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# Metabolism of Drugs. LXVII.[1] The Metabolic Fate of Isoprophenamine

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The *in vivo* metabolism of isoprophenamine was examined following oral administration to mice, rabbits and humans. In mice the drug was metabolized to *o*-chloromandelic acid which was excreted into urine as one of the main metabolites. Mice excreted also a considerable amount of unchanged compound and its basic metabolites. In rabbits and humans, on the other hand, the drug was converted to *o*-chlorobenzoic acid and *o*-chlorohippuric acid as acid metabolites and thus the metabolic patterns were resemble each other. The metabolic pathways of the drug and the relationship between metabolism and pharmacological effect are also discussed.

Isoprophenamine (1-*o*-chlorophenyl-2-isopropylaminoethanol) was shown by Powell, *et al.*[3] to possess a marked bronchodilating action, and has since been investigated clinically.

In the previous paper,[4] it was shown that the radioactivity was found almost in urine of mice and rabbits after administration of $^{14}C$- or $^{3}H$-labeled isoprophenamine. The tissue distribution of isoprophenamine[4] differed from that of dichloroisoproterenol which was reported by Mayer,[5] in spite of that both compounds were considered to have similar lipoid solubility. Namely, the latter entered the brain of dog, but the former did not enter those of mouse and rabbit. These facts suggest the difference of behavior between isoprophenamine and dichloroisoproterenol in animal body.

The present investigation was undertaken in order to explore metabolic pathway of isoprophenamine in mice, rabbits and humans utilizing radioactive compound. The results of these studies and also the discussion concerning the relationship between metabolism and pharmacological effect are described.

## Experimental

**Materials**——Isoprophenamine hydrochloride (aminoethanol-2-$^{14}C$) was synthesized by condensing nitromethane-$^{14}C$ and *o*-chlorobenzaldehyde, followed by hydrogenation over $PtO_2$ in acetone. Isoprophenamine-$^{3}H$ was prepared by exposure to tritium gas using the Wilzbach technique. The procedures were reported in detail previously.[4] The $\beta$-glucuronidase preparation was obtained from preputial glands of adult female rats and its activity was determined by using *p*-nitrophenyl-$\beta$-D-glucuronide as the substrate. In the present experiment was used the enzyme preparation possessing activity of 5000 Units/ml.

**Administration of Isoprophenamine**——Isoprophenamine-$^{14}C$ (specific activity, 0.27 $\mu$Ci/mg) was administered orally to male mice (CF-1 strain, 20—25 g) in a dose of 55 mg/kg (as HCl salt) and then each group, which consisted of two animals was housed in a glass metabolic cage. Isoprophenamine-$^{3}H$ (specific activity, 1.62 $\mu$Ci/mg) was also given to male rabbits (3.4—3.6 kg) in a dose of 5 mg/kg (as HCl salt). After medication, each animal placed in a individual metabolic cage. In the experiment designed for isolation of the metabolites rabbits were given orally 100 mg/kg/day of non-labeled compound over five days. The medicinal was dissolved in appropriate volume of water and then used. The animals were fasted overnight prior to use. Normal healthy human subjects (male) were received in a dose of 10 mg of $^{3}H$-labeled compound (specific activity, 2.79 $\mu$Ci/mg) as gelatin capsule.

1) Part LXVI: H. Yoshimura, H. Shiraki, K. Oguri and H. Tsukamoto, *Yakugaku Zasshi*, **90**, 364 (1970).
2) Location: *a*) *Katakasu, Fukuoka; b*) *Koishikawa* 4, *Bunkyo-ku, Tokyo.*
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**Thin-Layer Chromatography**——Thin-layer chromatography was carried out by use of silica gel plate (Kiesel gel G, Merck, 0.25 mm thick, activated for 30 min at 105°). The solvent systems used were 1) $CHCl_3$–MeOH–$Et_2NH$ (24:4:0.5); 2) $CHCl_3$–MeOH–conc. $NH_4OH$ (24:4:1); 3) EtOH–$H_2O$–conc. $NH_4OH$ (20:15:1); 4) AcOEt–hexane–AcOH (60:40:1). Basic and acidic compounds were visualized by spraying Dragendorff reagent and bromthymol blue reagent, respectively.

