METABOLIC STUDIES ON METIAZINIC ACID I
ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM IN RATS AND RABBITS

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Abstract - Absorption, distribution, excretion and metabolism after oral administration of H-labelled metiazinic acid were studied. The administered radioactivity was excreted through both the urinary and fecal routes. The maximum levels of concentration in blood and most tissues were shown within 3 hr after dosing. The highest radioactivity was found in the kidney throughout all experiments. Relatively high radioactivity was observed in inflammatory-treated parts in rats. Unchanged compound, metiazinic acid S-oxide and these conjugates were found in urine and feces. Approximately 60% of the unchanged form was observed in plasma after 6 hr.

Metaizinic acid (10-methyl-2-phenothiazinyl) acetic acid is a synthetic anti-inflammatory, antipyretic and analgesic drug which contains a phenothiazine ring.

\[
\text{\begin{tikzpicture}
\node at (0.5,0) {\text{CH}_2\text{COOH}};
\end{tikzpicture}}
\]

L. Julou (1) reported that metiazinic acid exhibited marked anti-inflammatory activity, which is at least equal to and, in some tests, 2 to 4 times higher than that of phenylbutazone, while being 3 times higher to 6 times lower than that of indomethacin.

K. Tsurumi et al. (2) confirmed the anti-inflammatory activities of metiazinic acid using various methods and concluded that metiazinic acid is clinically considered to be similar to phenylbutazone or flufenamic acid regarding acute and chronic inflammation.

Furthermore, K. Tsurumi et al. (3, 4) studied the general pharmacological actions and acute and subacute toxicities of metiazinic acid and reported slight analgesic action, few side effects, and in the acute toxicity tests, toxic symptoms were observed to be mainly respiratory inhibition. The LD50 value was 2 times and 1.5 times greater than that of phenylbutazone and flufenamic acid respectively, and that in the subacute toxicity test, no toxic symptoms were observed in either sex of treated groups of mice and rats.

H. Niwa et al. (5) reported absorption, distribution, metabolism and excretion of metiazinic acid using the chemical assay method.

The authors carried out detailed studies on absorption, distribution, metabolism and
excretion in rats and rabbits using $^3$H-labelled metiazinic acid and the results are reported herein.

**MATERIALS AND METHODS**

*Tritium labelled metiazinic acid*

Metiazinic acid labelled with $^3$H in the 8 position was prepared according to the following reaction:

\[
\begin{array}{c}
\text{Cl} \\
\text{N} \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_2\text{COOH}
\end{array} \xrightarrow{\text{Pd-C}} \begin{array}{c}
\text{S} \\
\text{N} \\
\text{CH}_3 \\
\text{CH}_3 \\
\text{CH}_2\text{COOH}
\end{array}
\]

(8-Chloro-10-methyl-2-phenothiazinyl) acetic acid, dissolved in 0.1N-NaOH, was reduced with tritium gas over paradiumcarbon. After removal of labile tritium, the crude product was purified by paper-chromatography using n-butanol saturated with water as developing solvent and finally recrystallized from ethanol-water.

The specific activity of the product was 310 mCi/m mole (1.14 mCi/mg) and the radiochemical purity was 99\% by reversed isotope dilution analysis, over 99\% by thin-layer chromatography. The material was diluted with non-labelled metiazinic acid before use.

**Animals and dosage**

Male Wistar rats weighing 140 to 160 g and male rabbits weighing 2 to 3 kg, fasted for 12 hr were employed.

$^3$H-Metiazinic acid with a specific activity of $2.54 \times 10^7$ dpm/mg was suspended in 2\% acacia and given orally to rats at a dose of 20 mg/kg in a concentration of 8 mg/ml, and to male rabbits at a dose of 20 mg/kg in a concentration of 25 mg/ml and a dose of 60 mg/kg in a concentration of 75 mg/ml. After administration, animals were fed and given water *ad libitum*.

**Measurement of radioactivity**

The radioactivity was measured by a liquid scintillation counter, ALOKA Model LSL-653 with automatic quenching monitor.

**Blood:** After administration of $^3$H-metiazinic acid in rabbits, a small volume of blood was obtained from the ear-vein, absorbed on the filter paper and burnt by an automatic sample oxidizer PACKARD Model 305.

**Urine and feces:** After administration, individual rabbits were placed in metabolic cages and naturally excreted urine and feces were collected at various times. An aliquot of urine was pipetted into the dioxane scintillation solution (PPO 7 g; dimethyl POPOP, 0.3 g; naphthalene, 100 g; dioxane, 900 ml). Feces were homogenized in water, and thereafter were subjected to combustion.

**Bile:** Bile was collected from the cannulated common bile duct of rabbits placed in restraining cages with free access to food and water. In the operative procedure for the biliary cannulae, rabbits were anesthetized with pentobarbital. $^3$H-metiazinic acid was administered after completion of the operation. An aliquot of bile was pipetted into the dioxane scintillation solution.

