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BIOCHEMICAL STUDIES ON POLDINE METHYL METHOSULPHATE

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Abstract—Spectrophotometric assays indicated that following repeated daily oral dosage, 70 per cent of the poldine methyl methosulphate or its metabolites was excreted in the faeces, while ^{14}C-poldine methyl methiodide experiments showed significant biliary excretion.

After intraperitoneal dosing, spectrophotometric assays showed that 11 per cent of the compound or its metabolites was excreted in the urine; with ^{14}C-poldine methyl methiodide 22–36 per cent of the radioactivity appeared in the bile, little appeared in the gastric juice and only a trace in expired CO_2.

Following intravenous dosing a rapid fall-off in blood level was demonstrated with peak liver and kidney levels at 3–5 min; with ^{14}C-poldine methyl methiodide about 90 per cent of the radioactivity was excreted within 48 hr.

Chromatographic/autoradiographic experiments suggested that several metabolites were excreted.

IN AN earlier paper[1] a spectrophotometric method for determination of poldine methyl methosulphate (Nacton, BRL.IS.499, a gastric secretion inhibitor) in aqueous solutions, urine, blood and tissues was described. The method is not specific for poldine and basic metabolites are likely to be determined at the same time. As tertiary and quaternary amines differ in their sensitivities to the reaction, the results can only be expressed in terms of the original compound.

The results obtained from applying this method to a study of the absorption, excretion and distribution of poldine are now presented. In addition limited radiochemical studies have been carried out using ^{14}C-poldine methyl methiodide. The experimental methods used and the results obtained are reported.

MATERIALS

(1) *List of apparatus*

Collection of urine, faeces and respired air. (a) Non-radioactive experiments: galvanized wire metabolism cages above polythene funnels adapted for separate collection of urine and faeces in tubes cooled with solid carbon dioxide. (b) Radioactive experiments: all-glass metabolism cages modified from the design of Roth.[2]

Spectrophotometric measurements. Unicam SP.600.

Chromatography. Two dimensional chromatography on Whatman No. 1 paper.

Autoradiography. Kodirex X-ray film.

Chromatogram autoscanning. Equipment described by Frank *et al.*[3]

Measurement of radioactivity. Nuclear Enterprise/Philips electronic counting equipment and Ekco scintillation equipment.

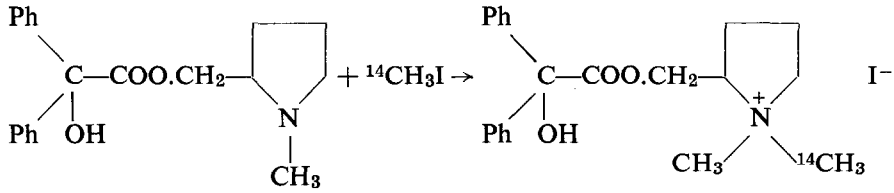
(2) *Reagents*

Spectrophotometric determinations as described earlier.[1]

Chromatography solvents. (1) isopentanol/acetone/water (2:2:1). (2) petroleum ether (100–120°)/isopentanol/ethanol/water (4:2:8:1).

Toluene scintillator. 0·8% 2,5-diphenyloxazole (PPO), 0·03% 1,4-bis-2-(5-phenyl-oxazolyl)-benzene (POPOP) in toluene.

Preparation of radioactive poldine methyl methiodide. Methyl iodide-C14 of spec. act. 4·7 mc/mM was obtained from the Radiochemical Centre, Amersham. ^{14}C-poldine methyl methiodide was prepared by the following reaction.



(1-Methyl-2 pyrrolidyl)methyl benzilate.