**Radioisotope Methods of Analysis**——The radioactivity of all samples was measured using a Beckman liquid scintillation spectrometer (Model DPM-100$^{T\cdot M\cdot}$) or an Aloka liquid scintillation spectrometer (Model 502), and was corrected for quenching by an internal standard method using $^{14}C$-or $^{3}H$-toluene standard. The samples of urine and aqueous solution were counted in a *p*-dioxane phosphor consisting 60 g naphthalene, 4 g PPO, 0.2 g POPOP, 100 ml methanol, 20 ml ethyleneglycol and *p*-dioxane to make 1 liter. The other materials were also counted in a toluene phosphor prepared by dissolving 4 g PPO and 0.1 g POPOP in 1 liter of toluene. Radioactivity on thin-layer radiochromatograms was determined by either an Aloka thin-layer radiochromatogram scanner (Japan Radiation & Medical Electronics, Inc., Tokyo) or a scintillation counter after silica gel on a plate was scraped at spaces of 0.5 cm into vials containing 10 ml of toluene phosphor.

**Extraction of Metabolites**——An aliquot (20 ml) of urine from mice was adjusted to pH 9 with conc. $NH_4OH$ and then extracted three times with 15 ml of AcOEt by shaking mechanically (basic fraction). The aqueous layer was adjusted to pH 2 with conc. HCl and again extracted three times with AcOEt (acidic fraction). The remaining aqueous layer was designated as aqueous fraction. The three fractions were obtained from urine of rabbits and humans similarly as above, but by continuous extraction with AcOEt for 20 hr.

**Quantitative Determination of Metabolites**——The urinary metabolites of mice were quantitatively determined by measuring the radioactivity with liquid scintillation spectrometer after separated by thin-layer chromatography. In rabbits and humans, the metabolites were determined by reversed dilution analysis as follows. To an aliquot of the basic extract was added exactly 100 mg of carrier isoprophenamine and 5 ml of 1% NaOH solution, and the mixture was extracted three times with equal volume of ether. The extract obtained after evaporation of the solvent was redissolved in isopropylalcohol-HCl solution, and again evaporated to dryness *in vacuo*. The crystalline residue was recrystallized repeatedly from AcOEt–MeOH, until its specific radioactivity became constant. The radioactivity of unchanged isoprophenamine excreted in urine was calculated by following equation:

$$AX \fallingdotseq AR \cdot \frac{MD}{MR}$$

$AX$ = radioactivity of original radioisotope
$AD$ = radioactivity of a part of purified radioisope after adding carrier
$MD$ = weight of nonlabeled compound added
$MR$ = weight of a part of purified radioisotope after adding carrier

Next, to the acidic fraction were added exactly 100 mg of *o*-chlorobenzoic acid and 100 mg of *o*-chlorohippuric acid. The mixture was dissolved in $CHCl_3$ containing a small volume of MeOH and submitted to silica gel column chromatography. *o*-Chlorobenzoic acid and *o*-chlorohippuric acid which were eluted with $CHCl_3$ as a mixture were separated each other by recrystallization from water. Each compound was further recrystallized from water until its specific activity reached a constant value. The radioactivity of the metabolites excreted in urine was calculated by equation described above.

## Results

### Excretion of Radioactivity in Human Urine

Three male human subjects were given orally with isoprophenamine-$^{3}H$. The urine was collected over a period of two days, and the aliquots of 0—12 hr, 12—24 hr and 24—48 hr urine were counted for radioactivity.

Table I. Percent Recovery of $^{3}H$ in Urine of Three Humans after Oral Administration of Isoprophenamine-$^{3}H$

| Time (hr) | Human 1 | Human 2 | Human 3 |
|---|---|---|---|
| 0—12 | 73.7 | 90.2 | 81.3 |
| 12—24 | 12.9 | 13.7 | 8.9 |
| 24—48 | trace | trace | trace |
| Total | 86.6 | 103.9 | 90.2 |

As can be seen in Table I, approximately 80% of the radioactivity administered was recovered within the first 12 hr. It was also shown that the major pathway of excretion was by way into urine, the result being similar to those in mice and rabbits.[4]

**Distribution of Radioactivity among Basic, Acidic and Aqueous Fractions**

The metabolite composition of 24 hr urine of mice and rabbits, and of 12 hr urine of humans dosed labeled isoprophenamine was investigated.