**Tissue, plasma and gastrointestinal contents:** Plasma, tissue and gastrointestinal
contents were collected at various time intervals from rabbits sacrificed by exsanguination from the common carotid artery. An aliquot of plasma was absorbed on the filter paper and burnt. Gastrointestinal contents were homogenized in water and aliquots were burnt. Tissues were dried at 40° for 24 hr and subjected to combustion.

Carrageenin edema: One hour after injection of 0.05 ml of 1% carrageenin solution suspended into saline in a rat's paw, 3H-metiazinic acid was administered orally. After administration, the rats were sacrificed at various time intervals by exsanguination from the common carotid artery. All tissues of rats located under calcaneus (Oscalesis) of paws were collected without bone. Radioactivity in a tissue was measured by the combustion method. As control, radioactivity in the untreated paw from the same rat was determined.

Ultraviolet-light Erythema: Rat's hair was removed from their backs by applying depilatory "Eba-cream (TOKYO TANABE Co.,)" and 2 hr later 3H-metiazinic acid was administered orally. Immediately after administration of 3H-metiazinic acid, the treated skin was brought into contact with a rubber board with a hole (diameter: about 5-7 mm). The target skin was then visualized under ultraviolet-light of a solar lamp (100 V, 500 W) for 150 sec. The rats were sacrificed by exsanguination from the common carotid artery at various times. The target and the control skin were collected and subjected to the combustion.

Whole body autoradiography

Rats were given 3H-metiazinic acid (20 mg/kg, 8 mCi/rat) orally. Whole body sections (60 μ thickness) were prepared according to the modified method of Ullberg (6) and exposed to X-ray film (SAKURA Type-N) for 20 days.

Identification of urinary and fecal metabolites

A sample of urine obtained within 24 hr from the rabbit dosed with 3H-metiazinic acid 60 mg/kg orally was spotted with mixture of authentic estimated metabolites on silica gel TLC plate F254 (Merck) and developed twice or three times in a solvent system of chloroform: methanol: formic acid (95: 5: 5 V/V). The specific activity of 3H-metiazinic acid administered was 4.75 · 10⁷ dpm/ml. The chromatogram was visualized under ultraviolet-light. For the measurement of radioactivity absorbed on silica gel, the silica gel corresponding to the spots was removed, thoroughly shaken with 0.1 N-NaOH (1 ml), mixed with dioxane scintillation solution and counted in liquid scintillation counter.

A hundred μl of urine was incubated at pH 5.2 for 24 hr at 37° with 20 μl of β-glucuronidase, arylsulphatase (IBF 160,000 UF/ml of β-glucuronidase, 1,000,000 UR/ml of arylsulphatase) at pH 5.5 and the mixture was chromatographed as described above.

A sample of feces was obtained during the period of 0-12 hr from a rabbit dosed with 3H-metiazinic acid 60 mg/kg orally.

Feces were homogenized with dilute alkali solution and centrifuged. The supernatant was then acidified with HCl and extracted three times with ethyl ether. The solvent was evaporated and the residue was chromatographed on TLC plate as described above.
Determination of the contents of unchanged metiazinic acid in plasma using reversed isotope dilution analysis.

The plasma (radioactivity: $3.38 \times 10^5$ dpm/ml, 1 ml) obtained from rabbits 6 hr after being dosed with $^3$H-metiazinic acid 60 mg/kg orally was added to a weighted quantity (about 100 mg) of non-labelled metiazinic acid. The resulting solution made alkaline with IN-NaOH was acidified with IN-HCl to precipitate metiazinic acid. The precipitate was collected by centrifugation and washed with water. After being resolved with ethanol, insoluble material was removed by centrifugation and water was added to the precipitate metiazinic acid. These procedures were repeated 6 times. Samples from the 3rd, 5th and 6th precipitation were dried in vacuo and used to determine the specific radioactivity of the material. The specific activities were constant for the last two procedures.

RESULTS

Blood and plasma levels

Figs. 1 and 2 show the concentration of radioactivity in the blood and plasma after oral administration of $^3$H-metiazinic acid. The maximum concentration of ra-

![Fig. 1. Blood levels after oral administration of $^3$H-metiazinic acid in rabbits.](image)

![Fig. 2. Plasma levels after oral administration of $^3$H-metiazinic acid (20 mg/kg) in rabbits.](image)

![Fig. 3. Cumulative biliary excretion of radioactivity after oral administration of $^3$H-metiazinic acid in rabbits.](image)
Radioactivity appeared in the blood at 3 hr after oral administration of 20 mg/kg and thereafter decreased. Plasma levels were higher than those of blood throughout all experimental periods. The concentration of radioactivity in the blood reached a maximum level at 1 hr after administration of 60 mg/kg.