It was calculated that poldine methyl methiodide of suitable activity would be obtained if the labelled methyl iodide was diluted with sufficient carrier to react with 0·25 g of the free base, (1-methyl-2-pyrrolidyl)methyl benzilate synthesized by the method of Doyle *et al.*[4] The quaternization was carried out in the ampoule in which the ^{14}C-methyl iodide was supplied. 5·0 ml of a solution of 0·5 g of free base in 10 ml dry acetone was transferred to the ampoule followed by 0·2 ml of 10 per cent v/v methyl iodide in acetone. The neck of the ampoule was washed with a little acetone and the mixture was left at 4°, when crystallization of the ^{14}C-poldine methyl methiodide occurred (m.p. 182–183·5°; spec. act. 6·49 μc/mg).

The labelled material used for injections was shown to be radiochemically and chromatographically pure by paper chromatography at 4° followed by autoradiography and radiochromatogram autoscanning.

METHODS

(a) *Urinary excretion following intraperitoneal dosage*

(i) Male Sprague-Dawley rats weighing 150–250 g were given single injections i.p. of poldine methyl methosulphate (25 mg/kg body wt.). Urine was collected continuously up to 6 days after the injection (with the exception of a 1-hr feeding period every 24 hr) and spectrophotometric determinations carried out to find the amount of poldine methyl methosulphate or metabolite excreted.

(ii) Two rats were injected i.p. with ^{14}C-poldine methyl methiodide (50 mg/kg body wt.) and urine collected for 24 hr subsequently. The urine samples were divided into three parts, which were adjusted to pH 5, pH 7 and pH 9 respectively and extracted three times with methyl ethyl ketone and three times with ether. After concentration by evaporation in a stream of oxygen-free nitrogen, chromatography, autoscanning and autoradiography were carried out on the methyl ethyl ketone extracts and ether extracts.

(b) *Urinary and faecal excretion following daily oral dosage*

Prior to repeated injections it was found to be advantageous to acclimatize the rats for 7 days to the routine of 1-hr feeding every 24 hr, as without this acclimatization they did not maintain their weight during the experiment. All injections were carried out immediately after the daily feeding period. For faecal determinations the method as used for tissues proved satisfactory with a Beer's Law relationship over the range 0–1000 μg poldine methyl methosulphate.

Fourteen rats in groups of two were given daily oral doses of poldine methyl methosulphate (50 mg/kg body wt.) for 12 days and the urine and faeces collected and assayed daily up to 48 hr after the last dose. The rats were then killed and the livers, brains, kidneys, spleens and gastrointestinal tracts were assayed for residual poldine methyl methosulphate.

(c) *Tissue and blood levels following intravenous dosage*

Rats were given a single intravenous dose of poldine methyl methosulphate (12·5 mg/kg body wt.) via the caudal vein. Immediately after sacrifice, blood samples were collected in tubes containing solid heparin as anticoagulant. The livers, brains, kidneys and spleens were immediately dissected out and homogenized prior to determination.

(d) *Urine and faeces levels following intravenous dosage*

After a single intravenous injection of ^{14}C-poldine methyl methiodide in saline (12·5 mg/kg body wt.), two rats were placed in all-glass metabolism cages and urine and faeces samples collected. Aliquots of the urine samples were freeze dried, oxidized with Van Slyke reagent[5] and the radioactivity measured by a Nuclear Chicago ion electrometer (Model 6000 Dynacon). Faecal samples were homogenized in water, freeze dried and assayed similarly to the urine samples.

(e) *Biliary excretion following oral dosage*

Following the cannulation of their bile ducts, four rats were dosed orally with ^{14}C-poldine methyl methiodide (approximately 50 mg/kg body wt.). Bile samples were collected and examined for radioactivity as above. Bile not used for these determinations was extracted with methyl ethyl ketone and concentrated extracts were subjected to chromatography. The resultant chromatograms were examined quantitatively by radiochromatogram autoscanners and qualitatively by autoradiography. Control chromatograms and autoradiographs were obtained by adding ^{14}C-poldine methyl methiodide to control bile obtained from an uninjected rat.