The solvent of basic and acidic fractions from above species was evaporated to dryness *in vacuo*, and the residue was redissolved in AcOEt to make 25 ml. An aliquot (0.2 ml) of this fraction was pipetted and counted in a toluene phosphor. The aqueous fraction remained after AcOEt extraction was diluted to appropriate volume with distilled water, and an aliquot (0.1 ml) was also counted in a dioxane phosphor. Table II shows the distribution of the radioactivity among these three fractions.

Table II. The Distribution of the Radioactivity among Basic, Acidic and Aqueous Fractions

| | Mouse[a] | Rabbit[b] | Human[c] |
|---|---|---|---|
| Basic fractions | 52.8% | 3.4% | 6.6% |
| Acidic fractions | 32.0% | 66.1% | 64.0% |
| Aqueous fractions | 15.0% | 30.5% | 29.4% |

*a*) mean of two groups *b*) mean of three rabbits
*c*) mean of three subjects

The pattern of distribution of the radioactivity was resemble in rabbits and humans, and the highest level was found in acidic fraction. On the contrary, in mice the radioactivity was higher in basic than in acidic fraction. The data shown in Table II suggest that the metabolic patterns in rabbits and humans are similar each other, but differ from that in mice. However, high radioactivity found in the basic fraction from mice urine is probably owing to administration of large amounts of isoprophenamine.

**Separation and Detection of the Metabolites in Mice**

The urine of mice given isoprophenamine-$^{14}$C was collected in five periods (0—3, 3—5, 5—8, 8—24 and 24—48 hr), and extracted with AcOEt as described in Methods.

**Basic Fraction**——This fraction was examined by thin-layer chromatography using solvent system 2. Fig. 1 shows the distribution of the radioactivity on the chromatogram of the extract of 0—3 hr urine. It shows three radioactive peaks (A, B and C) with *Rf* values of 0.88, 0.60 and 0.48. These peaks were also obtained in the extracts of four other periods, although the relative radioactivity among these peaks was somewhat different.

Peak A and B showed *Rf* values same to authentic isoprophenneami and 1-*o*-chlorophenyl-2-aminoethanol, respectively, which were spotted together with the extract and visualized by Dragendorff reagent. Peak C was remained unknown. In order to obtain further evidences for identification, peak A and B were eluted with MeOH from chromatogram, dissolved in isopropylalcohol-HCl solution and then evaporated to dryness *in vacuo*, respectively. The residue from peak A was recrystallized three times from AcOEt–MeOH, together with carrier isoprophenamine hydrochloride. At that time, the specific radioactivity of the crystals attained a constant value. Also, the residue of peak B was recrystallized together with carrier 1-*o*-chlorophenyl-2-aminoethanol hydrochloride. However, after the first recrystallization the radioactivity was not present in crystals, but remained in mother liquor. From these results, peak A was concluded to be unchanged isoprophenamine, but identity of peak B with 1-*o*-chlorophenyl-2-aminoethanol which was expected as one of the metabolites was negated. Herein, the metabolites corresponding to peaks B and C were designated as unknown I and II.

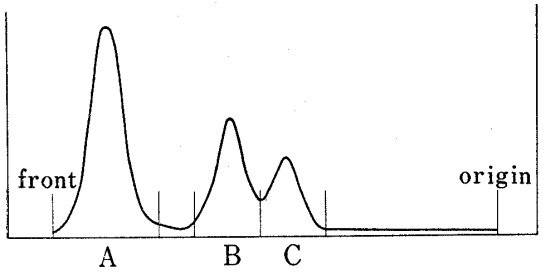


Fig. 1. Radioactive Scan of Thin-Layer Chromatogram of Basic Fraction from 0—3Hr Urine of Mice after Administration of Isoprophenamine-$^{14}$C

Solvent system 2 was used.