**Biliary excretion**

A percentage of 19.9% of radioactivity of dosing appeared in the bile within 24 hr. (Fig. 3)

**Distribution in gastrointestinal contents**

About 26% of dosing was observed in gastric contents at 1 hr after administration. The gastric contents at 24 hr included no more than 1%. Radioactivity in the intestinal contents was observed to be 6.97% of dosing at 3 hr after administration and increased to 19.4% at 6 hr. The intestinal contents decreased less than 2% at 48 hr. (Table 1)

**TABLE 1. Total radioactivity in gastrointestinal contents after oral administration of 3H-metiazinic acid in rabbits.**

| Values are percent of dose (mean ± standard error) |
|-----------------------------------------------|
| Dose 20 mg kg | 1 hr | 3 hr | 6 hr | 12 hr | 24 hr | 48 hr |
| Stomach | 26.20±6.25 | 10.70±8.90 | 4.73±4.69 | 2.74±1.70 | 0.97±0.46 | 0.12±0.06 |
| Intestine | 7.12±2.44 | 6.97±4.67 | 19.40±16.40 | 19.60±2.97 | 2.30±0.71 | 1.93±2.17 |

**Urinary and fecal excretion**

Following oral administration at the dose of 20 mg/kg and 60 mg/kg, radioactivity amounting to 42.9% and 54.6% respectively was rapidly excreted within 24 hr, and was 56.4% and 64.7% respectively within 120 hr. Elimination in the feces was slower the amounting being 21.3% and 26.6% respectively within 120 hr. (Fig. 4)

![Fig. 4. Cumulative urinary and fecal excretion of radioactivity after oral administration of 3H-metiazinic acid in rabbits.](image)

**Distribution in tissues**

Radioactivity expressed in dpm/g was found in tissue after administration at the dose of 20 mg/kg. (Table 2) The highest levels of radioactivity were noted in all tissues except...
### TABLE 2. Tissue distribution of radioactivity after oral administration of \(^3^H\)-metiazinic acid in rabbits.
Dose 20 mg kg

