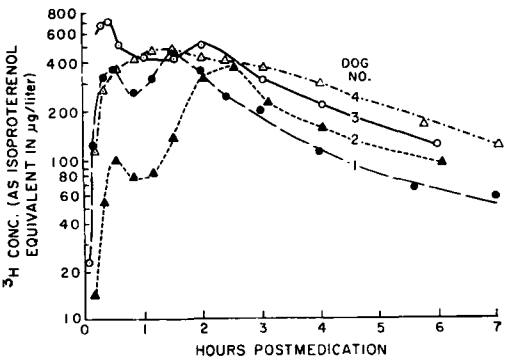

Fig. 4—Blood level of radioactivity following oral administration of isoproterenol-^{3}H to dogs as a solution of the hydrochloride salt. Key (dose, mg. base/kg.): ●, 0.78; ▲, 0.65; ○, 1.08; △, 0.82.

following oral medication indicated that the absorption from the intestinal tract was slow and prolonged. Slow absorption of oral medication was reflected also in the blood tritium level, which decreased at a considerably slower rate after oral than that after intravenous administration. At the third hour, when most of the cardiac effect had been terminated, the total blood tritium was still at a relatively high level of 270 mcg./l., reflecting mostly the total metabolites rather than active isoproterenol. In one experiment (dog No. 3) the plasma was analyzed by ion-exchange chromatography. Twenty minutes after medication, 5% of the radioactivity was in the form of OMI and 94% in the form of either neutral, acidic, or conjugated metabolites. Paper electrophoresis confirmed this and further indicated the presence of less than 5% in the form of acidic metabolites. Neither I nor NM was detected by either method. Estimation for tritiated water content of plasma indicated the conversion of 3% of the dose in 4 hr.

Urinary Excretion—The amount of radioactivity excreted in the urine of dogs within 6 hr. was 64, 45, 74, and 57%, respectively, of the administered dose. Chromatographic analysis of the urine from dog No. 3 (Fig. 5) indicated that less than 0.1% of the total dose was excreted as unchanged I. The major metabolite was conjugated I which accounted for 50% of the dose. Free OMI accounted for 5.8% of the dose and its conjugate accounted for another 6.2%. Less than 1% of either free or conjugated NM was found, and neutral and acidic metabolites, which have not been further identified, accounted for only 10% of the dose.

Plasma Levels After Intraduodenal Administration—The levels of radioactivity observed in three anesthetized dogs following direct injection of isoproterenol-^{3}H hydrochloride (in 2 ml. of water) into the duodenum are presented in Fig. 6. In contrast to the orally dosed dogs, only a single peak was observed. Chromatographic analysis of plasma samples indicated that the major metabolite at all times was conjugated I which, in one experiment, measured 70 mcg./l. at 10 min. and a maximum of 152 mcg./l. after 3 hr. Free drug levels ranging from 1 to 2.4 mcg./l. were measured in the

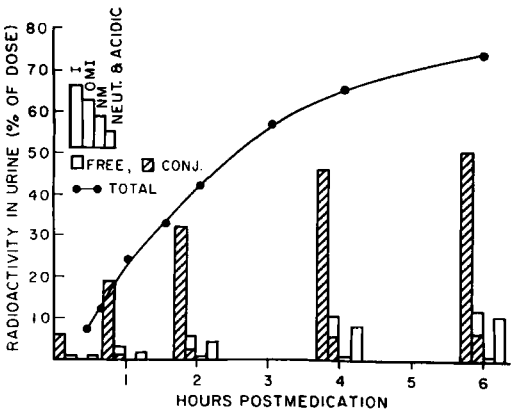

Fig. 5—Cumulative excretion of metabolites in the urine of dog No. 3 following oral administration of 1.08 mg./kg. of isoproterenol-^{3}H as the hydrochloride salt in solution.

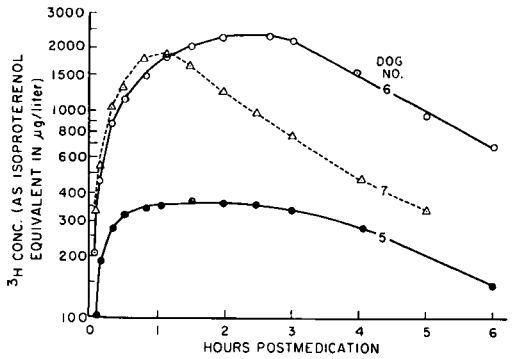

Fig. 6—Plasma level of radioactivity following intraduodenal administration of isoproterenol-^{3}H to anesthetized dogs. Key (mg. base/kg.): ●, 0.80; ○, 1.06; △, 1.07.