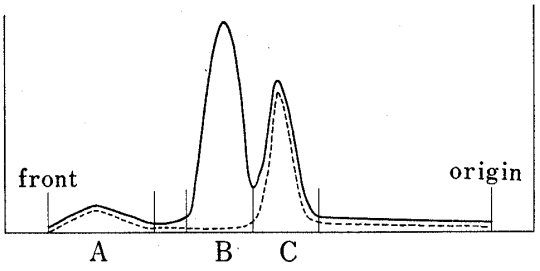


Fig. 2. Radioactive Scan of Thin-Layer Chromatogram after Hydrolysis of the Aqueous Fraction

—: chromatogram of basic fraction from complete incubation mixture
---: chromatogram of basic fraction from incubation mixture added saccharo-1,4-lactone
Solvent system 2 was used.

**Acidic Fraction**——The metabolites in this fraction was also examined by thin-layer chromatography using solvent system 1 and 3, respectively. Only one radioactive peak was observable in either solvent system and had the same mobility as that of authentic *o*-chloromandelic acid (solvent system 1:*Rf* 0.35, solvent system 3:*Rf* 0.81), color of which was developed by bromthymol blue reagent. The radioactive peak was eluted with MeOH from chromatogram after development using solvent system 3, dissolved in 1% HCl solution and extracted with AcOEt. The extract was evaporated to dryness *in vacuo* and recrystallized three times from benzene-petr.ether together with carrier *o*-chloromandelic acid. At that time, the specific radioactivity of crystals reached a constant value. This metabolite was thus identified as *o*-chloromandelic acid, which was a product of oxidative deamination of isoprophenamine.

**Aqueous Fraction**——The aqueous fraction of 0—3 hr urine remained after AcOEt extraction was treated with *β*-glucuronidase as follows. An aliquot (0.5 ml) of aqueous fraction was incubated at 37° for 16 hr in a mixture consisting of 0.5 ml of enzyme (2500 Units), 3 ml of 0.1M acetate buffer of pH 4.5 and water to make a final volume of 5 ml, with or without saccharo-1,4-lactone (final concentration, $1\times10^{-3}$M). In the control experiment was omitted the enzyme. The incubation mixture was extracted with AcOEt and separated to basic, acidic and aqueous fractions as described in Methods. Only in the experiment of the basic fraction with enzyme and without inhibitor, the radioactive scan of the thin-layer chromatogram using solvent system 2, revealed the presence of a new peak having *Rf* value which corresponded to that of peak B detected in basic fraction (Fig. 2).

The radioactivity of this peak accounted for about 16% of that of the incubation mixture. From the above results, it was evident that the aqueous fraction contained a small amount of *β*-glucuronides of unknown I.

**Determination of the Metabolites in Mice**

Table III shows the results of quantitative determination of the metabolites in basic, acidic and aqueous fraction of mice urine. After administration of isoprophenamine-$^{14}$C, 62.9% of the dose was recovered from the first 3 hr urine, of which unchanged compound accounted for 18.3% and its metabolites for 44.6%. The major degradated product was *o*-chloromandelic acid, which accounted for 20.8% of the dose. The urine of later sampling periods (3—48 hr) contained only a small amount of metabolites.

**Separation and Detection of the Metabolites in Rabbits and Humans**

The 24 hr urine of rabbits and the 12 hr urine of humans given isoprophenamine-$^{3}$H were collected and the extraction was carried out as described in Methods.

**Basic Fraction**——The basic fraction of urine from rabbits was examined by thin-layer chromatography using solvent system 1. As can be seen in Fig. 3, the chromatogram shows two radioactive peaks (A and D) with *Rf* values of 0.60 and 0.30.