| Tissue       | 1 hr       | 3 hr       | 6 hr       | 12 hr      | 24 hr      | 48 hr      |
|--------------|------------|------------|------------|------------|------------|------------|
| Blood        | (1.36 ± 1.25) × 10^6 | (2.00 ± 0.45) × 10^6 | (1.94 ± 0.53) × 10^6 | (8.95 ± 1.60) × 10^6 | (2.22 ± 0.49) × 10^6 | (9.31 ± 6.31) × 10^6 |
| Lungs        | (1.03 ± 0.74) × 10^6 | (3.94 ± 4.60) × 10^6 | (6.19 ± 1.16) × 10^6 | (2.37 ± 1.34) × 10^6 | (2.93 ± 1.64) × 10^6 | (6.55 ± 4.10) × 10^6 |
| Heart        | (8.78 ± 3.54) × 10^6 | (3.92 ± 4.48) × 10^6 | (7.39 ± 1.80) × 10^6 | (2.75 ± 1.56) × 10^6 | (1.44 ± 0.45) × 10^6 | (4.21 ± 2.71) × 10^6 |
| Liver        | (1.32 ± 1.02) × 10^6 | (1.98 ± 0.18) × 10^6 | (1.24 ± 5.60) × 10^6 | (5.23 ± 1.02) × 10^6 | (9.52 ± 0.43) × 10^6 | (2.41 ± 1.00) × 10^6 |
| Kidneys      | (4.68 ± 1.04) × 10^6 | (5.90 ± 0.88) × 10^6 | (4.99 ± 0.92) × 10^6 | (4.23 ± 4.70) × 10^6 | (8.03 ± 3.06) × 10^6 | (6.24 ± 4.58) × 10^6 |
| Adrenals     | (7.28 ± 3.31) × 10^6 | (1.34 ± 1.02) × 10^6 | (5.18 ± 1.34) × 10^6 | (2.49 ± 1.13) × 10^6 | (2.15 ± 0.62) × 10^6 | (2.82 ± 3.90) × 10^6 |
| Thymus       | (6.71 ± 6.54) × 10^6 | (5.18 ± 1.02) × 10^6 | (3.44 ± 0.33) × 10^6 | (1.19 ± 0.52) × 10^6 | (1.87 ± 1.48) × 10^6 | (2.90 ± 1.15) × 10^6 |
| Spleen       | (3.22 ± 3.23) × 10^6 | (6.58 ± 1.12) × 10^6 | (4.14 ± 0.74) × 10^6 | (2.59 ± 1.78) × 10^6 | (5.20 ± 4.22) × 10^6 | (1.16 ± 0.06) × 10^6 |
| Cerebrum     | (7.35 ± 4.94) × 10^6 | (6.66 ± 6.90) × 10^6 | (1.07 ± 0.32) × 10^6 | (5.56 ± 2.50) × 10^6 | (3.99 ± 2.80) × 10^6 | (1.33 ± 0.41) × 10^6 |
| Seminal vesica | (9.23 ± 3.87) × 10^6 | (3.02 ± 2.46) × 10^6 | (1.38 ± 0.53) × 10^6 | (7.50 ± 5.71) × 10^6 | (2.70 ± 1.96) × 10^6 | (2.70 ± 1.61) × 10^6 |
| Cerebellum   | (1.59 ± 0.73) × 10^6 | (1.74 ± 0.39) × 10^6 | (1.52 ± 1.04) × 10^6 | (6.94 ± 1.28) × 10^6 | (3.29 ± 1.43) × 10^6 | (2.09 ± 2.20) × 10^6 |
| Hypophysis   | (1.83 ± 1.15) × 10^6 | (1.69 ± 1.20) × 10^6 | (7.07 ± 3.24) × 10^6 | (8.73 ± 1.84) × 10^6 | (4.55 ± 1.06) × 10^6 | (6.31 ± 2.43) × 10^6 |
| Thyroid      | (8.64 ± 4.00) × 10^6 | (8.47 ± 2.99) × 10^6 | (6.21 ± 1.53) × 10^6 | (3.17 ± 2.21) × 10^6 | (2.29 ± 0.51) × 10^6 | (1.43 ± 0.82) × 10^6 |
| Testes       | (1.37 ± 1.38) × 10^6 | (7.51 ± 1.51) × 10^6 | (5.28 ± 1.06) × 10^6 | (2.03 ± 0.98) × 10^6 | (1.99 ± 0.98) × 10^6 | (3.82 ± 1.17) × 10^6 |
| Prostate     | (7.18 ± 2.56) × 10^6 | (1.07 ± 0.34) × 10^6 | (8.15 ± 9.20) × 10^6 | (1.22 ± 1.57) × 10^6 | (2.61 ± 1.41) × 10^6 | (2.73 ± 2.33) × 10^6 |
| Pancreas     | (6.13 ± 2.78) × 10^6 | (8.97 ± 3.25) × 10^6 | (4.09 ± 0.42) × 10^6 | (1.97 ± 0.68) × 10^6 | (8.48 ± 1.08) × 10^6 | (4.18 ± 2.01) × 10^6 |
| Muscle       | (2.58 ± 0.82) × 10^6 | (3.31 ± 0.25) × 10^6 | (1.69 ± 0.56) × 10^6 | (1.11 ± 0.15) × 10^6 | (5.06 ± 0.98) × 10^6 | (3.49 ± 0.58) × 10^6 |
| Fat          | (2.87 ± 1.37) × 10^6 | (1.26 ± 0.87) × 10^6 | (6.44 ± 0.76) × 10^6 | (4.00 ± 0.08) × 10^6 | (2.79 ± 1.43) × 10^6 | (1.34 ± 1.11) × 10^6 |
| Bone         | 6.01 × 10^6     | 7.88 × 10^5     | 9.85 × 10^5     | 1.40 × 10^5     | 2.36 × 10^6 |
| Cartilage    | 6.91 × 10^6     | 9.85 × 10^5     | 1.40 × 10^5     | 2.36 × 10^6     |
| Eyeball      | 2.88 × 10^6     | 3.54 × 10^5     | 3.54 × 10^5     | 3.54 × 10^5     |

Values are dpm/g wet tissue (mean ± standard error)
Table 3. Tissue distribution of radioactivity after oral administration of $^3$H-metiazinic acid in rabbits.
Dose 60 mg/kg
Values are dpm/g wet tissue (mean±standard error)