3-hr. period following medication, but these levels are so low as to be of doubtful significance. They do serve, however, to place an upper limit on the level of free drug to be expected under these conditions. From a level of 42 mcg./l. at 10 min., a maximum level of 66 mcg./l. of OMI was reached 30 min. after medication while its conjugates, which were labile to glucuronidase mixture A, attained a maximum of only 13 mcg./l. after 4 hr. NM was never present at a concentration above 2.4 mcg./l., but its conjugates were present at a concentration of 35 mcg./l. after 10 min. and a maximum concentration of 67 mcg./l. after 3 hr.

Urinary and Biliary Excretion—The excretion of radioactivity in the urine after intraduodenal administration ranged from 59 to 65% of the dose in 5–6 hr. after medication. However, biliary excretion was less than 2.6% of the dose. The drug was well absorbed under these conditions and after 5–6 hr. only 0.4–1.2% of the administered radioactivity remained unabsorbed from the small intestine.

Intraportal Infusion—In order to ascertain the fate of the drug after direct introduction into the portal system, isoproterenol-7-^{3}H in saline solution was infused into a branch of the mesenteric vein of dog No. 8 at a rate of 7.26 mcg. base/kg./min. for a period of 1 hr. (total dose 0.44 mg. base/kg.) in one experiment. This rate was felt to approximate that of absorption after oral medication.

The time course of radioactivity in plasma during and following the 1-hr. intraportal infusion of isoproterenol-7-^{3}H is given in Fig. 7. The effect on the heart rate is illustrated in Fig. 8. Although the level of radioactivity in the blood rose sixfold during the period of infusion, the heart rate, after the first 10 min., was found to be very constant at about 160 b.p.m. On cessation of the infusion, the heart rate returned to its preinfusion level within 10 min.

The cardiac effect during the steady state was relatively small considering the dose infused, indicating appreciable metabolic conversion of the drug on its passage through the liver. This was confirmed by estimation, using ion-exchange chromatography, of the plasma levels of free isoproterenol which were found to range from about 2–20 mcg./l. during the infusion. These levels were close to the limit of detection and should be regarded only as an order of magnitude. Only

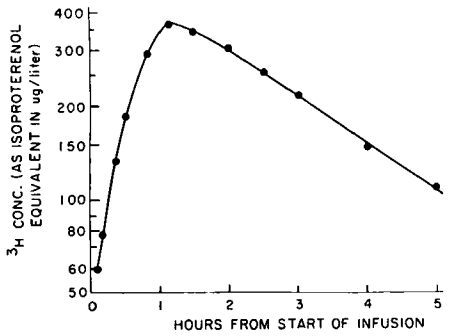

Fig. 7—Plasma level of radioactivity during and following infusion of isoproterenol-^{3}H into the mesenteric vein for 1 hr. at a rate of 0.44 mg. base/kg. body wt./hr. (dog No. 8).

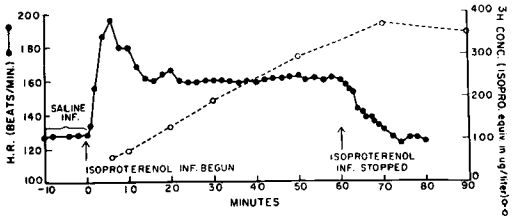

Fig. 8—Heart rate and plasma level of radioactivity during intraportal infusion of isoproterenol-^{3}H in anesthetized dog No. 8.

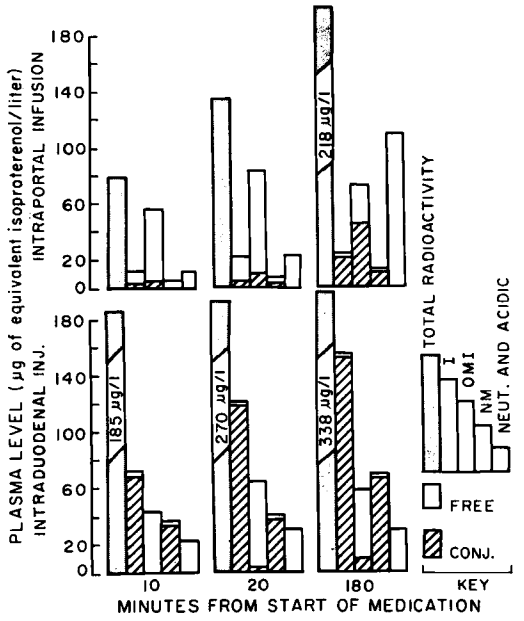

Fig. 9—Metabolites in plasma after intraduodenal administration (dog No. 5) and after the start of a 1-hr. intraportal infusion (dog No. 8).