Table III. The Metabolites in Basic, Acidic and Aqueous Fractions of Mice Urine

| Time (hr) | Basic metabolites | | | Acidic metabolite | Aqueous phase |
|---|---|---|---|---|---|
| | Unchange | Unknown I | Unknown II | *o*-chloro-mandelic acid | polar metabolite |
| 0— 3 | 18.3 | 15.6 | 2.5 | 20.8 | 5.7 |
| 3— 5 | 2.0 | 1.5 | 0.6 | 1.6 | 2.4 |
| 5— 8 | 0.9 | 0.8 | 0.7 | 2.6 | 2.9 |
| 8—24 | 0.7 | 0.3 | 0.5 | 1.9 | 1.8 |
| 24—48 | 0.4 | 0.4 | 0.2 | 0.6 | 0.4 |
| Total | 22.3 | 18.6 | 4.5 | 27.5 | 13.2 |

Peak A corresponded to authentic isoprophenamine and was identified by reversed dilution method. When the basic fraction from the first 3 hr urine of mice was developed on thin-layer plate using solvent system 1 in place of solvent system 2, two peaks with *Rf* 0.60 and 0.48 were observable. The former corresponded to unchanged compound, but the latter was not in agreement with peak D shown herein. From the results, it is concluded that the basic metabolites in rabbits differ from those in mice. The further investigation of peak D has not been undertaken and remained unidentified. The basic fraction of urine from humans was also examined by thin-layer chromatography using solvent system 1. The chromatogram shown in Fig. 4 indicated the presence of only one radioactive compound (peak A) with *Rf* value of 0.6 corresponding to unchanged isoprophenamine, not like rabbits in which two peaks were observed. This compound was finally identified by the same method as in rabbits.

**Acidic Fraction**——The acidic extract of urine from rabbits was submitted to thin-layer chromatography. Using solvent system 1, only one radioactive peak with *Rf* 0.42 was located on chromatogram, while using solvent system 4, three radioactive peaks with *Rf* 0.70, 0.20 and 0.00 were found as shown in Fig. 5. Peak E and F corresponded to authentic *o*-chlorobenzoic acid and *o*-chlorohippuric acid, respectively, and identified by reversed dilution method. Peak G was remained unidentified.

The similar study on the acidic fraction of urine from humans, indicated that *o*-chlorobenzoic acid and *o*-chlorohippuric acid were also the metabolites in humans.

**Aqueous Fraction**——The aqueous fraction of rabbits urine remained after extraction was treated with $\beta$-glucuronidase as described for that of mice urine. On the other hand, that of human urine was hydrolized by heating on boiling water bath for 1 hr after adding conc. HCl to make 5% concentration. However, any detectable radioactivity could not be extracted from either hydrolysate. From the above results, it was considered that the aqueous fraction from urine of rabbits or humans contained little glucuronide or sulfate.

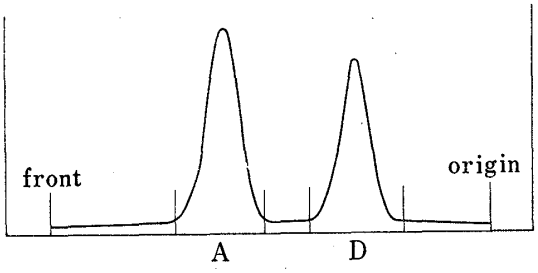


Fig. 3. Radioactive Scan of Thin-Layer Chromatogram of Basic Fraction from 24Hr Urine of Rabbits after Administration of Isoprophenamine-$^3$H

Solvent system 1 was uesd.

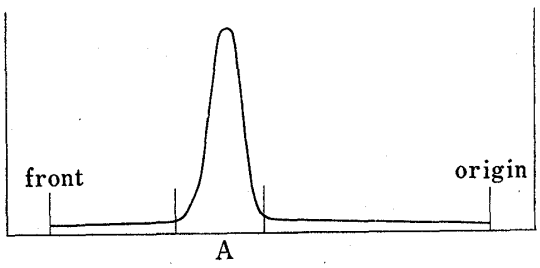


Fig. 4. Radioactive Scan of Thin-Layer Chromatogram of Basic Fraction from 12Hr Urine of Humans after Administration of Isoprophenamine-$^3$H

Solvent system 1 was used.

