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The action and fate of sodium pyridinethione when applied topically to the rabbit

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Synopsis—TOXICOLOGICAL studies of SODIUM PYRIDINETHIONE have warranted a study of absorption, distribution and excretion following topical therapy to RABBITS.

The extent of percutaneous absorption of sulphur–35 labelled sodium pyridinethione has been related to the skin's structural integrity by RADIOMETRIC assay for sulphur–35 analysis.

The distribution of the drug and METABOLITES for the mjaor tissues and body fluids has been established, and the metabolic fate of the drug has been determined using a RADIOCHROMATOGRAPHIC technique.

The safety evaluation of sodium pyridinethione needs to include the study of absorption, distribution and excretion following topical therapy. Using a radiometric assay for ^{35}S, the extent of percutaneous absorption of ^{35}S-labelled sodium pyridinethione has been examined in relation to the skin's structural integrity. Distribution of the drug and metabolites in the major tissues and body fluids has been established, and the metabolic fate of the drug determined using a radiochromatographic technique.

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The heavy metal salts of 2-pyridinethiol-1-oxide first synthesized by Shaw *et al.* (1) were first demonstrated to have antibacterial properties by Cox (2). Safety of the zinc salt was extensively studied by Brauer, Opdyke and Burnett (3) and by Opdyke *et al.* (4) and this compound is currently incorporated into several formulations for application to the scalp. Unlike the zinc salt, the sodium salt is readily water soluble, a property which, according to Davson and Danielli (5) can influence percutaneous absorption. The study reported here was intended:

(1) to establish the threshold dose levels for signs indicative of sodium pyridinethione toxicity in acute studies;
(2) to determine the extent of percutaneous absorption and ensuing toxicity of sodium pyridinethione applied to abraded and intact skin;
(3) to record the distribution and metabolic fate of sodium pyridinethione following dermal application.

MATERIALS AND METHODS

Materials

Sodium pyridinethione and pyridine-N-oxide-2-sulphonic acid were provided by Olin Research Centre, New Haven, Conn. Sodium pyridinethione labelled with ^{35}S was synthesized at Beecham Research Laboratories, Betchworth, Surrey, as a white powder, shown by tlc and ir examination to be 90% pure. All other chemicals and solvents were of analytical reagent grade and were used without further purification. Throughout the experimental work polyethylene apparatus was used to reduce drug losses from adsorption during recovery operations.

Preparation of animals

Acute toxicity studies

Two groups of five New Zealand white rabbits (1–1.5 kg) received an anaesthetic dose of sodium pentobarbitone 35 mg kg^{-1}, before i.v. infusion of sodium pyridinethione as a 4% w/v aqueous solution, at a constant rate of 20 mg (0.5 ml) per min into the cannulated jugular vein. The carotid artery was cannulated for blood pressure studies and the heart rate calculated from the electrocardiograph. One group of anaesthetized rabbits received artificial respiration whereas the other groups breathed spontaneously for the duration of the infusion. A control group of rabbits anaesthetized, but breathing spontaneously, was infused with a 1.8% w/v saline solution (equivalent in tonicity to 4.0% w/v sodium pyridinethione) at a rate of 0.5 ml min^{-1} for a period of time in excess of the time required to reach the lethal dose for any rabbit in either of the test groups. This experimental arrangement permitted the respiratory and cardiovascular systems to be monitored for the detection of functional changes at threshold dose levels.

Dermal application studies

All dermal applications were carried out on female New Zealand white rabbits weighing 1.6–2.5 kg. The rabbits were restrained in stocks to isolate the application site in the dorsal-lateral lumbar region. The application site was shaved 24 h prior to dermal administration and the aqueous topical application confined to an area of skin measuring 7.9 cm^2 using a moulded perspex occlusive device (*Fig. 1*). This device was secured to the skin by means of Stomaseal adhesive discs (Medical Products Division, 3M Company, St Paul, Minnesota) and Ostomy adhesive solution (Salt & Son Ltd, Birmingham). The entire device was harnessed to the animal by an elasticated sleeve positioned around the animal's trunk. An application volume of 4.2 ml was introduced into the device through the aperture designed to accommodate a size 12 hypodermic needle.