(f) *Gastric juice levels and biliary excretion following intraperitoneal dosage*

Six-hour pyloric-ligated rats[6] were injected i.p. with ^{14}C-poldine methyl methiodide (5 mg/kg body wt.), the low dose being necessary as at higher doses no gastric juice was secreted. After 6 hr, aliquots of the gastric juice samples were freeze dried, oxidized and the radioactivity determined as above. For examination of bile, two rats with cannulated bile ducts were injected intraperitoneally with ^{14}C-poldine methyl methiodide (25 mg/kg body wt.). Using a fraction collector, hourly samples were collected over a period of 24 hr. The samples were freeze dried, oxidized and the radioactivity measured as above.

(g) *Examination of respired carbon dioxide following intraperitoneal dosage*

After injection of ^{14}C-poldine methyl methiodide (50 mg/kg body wt.) i.p., a rat was placed in an all-glass metabolism cage with access to food and water for the 24-hr duration of the experiment. Carbon dioxide-free air was drawn through the chamber and then through twenty-four tubes each containing a mixture of 3 ml of ethanolamine and 21 ml of ethylene glycol monomethyl ether, which absorbed all the expired CO_2. Eight ml of each solution were added to 6 ml of toluene scintillator and the samples were then counted for radioactivity by scintillation techniques using Nuclear Enterprises/Philips electronic counting equipment and Ekco scintillation equipment. The percentage of radioactivity in the respired air was calculated.

RESULTS

(a) *Urinary excretion following intraperitoneal dosage*

(i) The results are shown graphically in Fig. 1 and indicate that approximately 11 per cent of the administered dose was accounted for as unchanged compound or

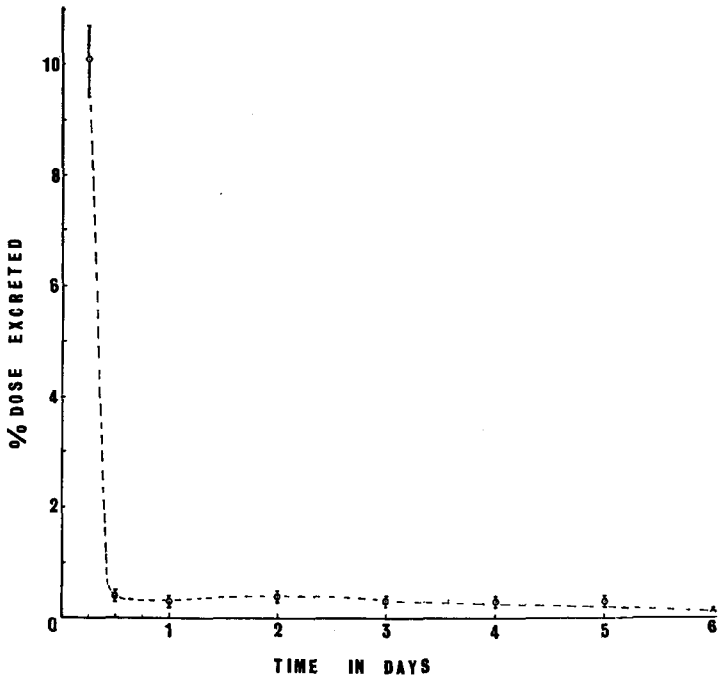


FIG. 1. Urinary excretion of poldine methyl methosulphate or metabolite after intraperitoneal injection of 25 mg/kg (mean values with S.E.M.)

metabolites in the urine, almost entirely in the first 12 hr, with little of significance beyond 24 hr. The lowest levels were confirmed by testing concentrated urine extracts for mydriatic effects in mice.