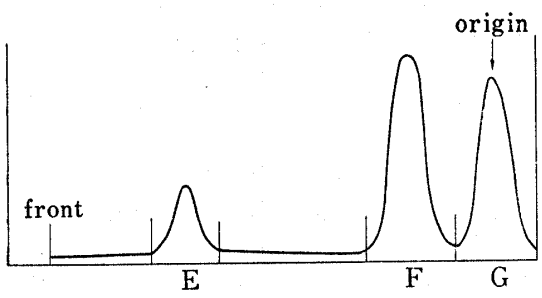


Fig. 5. Radioactive Scan of Thin-Layer Chromatogram of Acidic Fraction from Urine of Rabbits

Solvent system 4 was used.

### Determination of Urinary Metabolites in Rabbits and Humans

Table IV shows the results of the quantitative determination of the metabolites in urine of rabbits and humans.

As can be seen from Table IV, metabolic patterns in urine of rabbits and humans were resemble quantitatively and differed very much from that of mice.

### Isolation and Identification of *o*-Chlorobenzoic Acid as Acidic Metabolite of Isoprophenamine administered orally in Rabbits

Urine of the rabbits given cold isoprophenamine for 5 days (100 mg/kg/day) was collected and extracted with AcOEt according to the method described above. The acidic fraction obtained was evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ and then chromatographed on silica gel column. The column was eluted with $CHCl_3$ and each fraction (25 ml) was examined by thin-layer chromatography using solvent system 3. *o*-Chloroben zoic acid was obtained from fraction No. 24—48 as colorless crystals, which showed mp 142° by recrystallization from $H_2O$. Its ultraviolet (UV) and infrared (IR) spectra were entirely identical with those of the authentic compound.

Table IV. Percent Recovery of $^3H$ in Metabolites of Urine from Rabbits and Humans

| | Basic fraction | | Acidic fraction | | |
|---|---|---|---|---|---|
| | Isoprophenamine | Unknown | *o*-Chlorobenzoic acid | *o*-Chlorohippuric acid | Unknown |
| Rabbit 1 | 0.05 | 1.2 | 2.7 | — | 39.5 |
| Rabbit 2 | 0.07 | 2.4 | 5.7 | 5.2 | 41.7 |
| Rabbit 3 | 0.07 | 3.8 | 5.4 | 10.8 | 38.4 |
| Human 1 | 0.4 | 3.2 | 3.1 | 6.9 | 40.9 |
| Human 2 | 0.8 | 4.8 | 7.6 | 11.6 | 35.9 |
| Human 3 | 2.5 | 4.8 | 3.1 | 10.9 | 36.3 |

## Discussion

The present results show that isoprophenamine is rapidly metabolized and then metabolites are largely excreted in urine, following oral administration. After oral administration of isoprophenamine-$^{14}C$ (55 mg/kg) to mice, it was found that, in addition to 22% of the dose as unchanged compound, 48 hr urine contains 28% of the dose as *o*-chloromandelic acid and 36% as unidentified metabolites which consisted of 23.1% as basic or neutral products, and 13.2% as polar products. In contrast, after oral administration of isoprophenamine-$^3H$ (5 mg/kg) to rabbits and 0.2 mg/kg to humans, unchanged compound excreted in urine was 0.5—2.5% of the dosed radioactivity which was very little as compared to that of mice. This difference might be due to the difference of dose level. On the other hand, acidic metabolites, *o*-chlorobenzoic acid and *o*-chlorohippuric acid excreted in urine of both rabbits and humans accounted for 2.7—7.6% and 5.2—11.6% of the dose. The conjugated compound was detected in mice, but not in rabbits and humans. From above results, the metabolic pattern of isoprophenamine in rabbits and humans was similar each other, but differed from that of mice.

It must be remembered that tritium labeled compound used in rabbits and humans was prepared by contact of cold isoprophenamine with tritium gas, according to Wilzbach technique. Therefore, it is considered that $^{3}H$ is scattered in molecule of isoprophenamine and the degradated products loose considerable radioactivity as compared to original compound. Accordingly, the excretion rate of *o*-chlorobenzoic acid and *o*-chlorohippuric acid should be larger than the values shown in the present paper.