Abrasion of the application site was produced using a stripping technique employing cellophane tape (6). The net effect was to produce an application site which was erythematous without showing signs of capillary bleeding.

The dermal dose of ^{35}S-labelled sodium pyridinethione was 0.11 g kg^{-1} (specific activity 0.4 μCi mg^{-1}) in a constant volume of 4.2 ml. The topical application was left in contact with the skin for 4, 8, 12, 16, 20 and 24 h experiments, during which time blood samples were taken at 30 min intervals from the marginal ear vein for quantitative studies of drug serum levels. The animals were killed by cervical dislocation and exsanguinated. Percutaneous absorption of ^{35}S-labelled sodium pyridinethione from the site of application was quantified by a disappearance technique based on the method used by Parekh and co-workers (7). Tissues were removed, blotted and weighed in preparation for radiometric analysis.

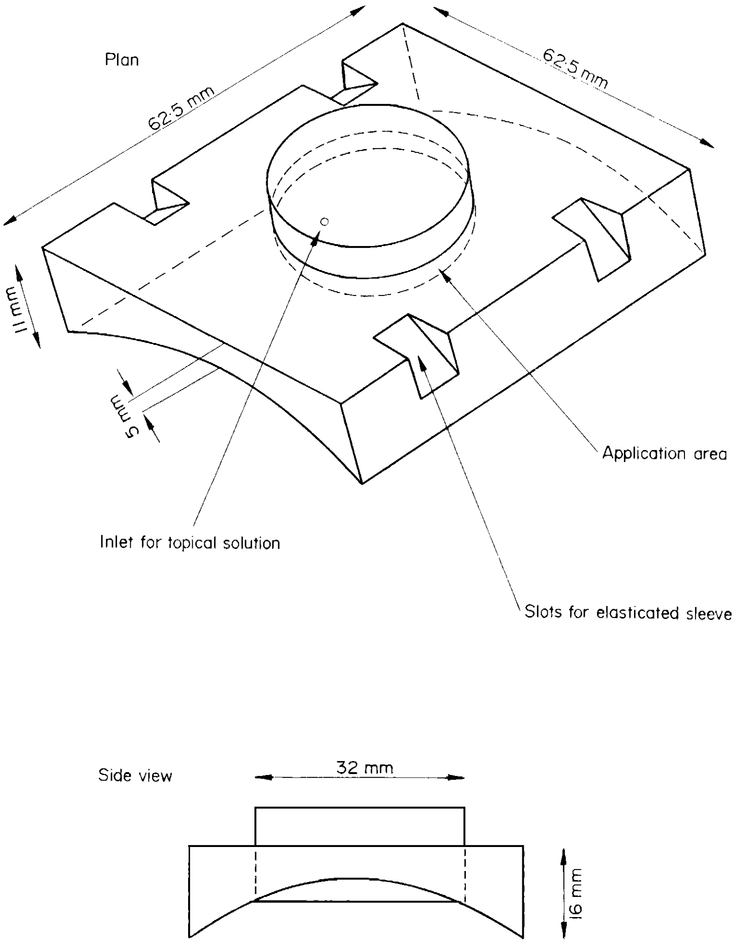

Figure 1. Occlusive device.

Radiometric methods

For distribution studies, 500 mg tissue samples or 0.5 ml of biological fluids were digested in 3 ml of 0.5 M solution of tissue solubilizer Protosol (N.E.N. Boston, Mass.) at 50° for 18 h. The ^{35}S radioactivity in the solubilized media was measured in plastic vials containing 14.5 ml of toluene-based scintillator containing 0.8% 2,5-diphenyloxazole (PPO) and 0.01% *p*-bis-(2,5-phenyloxazole) benzene (POPOP). The radiometric assay was performed on a Tri-Carb 2000, model no. 3003 liquid scintillation spectrometer (Packard Instrument Co.) under conditions suitable for measuring ^{35}S. The channels ratio method of standardization employing ^{14}C as a secondary counting standard (8) was used to calculate the absolute disintegration rates for ^{35}S.