slightly higher amounts of conjugated I were present ranging from 3 mcg./l. at 6 min. to 24 mcg./l. at 50 min. The levels of conjugated NM were also low each ranging from less than 3 mcg./l. at 6 min. to less than 11 mcg./l. at 50 min. The major metabolite present at all times was free OMI which rose from 36 mcg./l. at 6 min. to 108

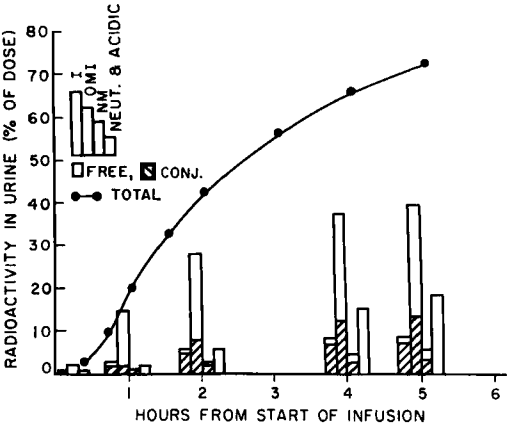

Fig. 10—Cumulative excretion of metabolites in the urine of dog No. 8 during and after infusion of isoproterenol-^{3}H into the mesenteric vein for 1 hr. at a rate of 0.073 mg. base/kg./min.

mcg./l. at 50 min. The level of conjugated OMI rose from 0 at 6 min. to 11 mcg./l. at 30 min. to 58 mcg./l. at 50 min. Unidentified neutral and acidic metabolites rose from 14 mcg./l. at 6 min. to 67 mcg./l. at 50 min.

A graphic comparison of the metabolites in plasma at 10, 20, and 180 min. after intraduodenal administration (dog No. 5) and after the start of the 1-hr. portal infusion (dog No. 8) of isoproterenol into the mesenteric vein is given in Fig. 9. It is readily apparent that the major metabolite in plasma at early times after intraduodenal administration was conjugated isoproterenol while after intraportal administration it was OMI.

Urinary and Biliary Excretion—Over a 5-hr. period from the start of portal infusion, 72% of the dose was excreted in the urine (Fig. 10). An additional 2.3% was excreted in bile. The major metabolites excreted in urine were OMI and its conjugates which together accounted for 40% of the dose. A comparison of the metabolites excreted in urine during the 0–4 hr. postmedication period with those excreted following oral administration and injection into the femoral vein is given in Fig. 11. It emphasizes the similarity of the excretion

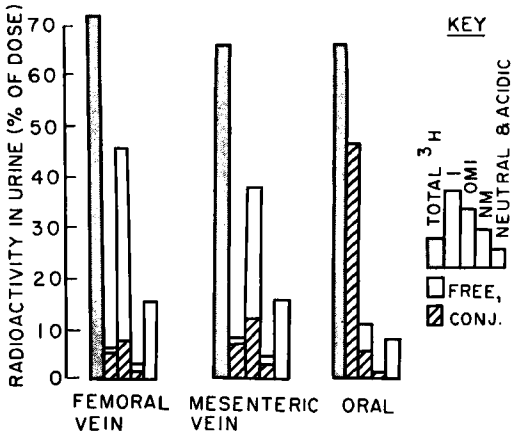

Fig. 11—Comparison of metabolites of isoproterenol-^{3}H excreted in the 0–4 hr. postmedication period after various routes of administration.

TABLE I—HYDROLYSIS WITH VARIOUS ENZYME PREPARATIONS
30–60 min. URINE, DOG NO. 3

Enzyme	Buffer	Enzyme Concn. (Effective Units/ml.) β-Glucuronidase	Sulfatase	Chromatographic Fractions, (% of ^3H in Sample) Neut. Acidic Conj.	OMI	I	NM
None	—	0	0	91.3	7.3	0.2	1.1
Glucuronidase mix. B[a]	Acetate	12	0	91.5	7.2	0.2	0.9
Liver β-gluc.	Acetate	29	0	90.5	8.1	0.2	0.6
Glucuronidase mix. A[b]	PO_4 (0.5 *M*)	35	11	78.7	9.4	8.0	3.7
Glucuronidase mix. A[b]	Acetate	77	560	5.3	9.5	82.3	2.2

[a] Ketodase. [b] Glusulase.

pattern after administration *via* either the femoral or mesenteric vein where OMI is the major metabolite and contrasts this with the pattern obtained after oral administration where mainly conjugated isoproterenol is excreted.