In the previous paper,[4] it was reported that in mice the radioactivity derived from isoprophenamine (aminoethanol-2-$^{14}C$) is excreted almost entirely in the urine, a small amount appears in the feces and a negligible amount is excreted as $^{14}CO_2$. The fact was consistent with the present observation that isoprophenamine dosed to mice was converted to radioactive *o*-chloromandelic acid, but not to further degradated product, *o*-chlorobenzoic acid which should be radioinactive. On the basis of these results, it seemed reasonable to assume that the enzyme activity responsible for the formation of the latter from the former is very low.

A postulated scheme for the metabolism of isoprophenamine illustrates that this drug may be metabolized to 1-*o*-chlorophenyl-2-aminoethanol which in turn is metabolized to acid derivatives (Chart 1).

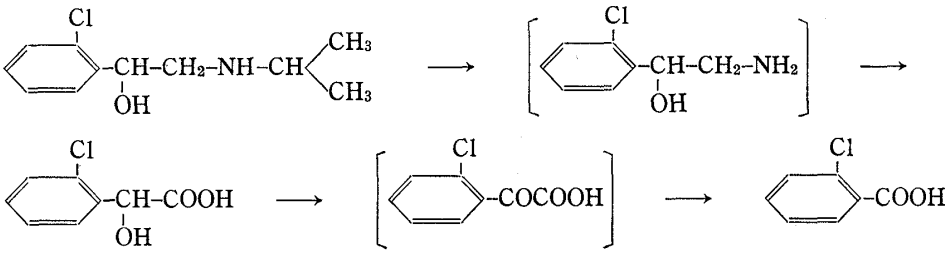

Chart 1. Scheme for the Metabolism of Isoprophenamine

Bond and How[6] investigated the metabolism of pronethalol, 2-isopropylamino-1-(2-naphthyl)ethanol and proposed the same metabolic pathway.

It has been known that the physiological activity of epinephrine decreases extremely in oral administration as compared to intravenous injection. On the other hand, the bronchodilating effect of isoproterenol and isoprophenamine was compared using guinea pig, in which depression of respiration was made by inhalation of histamine, acetylcholine and serotonine. As the results, $ED_{50}$ of isoproterenol was 100 times higher in oral dose than in subcutaneous injection, but $ED_{50}$ of isoprophenamine was not different in both routes.[7]

Concerning the metabolism of these compounds, following evidences have been known. Oral doses of epinephrine administered to human have been reported to be inactivated by sulfate formation,[8,9] and in the case of rabbits by glucuronide formation.[10,11] In contrast, after intravenous infusion of epinephrine in humans, urinary excretion of metanephrine or its conjugated form was reported to account for almost half the dose while most of the remainder was excreted in the form of deaminated product. Little free or conjugated epinephrine was excreted.[12,13] Also, Haggendal[14] came to the same conclusion as above by the experiments of insulin administration, performances of muscular work and oral administration of epinephrine.

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On the other hand, Conway, *et al.*[15] demonstated that while isoproterenol given intravenously to dogs is metabolized largely by O-methylation, the orally administered drug is converted mainly to sulfate. The fact that virtually identical patterns of urinary metablites were found following either intraportal infusion of isoproterenol or injection into femoral vein in dogs indicated that in the oral medication it is metabolized by conjugation to sulfate either within the intestinal tract or during its passage through the intestinal wall. Davies, *et al.*[16] obtained the similar result as above by oral administration of isoproterenol to dog and man.

From these evidences, it is reasonable to consider that physiological activity of epinephrine or isoproterenol decreases in oral administration because these drugs are inactivated by conjugation with sulfuric acid or glucuronic acid in the process of absorption from intestinal tract and effective concentration of these drugs are not obtained in plasma. On the contrary, from the present observation that isoprophenamine is not almost conjugated in animal body after oral administration, except for small amount of conjugate in mice, it was suggested that a considerable amount of isoprophenamine could be absorbed unchanged from the intestinal tract and showed the same physiological effect as in parenteral routs.

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