Excretion and metabolism studies

Urine and faeces were collected separately from rabbits, the former being collectively summated with urine withdrawn from the bladder at death. A radiometric assay of urine provided information for excretion rate studies, whereas urine subjected to solvent extraction and chromatographic techniques provided the means of establishing the metabolic fate of sodium pyridinethione following dermal administration.

One millilitre aliquots of urine were withdrawn from the bladder of rabbits exposed to sodium pyridinethione for 24 h and introduced directly onto a polyethylene column (1.5 × 100 cm) containing Sephadex G10 (Pharmacia Fine Chemicals Co.) and eluted with distilled water. This procedure was repeated for urine acidified to pH 4 with dilute hydrochloric acid and extracted with chloroform. Twenty-six fractions of 5 ml were collected automatically and assayed radiometrically. Fractions containing activity were summated and concentrated by freeze-drying; samples of the concentrated aqueous residue underwent paper chromatography (9) followed by radiochromatographic scanning. The Rf values were calculated. A similar analysis was performed for ^{35}S-labelled components in tissue extracts. Tissues were prepared as a protein-free aqueous filtrate by the Valov method. This filtrate was concentrated by freeze-drying and the concentrated tissue extract applied to the Sephadex column. The radioactive spots were eluted with water and examined in a Unicam SP 800 spectrophotometer.

RESULTS AND DISCUSSION

Acute toxicity

Rabbits received sodium pyridinethione by i.v. infusion 20 mg min^{-1} until a lethal dose was administered as described above. Results are given in *Table I.*

Table I. Lethal dose of sodium pyridinethione in rabbits with and without assisted respiration

Condition of rabbits	Group size	Mean lethal dose (mg kg^{-1})
Anaesthetized and artificially ventilated	5	1608 ±230
Anaesthetized and breathing spontaneously	5	1041 ±196
Conscious	5	1159 ±228

The mean lethal dose for rabbits with assisted respiration was significantly greater than the other LD values ($P = 0.05$), i.e. assisted respiration aided the rabbit's tolerance for sodium pyridinethione.

Further respiration studies on anaesthetized rabbits breathing spontaneously (*Fig. 2*) indicated a 20% increase in respiration rate during the

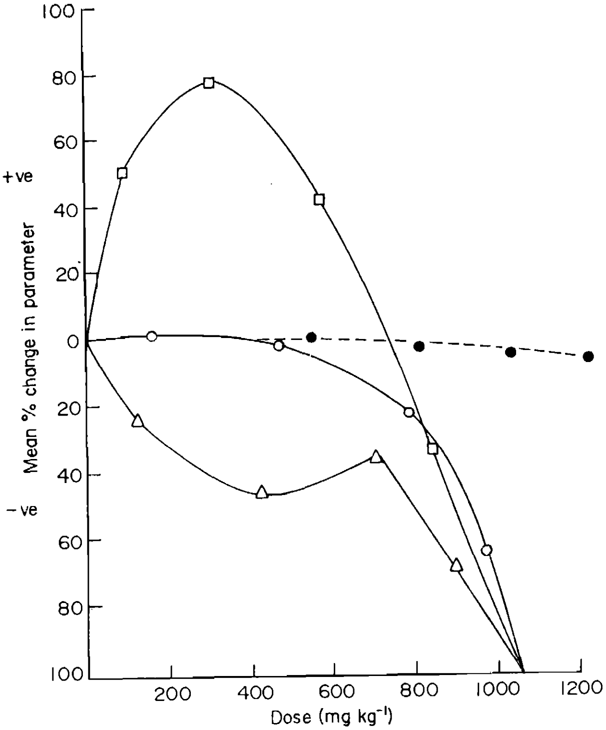

Figure 2. The mean effects upon respiration, blood pressure and heart rate of infusing sodium pyridinethione intravenously into the marginal ear vein of five anaesthetized rabbits. □, Respiration rate; ○, heart rate; △, blood pressure; ●, control.