(ii) Numerous radioactive compounds were shown to be present in the extracts at each pH. The most highly active spot was extracted by the methyl ethyl ketone. Paper

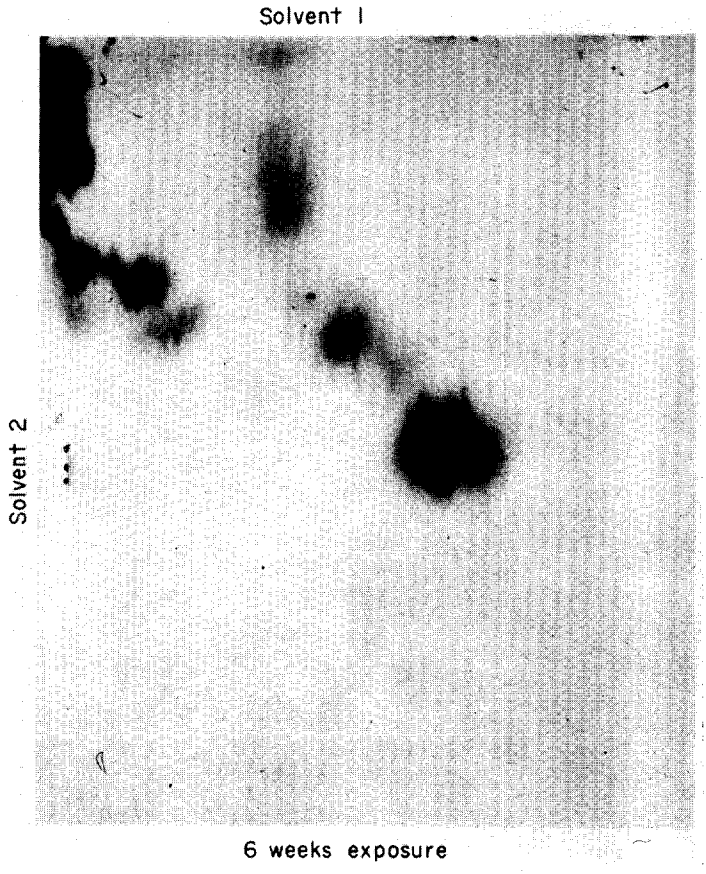


FIG. 2. Autoradiogram of chromatogram of the pH7 ether extract of test urine.

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chromatography and autoradiography gave a zone of increased size on addition of ^{14}C-poldine methyl methiodide to the methyl ethyl ketone extract, and therefore it seems likely that this spot was unchanged ^{14}C-poldine methyl methiodide, particularly as four other solvents failed to resolve this enlarged zone. A typical autoradiograph is shown in Fig. 2.

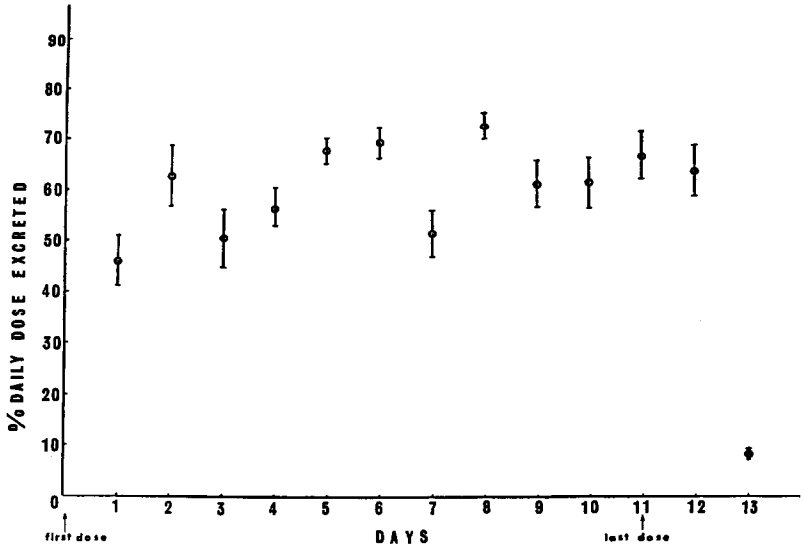

FIG. 3. Faecal excretion of poldine methyl methosulphate or metabolite after repeated daily oral dosage of 50 mg/kg (mean values with S.E.M.).