| Tissue                        | 1 hr                  | 6 hr                  |
|-------------------------------|-----------------------|-----------------------|
| Blood                         | $(3.77\pm 2.48) \times 10^6$ | $(1.94\pm 0.53) \times 10^6$ |
| Lungs                         | $(2.71\pm 0.96) \times 10^5$ | $(4.93\pm 2.65) \times 10^5$ |
| Heart                         | $(2.86\pm 1.39) \times 10^5$ | $(2.76\pm 0.86) \times 10^5$ |
| Liver                         | $(5.17\pm 3.71) \times 10^5$ | $(2.96\pm 2.06) \times 10^5$ |
| Kidneys                       | $(1.24\pm 0.54) \times 10^6$ | $(9.74\pm 3.75) \times 10^5$ |
| Adrenals                      | $(1.82\pm 0.36) \times 10^5$ | $(9.83\pm 2.36) \times 10^5$ |
| Thymus                        | $(1.45\pm 0.34) \times 10^5$ | $(3.45\pm 2.69) \times 10^5$ |
| Spleen                        | $(1.97\pm 0.68) \times 10^5$ | $(2.88\pm 1.16) \times 10^5$ |
| Cerebellum                    | $(6.66\pm 2.28) \times 10^4$ | $(7.93\pm 4.90) \times 10^4$ |
| Cerebrum                      | $(8.43\pm 5.53) \times 10^4$ | $(1.21\pm 8.40) \times 10^5$ |
| Seminal vesicula              | $(3.77\pm 3.43) \times 10^4$ | $(4.00\pm 2.73) \times 10^4$ |
| Hypophysis                    | $(5.24\pm 2.89) \times 10^4$ | $(5.36\pm 2.07) \times 10^4$ |
| Thyroid                       | $(3.03\pm 2.05) \times 10^4$ | $(4.81\pm 2.26) \times 10^4$ |
| Testes                        | $(1.44\pm 0.54) \times 10^3$ | $(1.22\pm 0.16) \times 10^3$ |
| Prostate                      | $(1.76\pm 0.92) \times 10^3$ | $(1.43\pm 0.40) \times 10^3$ |
| Pancreas                      | $(2.07\pm 1.92) \times 10^3$ | $(4.54\pm 2.78) \times 10^4$ |
| Muscle                        | $(1.67\pm 0.48) \times 10^4$ | $(1.91\pm 0.37) \times 10^2$ |
| Fat                           | $(3.82\pm 2.41) \times 10^4$ | $(2.00\pm 1.59) \times 10^5$ |
| Bone                          | $5.72 \times 10^4$       | $7.13 \times 10^4$       |
| Cartilage                     | $1.31 \times 10^3$       | $2.26 \times 10^4$       |
| Eyeball                       | $1.67 \times 10^4$       | $4.19 \times 10^4$       |

n = 3

Table 4. Percent distribution in tissues after oral administration of $^3$H-metiazinic acid in rabbits.
Dose 20 mg/kg
Values are percent of dose (mean±standard error)

| Tissue                        | 1 hr                  | 3 hr                  | 6 hr                  |
|-------------------------------|-----------------------|-----------------------|-----------------------|
| Lungs                         | $(6.10\pm 5.02) \times 10^{-2}$ | $(3.14\pm 3.70) \times 10^{-1}$ | $(5.67\pm 1.37) \times 10^{-2}$ |
| Heart                         | $(1.64\pm 3.40) \times 10^{-1}$ | $(6.48\pm 2.08) \times 10^{-2}$ | $(5.31\pm 1.29) \times 10^{-2}$ |
| Liver                         | $(9.31\pm 7.21) \times 10^{-1}$ | $1.01 \pm 0.80$       | $(8.40\pm 4.30) \times 10^{-1}$ |
| Kidneys                       | $(6.98\pm 2.17) \times 10^{-1}$ | $(6.59\pm 0.23) \times 10^{-1}$ | $(1.71\pm 2.05) \times 10^{-1}$ |
| Adrenals                      | $(2.78\pm 3.33) \times 10^{-2}$ | $(8.21\pm 1.23) \times 10^{-1}$ | $(3.23\pm 4.62) \times 10^{-2}$ |
| Thymus                        | $(3.10\pm 5.10) \times 10^{-1}$ | $(1.80\pm 1.44) \times 10^{-2}$ | $(1.26\pm 0.82) \times 10^{-2}$ |
| Spleen                        | $(6.41\pm 4.91) \times 10^{-1}$ | $(1.03\pm 0.49) \times 10^{-2}$ | $(1.06\pm 0.76) \times 10^{-2}$ |
| Cerebrum                      | $(4.44\pm 3.01) \times 10^{-1}$ | $(3.36\pm 2.91) \times 10^{-1}$ | $(6.41\pm 0.33) \times 10^{-2}$ |
| Seminal vesicula              | $(1.74\pm 1.02) \times 10^{-2}$ | $(8.43\pm 8.86) \times 10^{-3}$ | $(2.52\pm 3.09) \times 10^{-3}$ |
| Cerebellum                    | $(7.47\pm 7.60) \times 10^{-2}$ | $(2.81\pm 2.79) \times 10^{-3}$ | $(4.30\pm 2.89) \times 10^{-3}$ |
| Hypophysis                    | $(2.88\pm 1.83) \times 10^{-4}$ | $(2.67\pm 1.90) \times 10^{-4}$ | $(1.14\pm 0.50) \times 10^{-4}$ |
| Thyroid                       | $(6.52\pm 8.56) \times 10^{-4}$ | $(2.01\pm 1.91) \times 10^{-3}$ | $(6.30\pm 3.15) \times 10^{-4}$ |
| Testes                        | $(5.67\pm 2.53) \times 10^{-2}$ | $(1.97\pm 1.73) \times 10^{-3}$ | $(1.27\pm 0.61) \times 10^{-2}$ |
| Prostate                      | $(4.68\pm 3.87) \times 10^{-3}$ | $(2.70\pm 4.30) \times 10^{-2}$ | $(1.64\pm 2.74) \times 10^{-2}$ |
| Eyeball                       | $5.75 \times 10^{-3}$       |                       | $1.85 \times 10^{-3}$       |
the hypophysis within 3 hr, the maximum level in the hypophysis was observed within 1 hr. The specific radioactivity in the kidneys, lungs, heart, hypophysis, liver, adrenals, pancreas, testes, prostate and seminal vesicle was higher than that in other tissues, and the levels at 3 hr in the lungs, kidneys, heart, and seminal vesicle were higher than those in the blood at 3 hr.