infusion of 50 mg kg^{-1} sodium pyridinethione, which remained elevated until 700 mg kg^{-1} was infused. At this point respiration rate diminished dramatically as did the blood pressure and heart rate. The respiration rhythm was impaired at doses of 500 mg kg^{-1} and eventual collapse was climaxed by anoxic convulsions. It would appear from *Fig. 2* that infusion of sodium pyridinethione had a primary effect on blood pressure rather than heart rate, a hypotensive effect being recorded at the 50 mg kg^{-1} dose level. Results from the control group of rabbits indicated no gross changes in respiration, blood pressure or heart rate arising from the infusion, surgery or the anaesthetic during the time taken to infuse the lethal dose in the test group of rabbits. These studies showed that systemic levels of 50 mg kg^{-1} precipitated functional changes in the respiratory and cardiovascular systems of the anaesthetized rabbit.

Percutaneous absorption

Percutaneous absorption studies were carried out using ^{35}S-labelled sodium pyridinethione as described above. A dermal dose in excess of the threshold systemic toxic dose, namely 0.11 g kg^{-1}, was applied to intact and

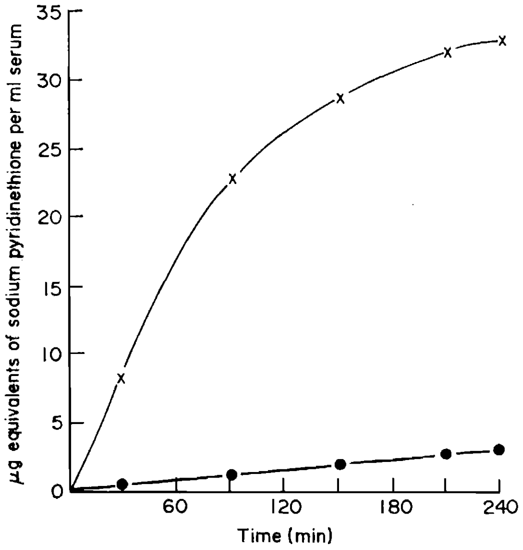

Figure 3. Serum levels of sodium pyridinethione for rabbits with (O) intact and (×) abraded skin.

abraded skin as an aqueous solution. During the 4 h exposure time, a profile of the blood serum levels was obtained by radiometric assay (*Fig. 3*). There was a twenty-five-fold increase in drug serum levels between abraded and intact skin after an exposure time of 2 h. These results emphasized the barrier function of skin and support well-documented evidence, e.g. Cronin and Stoughton (10), showing that abrasion facilitates penetration and percutaneous absorption. The extent of percutaneous absorption in the groups of rabbits with intact and abraded skin was quantified radiometrically by estimating the difference between the activity applied to the site and the total activity recovered from the application solution and excised skin (*Table II*).

Table II. Percutaneous absorption through abraded and intact skin

Condition of rabbit skin	No. of rabbits	Mean quantity absorbed (mg kg^{-1})	$\frac{\text{Quantity applied}}{\text{Quantity absorbed}}$ %
Intact	5	1.346 ±0.795	1.20
Abraded	4	18.363 ±4.012	16.42

Table II indicates a fourteen-fold increase in percutaneous absorption by applying sodium pyridinethione to broken skin, thereby greatly reducing the safety margin before toxic systemic levels are reached.

Distribution of labelled sodium pyridinethione

^{35}S-labelled sodium pyridinethione was administered to six groups of rabbits as a 0.11 g kg^{-1} aqueous solution for 4, 8, 12, 16, 20 and 24 h respectively for distribution studies as described above. *Table III* summarizes the results. Mean concentrations expressed as mg kg^{-1} were estimated for each group of five rabbits and this figure in *Table III* was taken as the representative figure for analysis. These results suggested that rapid urinary excretion of the ^{35}S-labelled parent compound and metabolites takes precedence over tissue concentration. A three-fold increase in concentrations was noted in the liver, kidney and lungs over 24 h but was not surprising because blood serum levels similarly increased with the rate of percutaneous absorption. The increased uptake rate of drug in the bile and small intestine, with no detectable levels in the faeces or colon, suggested the existence of a biliary