Nature of Conjugated Isoproterenol—As described under *Methods*, the various samples of urine and plasmas were hydrolyzed with glucuronidase mixture A, which contains both β-glucuronidase and sulfatase, in order to determine the relative amounts of free and conjugated material.

Hydrolysis of all urine samples from dog No. 3 with glucuronidase mixture B,[3] which contains glucuronidase, released no isoproterenol, indicating that the conjugate was a sulfuric acid ester. But since glucuronidase mixture B is a much less potent source of glucuronidase than is glucuronidase mixture A, the 30–60 min. urine sample from dog No. 3 was hydrolyzed with various sources of glucuronidase as summarized in Table I. In addition to glucuronidase mixture B, a liver glucuronidase preparation did not hydrolyze the conjugate, nor did glucuronidase mixture A in the presence of 0.5 *M* phosphate buffer, which inhibits 98% of the sulfatase activity while depressing glucuronidase by only half. Glucuronidase mixture A in the acetate buffer was the only effective agent, indicating that the conjugate is a sulfate.

Gastric Emptying Time—Enterohepatic recirculation was ruled out as the explanation of the double peaks observed in the blood level *versus* time curves after oral medication (Fig. 4), because it was found that only a small percent of the dose was excreted in bile (dogs No. 5–8). Preliminary studies have been carried out in three additional experiments to determine the location of the unabsorbed drug in the gastrointestinal tract at various times after oral medication. In two of the animals, which were sacrificed 1 hr. after medication, 55 and 29% of the administered radioactivity was still retained in the stomach. The stomach of the third dog sacrificed 2 hr. after medication contained only 1% of the administered radioactivity. In all cases less than 14% of the dose was found in the small intestine, indicating that the drug is readily absorbed from this area when it is released from the stomach.

DISCUSSION

The absorption and elimination profile of isoproterenol in dogs has been studied previously using the increase in heart rate as an indirect measure of the plasma level (1, 2). Only two other studies (5, 6) on the metabolic fate of the drug have been reported. However, pertinent metabolic studies have been carried out on the related drugs *dl*-nordefrin[4] and 4-(2-methyl-aminoethyl)pyrocatechol,[5] and several studies on epinephrine have been reported and will be reviewed in the following discussion.

Sjoerdsma (5) has reported that, when intravenously administered, the *d*-isomer of isoproterenol is converted to its 3-*O*-methyl derivative and has utilized this reaction to measure catechol *O*-methylating activity in man. No other metabolic studies in humans have been reported.

After intravenous administration of approximately 23 mcg. of isoproterenol-^3H/kg. to rats, Hertting (6) found that one-half of the administered radioactivity was excreted in urine over an 8-hr. period and 77% within 24 hr. The remainder was accounted for in the bile in a form which was not readily absorbed from the intestine. In addition to 10% of the dose in unchanged form, he found the urine to contain 18% as isoproterenol glucuronide, 14% as the *O*-methyl derivative, and 35% as the glucuronide conjugate of OMI. He was unable to detect any deaminated metabolites and concluded that the drug was metabolized exclusively by *O*-methylation and conjugation.

These results show that while isoproterenol given intravenously to dogs is metabolized largely by *O*-methylation, the orally administered drug is converted mainly to a sulfuric acid ester. Only traces of unchanged drug were excreted after i.v. administration and none after oral medication. The excretion of 10–20% of the dose as unidentified neutral and acidic products and up to 3% as normetanephrine or its conjugates indicates that monoamine oxidase plays a secondary role in the metabolism of the drug by the dog.

Haggendal (16) found that conjugation did not play a significant role in the metabolism of epinephrine and norepinephrine by humans when the plasma levels of these hormones were increased either by insulin administration or performance of muscular work. His conclusion was based on the fact that there was no concomitant increase in the levels of their conjugates. In contrast, after oral administration of epinephrine he found conjugation to be the predominant metabolic pathway. Since previous workers had proposed that circulating catecholamines are metabolized by *O*-methylation, primarily in liver tissue (17, 18), Haggendal suggested that the oral medication may be con-

[3] Ketodase, Warner-Chilcott, Morris Plains, N. J.

[4] Corbasil.

[5] Epinine.

jugated by the intestinal flora or in the intestinal mucosa.