|                | 12 hr                      | 24 hr                      | 48 hr                      |
|----------------|----------------------------|----------------------------|----------------------------|
| Lungs          | (1.60 ± 0.91) × 10^{-4}    | (2.55 ± 1.94) × 10^{-4}    | (3.53 ± 2.19) × 10^{-3}    |
| Heart          | (9.76 ± 5.60) × 10^{-3}    | (3.50 ± 2.26) × 10^{-3}    | (3.49 ± 1.40) × 10^{-4}    |
| Liver          | (3.21 ± 0.35) × 10^{-1}    | (5.64 ± 3.14) × 10^{-1}    | (1.47 ± 0.39) × 10^{-1}    |
| Kidneys        | (9.83 ± 9.10) × 10^{-3}    | (6.62 ± 2.40) × 10^{-3}    | (4.44 ± 2.90) × 10^{-3}    |
| Adrenals       | (1.24 ± 1.64) × 10^{-3}    | (3.21 ± 4.34) × 10^{-3}    | (4.03 ± 4.50) × 10^{-4}    |
| Thymus         | (2.19 ± 0.96) × 10^{-3}    | (4.10 ± 3.76) × 10^{-3}    | (1.13 ± 0.31) × 10^{-2}    |
| Spleen         | (9.12 ± 0.37) × 10^{-4}    | (2.73 ± 3.72) × 10^{-3}    | (6.64 ± 1.73) × 10^{-4}    |
| Cerebrum       | (2.98 ± 1.28) × 10^{-3}    | (1.31 ± 1.71) × 10^{-3}    | (8.52 ± 1.77) × 10^{-4}    |
| Seminal vesicula | (1.29 ± 8.30) × 10^{-3}  | (3.75 ± 0.57) × 10^{-4}    | (5.27 ± 5.41) × 10^{-4}    |
| Cerebellum     | (4.16 ± 1.65) × 10^{-4}    | (9.64 ± 7.90) × 10^{-3}    | (1.29 ± 0.92) × 10^{-4}    |
| Hypophysis     | (1.57 ± 0.28) × 10^{-1}    | (7.14 ± 1.13) × 10^{-3}    | (1.02 ± 0.38) × 10^{-4}    |
| Thyroid        | (3.67 ± 3.03) × 10^{-4}    | (1.69 ± 1.36) × 10^{-4}    | (1.88 ± 1.15) × 10^{-4}    |
| Testes         | (1.88 ± 1.11) × 10^{-3}    | (4.35 ± 5.64) × 10^{-3}    | (7.87 ± 7.83) × 10^{-4}    |
| Prostate       | (3.47 ± 1.80) × 10^{-3}    | (2.13 ± 0.72) × 10^{-4}    | (2.25 ± 2.90) × 10^{-4}    |
| Eyeball        | 1.26 × 10^{-4}             |                           |                           |

TABLE 5. Percent distribution in tissues after oral administration of 'H-metiazinic acid in rabbits.

Dose 60 mg.kg

Values are percent of dose (mean ± standard error)

|                | 1 hr                      | 6 hr                       |
|----------------|----------------------------|----------------------------|
| Lungs          | (7.76 ± 5.10) × 10^{-4}    | (1.13 ± 0.50) × 10^{-4}    |
| Heart          | (4.50 ± 2.21) × 10^{-4}    | (4.47 ± 1.15) × 10^{-4}    |
| Liver          | (1.03 ± 0.80) × 10^{-4}    | (6.65 ± 5.05) × 10^{-4}    |
| Kidneys        | (2.50 ± 1.16) × 10^{-3}    | (6.29 ± 7.39) × 10^{-3}    |
| Adrenals       | (6.46 ± 2.18) × 10^{-4}    | (5.16 ± 1.93) × 10^{-4}    |
| Thymus         | (1.43 ± 0.16) × 10^{-4}    | (3.11 ± 3.53) × 10^{-4}    |
| Spleen         | (5.29 ± 2.30) × 10^{-4}    | (9.76 ± 5.43) × 10^{-4}    |
| Cerebrum       | (6.06 ± 1.28) × 10^{-4}    | (1.97 ± 1.48) × 10^{-3}    |
| Seminal vesicula | (4.68 ± 5.29) × 10^{-4}  | (2.10 ± 2.33) × 10^{-3}    |
| Cerebellum     | (1.82 ± 0.21) × 10^{-4}    | (5.64 ± 3.77) × 10^{-4}    |
| Hypophysis     | (2.47 ± 1.45) × 10^{-4}    | (2.44 ± 0.91) × 10^{-4}    |
| Thyroid        | (4.33 ± 2.79) × 10^{-4}    | (7.38 ± 0.84) × 10^{-4}    |
| Testes         | (1.16 ± 1.04) × 10^{-4}    | (3.58 ± 2.27) × 10^{-4}    |
| Prostate       | (1.23 ± 0.41) × 10^{-4}    | (8.06 ± 0.34) × 10^{-4}    |
| Eyeball        | 2.33 × 10^{-2}             | 6.46 × 10^{-4}             |
Regarding specific radioactivity found in tissues after administration at the dose of 60 mg/kg, the pattern of distribution was almost similar to that observed with the dose of 20 mg/kg. (Table 3)