Table III. Distribution of ^{35}S-labelled material

Site	Exposure time (h)					
	4	8	12	16	20	24
Urine	0.49	1.33	2.23	3.88	5.67	6.94
Serum	0.057	0.11	0.14	0.16	0.15	0.16
Liver	0.10	0.15	0.19	0.25	0.22	0.32
Kidney	0.027	0.043	0.043	0.053	0.056	0.10
Lung	0.0052	0.0062	0.0070	0.0071	0.0067	0.014
Heart	0.0025	0.0028	0.0031	0.0046	0.0034	0.0037
Spleen	0.0006	0.0007	0.0006	0.0005	0.0005	0.0006
Brain	0.0014	0.0031	0.0044	0.0080	0.0089	0.0142
Bile	0.0011	0.0021	0.0026	0.0034	0.0041	0.0054
Skeletal muscle/G.	0.0004	0.0003	0.0008	0.0010	0.0009	0.0017
Pancreas/G.	0.0004	0.0006	0.0013	0.0016	0.0012	0.0017
Duodenum/G.	0.0009	0.0006	0.0039	0.0058	0.0059	0.0057
Small intestine/G.	0.0010	0.0009	0.0036	0.0039	0.0054	0.0056

enterohepatic shunt. No significant activity was located in the adrenal glands, gonads, skeletal tissue or adipose tissue.

Excretion studies

The excretion of ^{35}S-labelled material in these six groups of rabbits was mainly via the urine. *Fig. 4* shows that the excretion and absorption profiles after 12 h exposure to ^{35}S-labelled sodium pyridinethione were linear and parallel, implying that urinary excretion took precedence over tissue concentration and could be used as a parameter to quantify percutaneous absorption. *Fig. 5* revealed that drug serum levels reach a plateau about 12 h. During the initial 12 h exposure period, factors relating to skin penetration and tissue distribution would be expected to influence systemic drug levels, thus explaining the initial rise in serum levels.

Thirty rabbits received 0.11 g kg^{-1} of ^{35}S-labelled sodium pyridinethione dermally. Five rabbits were killed at 4 h intervals. Tissues were assayed for total activity. Total serum activity was calculated assuming the serum volume was 3% of the body-weight. The activity was converted to mg equivalents of drug from the specific activity and expressed as mg kg^{-1}. Mean values were calculated for each group of five rabbits and appear in *Table III*.

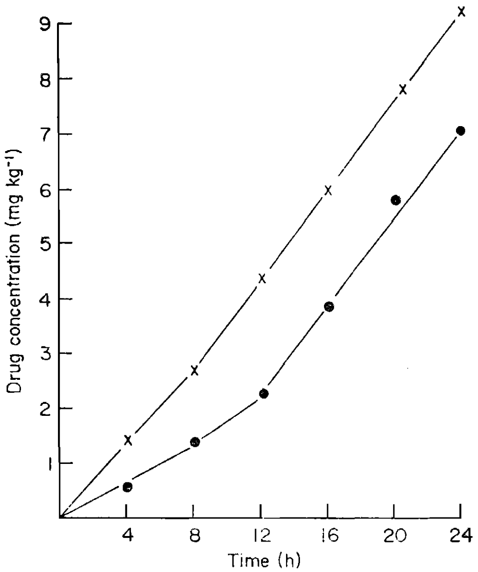

Figure 4. Absorption (×) and excretion (O) rates of sulphur-35 labelled sodium pyridinethione and metabolites over 24 h exposure time.