Oral doses of epinephrine administered to humans have been reported to be inactivated by formation of the sulfuric acid conjugate (7–9) and after oral administration to rabbits by formation of the glucuronic acid conjugate (10, 11). Likewise more than half of the 50-mg. oral doses of the related drugs *dl*-nordefrin, $3,4\text{-}(OH)_2C_6H_3CHOHCH(NH_2)CH_3$, and 4-(2-methyl-aminoethyl)pyrocatechol, $3,4\text{-}(OH)_2C_6H_3CH_2CH_2HNCH_3$, were eliminated in conjugated form in the urine of humans and dogs within 9 hr. (7, 9).

In contrast, after intravenous infusion of epinephrine in humans (approximately 0.18–0.3 mcg./kg./min. for 0.5 to 1 hr.), urinary excretion of metanephrine or its conjugates was reported to account for almost half the dose while most of the remainder was excreted in the form of deaminated products (mainly 3-methoxy-4-hydroxymandelic acid). Little free or conjugated epinephrine was excreted (12, 13). In a particularly detailed study of the excretion products after intravenous injection (approximately 0.07 mcg./kg.) of epinephrine, only a small percent of the dose was excreted within 24 hr. as epinephrine or its conjugates whereas 23% was excreted as methoxyhydroxymandelic acid and 26% as the sulfuric acid conjugate of metanephrine (14).

The conclusion by Resnick (15) that *O*-methylation represented the predominant pathway in the metabolism of orally ingested epinephrine was unwarranted since he examined the metabolites only after subjecting the urine to acid hydrolysis and did not measure the amount of unconjugated metabolites prior to hydrolysis. In fact his identification of the hydrolysis products, epinephrine and metanephrine, were only qualitative and he did not determine their relative amounts.

The inactivation of orally administered isoproterenol by conjugation with sulfate is therefore consistent with the reported findings for related catecholamines. The fact that virtually identical patterns of urinary metabolites were found following either intraportal infusion of isoproterenol or injection into the femoral vein in dogs indicates that the oral medication is metabolized by conjugation to sulfate either within the intestinal tract or during its passage through the intestinal wall.

The observation of a biphasic absorption of the oral medication as indicated by the double peak in the plasma level and heart rate *versus* time curves at first suggested enterohepatic circulation of the drug. But this was ruled out by the demonstration that only a small amount was excreted in the bile. A biphasic curve was not obtained after intravenous or intraduodenal administration. Preliminary studies of the distribution of radioactivity in the gastrointestinal tract of dogs after oral medication showed a significant proportion of the dose remaining in the stomach 1 hr. after medication. This suggested that a gastric emptying cycle may be responsible for the biphasic absorption. However, the exact cause of the phenomenon is uncertain at present.

Attempts to extend these studies to the direct measurement of isoproterenol and its metabolites in plasma were only partially successful. It was possible to demonstrate that the major metabolite in plasma after intraduodenal administration was a sulfuric acid ester of isoproterenol, while after intraportal infusion the major metabolite was 3-*O*-methylisoproterenol. These results are analogous to the authors' analysis of urinary metabolites after oral and intravenous medication, respectively. Metabolism of the drug was demonstrated to be quite rapid as evidenced by the finding that the unchanged drug constituted less than 1% of the radioactivity in plasma 1 min. after an intravenous injection.

Isoproterenol disappears from the circulation with a half-life of a few minutes as indicated by the decrease in the induced tachycardia following intravenous administration of the drug. Initial disappearance of radioactivity from the blood occurs at a significantly slower rate. The initial regression of tachycardia results mainly from metabolic conversion of isoproterenol to inactive products while the slower elimination of total radioactivity reflects the slow excretion of these metabolites.

Orally administered isoproterenol is slowly absorbed and is less efficient than intravenous medication in inducing tachycardia in dogs. This lower efficiency results from inactivation of a large portion of the medication by conversion to a sulfuric acid ester either before or during absorption from the small intestine. Even that fraction of the drug which escapes this conjugation is then subjected to deactivation by conversion to the 3-*O*-methyl derivative on passage through the liver. Thus, only a very small fraction of an oral dose reaches the general circulation in an active form even on the first circuit of the blood through the body.

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Keyphrases

Isoproterenol—absorption, elimination
dl-Isoproterenol-7-^{3}H—metabolic fate
Metabolites—administration route effect
Column chromatography—separation
Radioactivity distribution—blood, urine
Electrophoresis, paper—identity, metabolites
UV spectrophotometry—analysis