About 1% of radioactivity was found in the 20 mg/kg group, 10% in the 60 mg/kg group located in the liver at 1 hr and thereafter decreased to 0.15% and 0.7% respectively.

**Fig. 5.** Autoradiograms showing the distribution of radioactivity at 2 hr after oral administration of H-metizinic acid.
Levels in other tissues were below 0.7", at 1 hr. After 72 hr no appreciable residue of radioactivity was observed. (Table 4.5)

Whole body autoradiography

Results of whole body autoradiograms, were obtained with male rats at 2 and 4 hr after administration at the dose of 20 mg/kg.

The results obtained from autograms were compatible with the distribution in rabbits. (Fig. 5)

Distribution in the case of edema-induced paws

Radioactivity in edema-induced paws was greater than that in control, and the ratio of radioactivity to that in control decreased with time. (Table 6)

| Table 6 | Radioactive distribution in edema-induced paws after oral administration of "H-metiazinic acid in rats. |
|---------|--------------------------------------------------------------------------------------|
| A       | edema-induced paws                                                                  |
| B       | non-treated paws                                                                    |
| C       | ratios of edema-induced paws to non-treated paws                                     |
| Values  | of A and B are percent of dose (mean - standard error)                               |
|---------|--------------------------------------------------------------------------------------|
| 30 min  | 1 hr                                   | 3 hr                                   |
| A       | (3.16 ± 1.01)×10⁻²                     | (4.29 ± 0.36)×10⁻²                     | (4.87 ± 1.09)×10⁻²                     |
| B       | (4.47 ± 0.62)×10⁻¹                     | (2.24 ± 0.89)×10⁻¹                     | (2.97 ± 1.44)×10⁻¹                     |
| C       | 6.96 ± 1.88                           | 2.03 ± 0.67                           | 1.86 ± 0.71                           |
| n=3     |                                                                                     |                                                                                      |

| Table 7 | Radioactive distribution of ultraviolet erythema-formed skin after oral administration of ³H-metiazinic acid in rats. |
|---------|--------------------------------------------------------------------------------------|
| A       | ultraviolet erythema-formed skin                                                    |
| B       | non-treated skin                                                                    |
| C       | ratios of ultraviolet erythema-formed skin to non-treated skin                      |
| Values  | of A and B are dpm/g (mean - standard error)                                         |
|---------|--------------------------------------------------------------------------------------|
| 30 min  | 1 hr                                   | 3 hr                                   |
| A       | (1.29 ± 0.89)×10⁹                     | (1.85 ± 1.18)×10⁹                     | (1.24 ± 0.59)×10⁹                     |
| B       | (4.96 ± 3.68)×10⁷                     | (1.04 ± 0.69)×10⁷                     | (1.00 ± 0.48)×10⁷                     |
| C       | 2.47 ± 0.25                           | 1.72 ± 0.11                           | 1.23 ± 0.04                           |
| n=3     |                                                                                     |                                                                                      |

Distribution in the case of ultraviolet light erythema

Levels in both erythema and control reached a maximum within 1 hr. The ratio of radioactivity of erythema to that in control decreased with time. (Table 7)

Metabolites in the urine and feces

The mixture of authentic estimated metabolites consisted of the following compounds: metiazinic acid, 2-phenothiazinyl acetic acid, 2,10-dimethyl-phenothiazine, metiazinic acid S-oxide, metiazinic acid amide and 7-hydroxy metiazinic acid S-oxide. Fig. 6 shows the thin-layer chromatogram of this mixture.
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**Fig. 6.** Thin-layer chromatogram of metiazinic acid and its related compounds

Solvent: CHCl₃-CH₃OH-HCOOH (95:5:5 v/v)

- A: 2-phenothiazinyl acetic acid
- B: 2, 10-dimethylphenothiazine
- C: metiazinic acid S-oxide
- D: metiazinic acid amide
- E: 7-hydroxymetiazinic acid S-oxide
- F: metiazinic acid