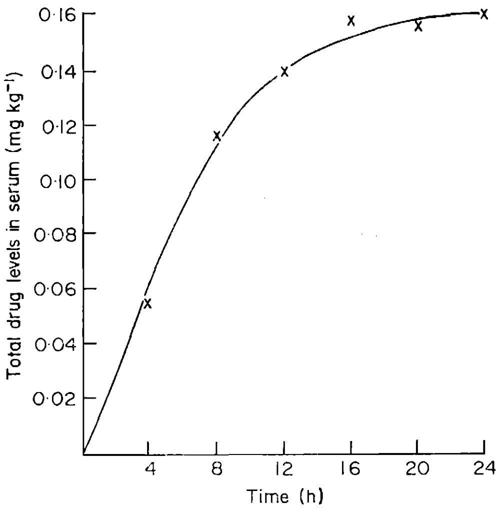

Figure 5. Serum levels of sulphur-35 labelled sodium pyridinethione and metabolites over 24 h exposure time.

Further evidence that excretion of the drug took place in preference to tissue concentration was gained when the ratio of urine, serum, liver and kidney levels were determined for the six groups of rabbits (*Table IV*).

Table IV. Ratio of ^{35}S-labelled material in urine, serum, liver, kidney

Location	Exposure time (h)					
	4	8	12	16	20	24
Urine	8.7	11.7	16.1	24.4	33.1	44.2
Serum	1.0	1.0	1.0	1.0	1.0	1.0
Liver	1.8	1.3	1.4	1.5	1.5	2.0
Kidney	0.5	0.4	0.3	0.3	0.4	0.6

The only amounts changing significantly with extended exposure were the urine levels.

Metabolic rate of sodium pyridinethione

In accordance with the method outlined, urine and tissues were subjected to a chromatographic and spectrophotometric analysis with the aim of identifying the metabolites following percutaneous absorption. *Table V* records the Rf values for reference compounds and components of urine and tissues with ^{35}S activity.

Table V shows that the urine and tissues examined contained pyridine-N-oxide 2 sulphonic acid; the urine and liver also had detectable levels of

Table V. Rf values for reference compounds and components of urine and tissues with ^{35}S activity

Reference compounds and materials examined	Solvent systems			
	Isobutanol 1: Propanol 4: Ammonia 2		N-Butanol 5: Acetic acid 1: Water 2	
Sodium pyridinethione	0.42		0.85	
Pyridine-N-oxide 2 sulphonic acid		0.25		0.18
Urine components	0.42	0.27	0.86	0.16
Tissue components: Liver	0.43	0.26	0.86	0.17
Kidney		0.26		0.16
Lung		0.25		0.16
Brain		0.24		0.16
Heart		0.26		0.17

sodium pyridinethione. Elution of the chromatographic paper spots with Rf value of 0.25 for spectrophotometric analysis, provided uv spectra with absorption peaks at wavelengths 217 and 265 mμm which was in agreement with the reference compound pyridine-N-oxide 2 sulphonic acid. Those spots with an Rf value of 0.42 absorbed at wavelengths 243 mμm, 282 mμm and 332 mμm in the uv spectrum confirming that the parent compound was excreted unchanged. Finally, the urine was assayed radiometrically before and after chloroform extraction to separate sodium pyridinethione from the aqueous soluble acid. Results from this separation indicated pyridine-N-oxide 2 sulphonic acid to be the major excretory product along with sodium pyridinethione in an 8:1 ratio.

These findings regarding the metabolic fate of sodium pyridinethione in the rabbit support the conclusions of Min *et al.* (11) following a dermal study on rats and monkeys. However, there was no evidence to support the findings of Kabacoff and his co-workers (12) whose studies proposed a conjugated mechanism with glucuronic acid as the major metabolic pathway for intravenously-administered sodium pyridinethione to rabbits.

SUMMARY

Sodium pyridinethione was lethal to rabbits at 1 g kg^{-1} by i.v. infusion, the prime cause of death being respiratory failure. Respiratory and cardiovascular effects were evident at 50 mg kg^{-1}, a systemic dose level that was not approached by dermal application of 110 mg kg^{-1} to intact skin. From the findings on absorption, distribution and excretion there appeared to be no tissue concentration and the major proportion of material was excreted via the urine. The metabolic fate of sodium pyridinethione following percutaneous absorption in rabbits appears to be oxidation to pyridine-N-oxide-2-sulphonic acid.

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