**Fig. 7.** Radiochromatogram of rabbit urine administered ^3^H-metiazinic acid orally. (60 mg kg, 0-24 hr urine)

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**Table 8.** The ratio of metabolites before and after hydrolysis of urine of rabbit administered ^3^H-metiazinic acid orally. (60 mg kg, 0-24 hr urine)

|           | C    | E    | A | D | F       | B |
|-----------|------|------|---|---|---------|---|
| before    | 24.2 | 29.2 | 1 | 1 | 5.8     | 1 |
| after     | 14.2 | 49.0 | 1 | 1 |         | 8.4|

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**Fig. 8.** Chromatogram of rabbit urine administered ^3^H-metiazinic acid. Refer to Fig. 6.
Thin-layer chromatograms before and after enzymatic hydrolysis of 0-24 hr urine are seen in Fig. 7-8 and Table 8 shows the ratio of metabolites. In the urine before hydrolysis, 5.8% and 29.2% radioactivity was found in unchanged metiazinic acid and metiazinic acid S-oxide. On the other hand, in urine after hydrolysis, these rates were increased to 8.4% and 49.1% respectively. It is thus estimated that metabolites in the urine were partly presented as the conjugated form.

Other radioactivity was found mainly at the original point and the unknown spot at Fr. No. 4 in Fig. 7 and Fr. No. 2 and 3 in Fig. 8. The latter spot appeared to be a hydroxy derivative of metiazinic acid but could not be identified.

Thin-layer chromatogram of 0-12 hr feces, when $^3$H-metiazinic acid was administered orally to rabbits is seen in Fig. 9. Metabolites in the feces were similar to those in the urine but the rate of S-oxide metabolite was much larger and unchanged metiazinic acid was less than in the urine. (Compare Fig. 7 and Fig. 9).
Determination of the content of unchanged metiazinic acid in plasma

The content of unchanged ³H-metiazinic acid in plasma at 6 hr after oral administration of ³H-metiazinic acid was 59.7% when reversed isotope dilution analysis was employed.

DISCUSSION

A greater part of the orally dosed ³H-metiazinic acid was assumed to be rapidly absorbed from the gastrointestinal tract. The maximum levels of radioactivity in the blood and tissues were observed within 3 hr after 20 mg/kg and 60 mg/kg dosing and at this time, the gastrointestinal contents remained about 7% of dose. Niwa et al. (5) reported that the maximum blood level was observed at 30 min in rabbits and at 1 hr in rats and the maximum tissue levels were observed at 1 hr in liver, at 3 hr in kidneys and spleen in rats after 100 mg/kg oral dosing.

Results of the present experiments show that the absorbed radioactivity was distributed mainly in the kidneys, lungs, heart, hypophysis, seminal vesicles, liver and adrenals, and the distribution in the eye was also observed by whole body autoradiography. Most radioactivity was eliminated through the urine. The fecal excretion was about half that of the urine which is assumed to be excreted via bile.

It is interesting that a relatively high concentration was demonstrated in the hypophysis and adrenals. Such a result suggests that the radioactive substances accumulating in the hypophysis release ACTH, followed by steroidogenesis in the adrenal cortex or the radioactive substances distributed in the adrenal stimulate directly steroidogenesis. It is suggested, therefore, that one of anti-inflammatory actions of metiazinic acid may be mediated by way of the adrenal gland.

L. Julou, et al. (1) reported that dogs and rats on a daily dose of 6.18 mg kg exhibited ulcerations and hemorrhages of gastrointestinal mucosa. This gastrointestinal toxicity is a general property of potent anti-inflammatory drugs. However, according to this same author, this toxicity is especially weak in the case of metiazinic acid. Relatively high distribution in the gastrointestinal tract wall was however observed in the autoradiograms of rat. This evidence may be related to the weak gastrointestinal toxicity of metiazinic acid as reported by the above mentioned author.

According to the same report, dog and mouse administered toxic doses exhibited vomiting and dyspnea, while a relatively high radioactivity was rapidly observed in the brain of rabbits. It is assumed, therefore, that substances which quickly pass through the blood-brain barrier act on the vomiting and respiratory centers.

The urinary metabolites of rabbit were mainly unchanged metiazinic acid, metiazinic acid S-oxide, unknown compounds and these conjugates. The fecal metabolites were similar to those found in the urine. One of the unknown compounds was estimated to be the hydroxy derivative of metiazinic acid and a trace was excreted but was not actually identified. These results concerning the metabolites of metiazinic acid support the data by P. Populaire et al. (7). The content of unchanged metiazinic acid in plasma at 6 hr after dosing was 59.7%.
The high distribution in carrageenin edema and ultraviolet light erythema facilitate illustration of the effect of this drug.

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