(b) *Urinary and faecal excretion following daily oral dosage.*

The results of the faecal determinations shown in Fig. 3 indicate that the faecal excretion reached a maximum of 50–70 per cent 2–3 days after the first oral dose and this level was maintained until 24 hr after the last dose, the mean daily faecal excretion being 62 per cent. The total poldine methyl methosulphate or metabolites excreted in the faeces during the 13 days of the experiment varied between 54 and 75 per cent (mean 66 per cent) in the seven pairs of rats. Less than 1 per cent of the daily oral dose was excreted in the urine. Forty-eight hr after the final injection, approximately 3 per cent of the daily dose was present in the gastrointestinal tract and contents, and only 0·1–0·2 per cent in the kidneys. None was detected in the livers, brains or spleens.

(c) *Tissue and blood levels following intravenous dosage*

(i) Blood levels. The results of determinations at intervals up to 30 min after injection are shown in Fig. 4. Only 15 per cent of the compound was detectable 30 sec after injection and this level fell rapidly to 3 per cent after 4 min and then more slowly until by 30 min none was detected.

(ii) Liver levels. Figure 4 also indicates the liver levels found for poldine methyl methosulphate, with a peak level of about 16 per cent of the administered dose being evident at 4–5 min after intravenous injection. A steady fall in concentration then occurred until at 45 min only 2 per cent of the dose remained, and after 1 hr none was detected.

(iii) Kidney levels. As with the liver, a rapid build up to a peak of about 10 per cent of the administered dose at 3 min is evident in Fig. 4. The decrease in concentration was however more rapid than with the liver and at 15 min after administration little more than 1 per cent remained.

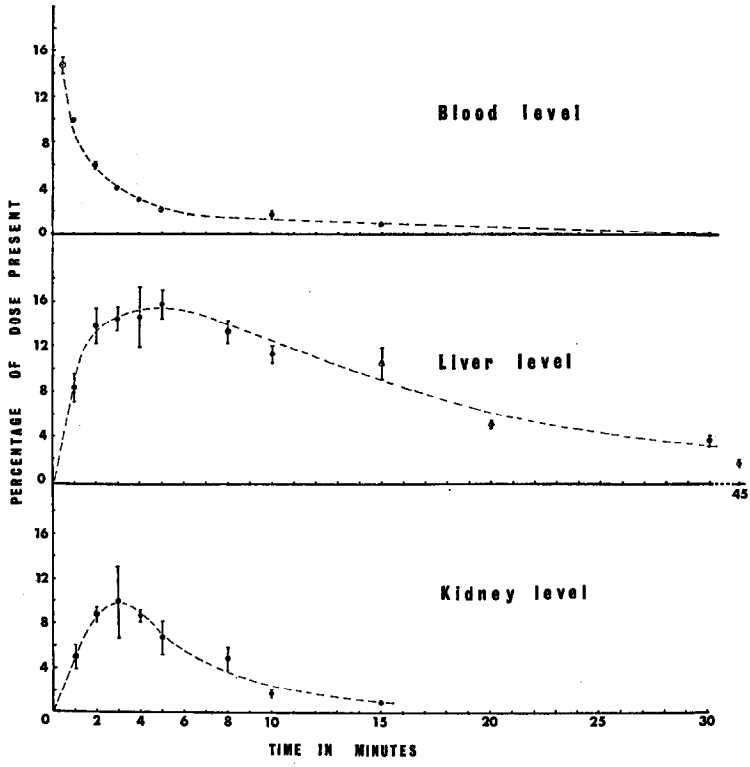

FIG. 4(a). Blood level of poldine methyl methosulphate or metabolite after intravenous injection of 12·5 mg/kg. (b) Liver levels of poldine methyl methosulphate or metabolite after intravenous injection of 12·5 mg/kg. (c) Kidney levels of poldine methyl methosulphate or metabolite after intravenous injection of 12·5 mg/kg (mean values with S.E.M.).

(iv) Brain and spleen levels. All determinations of poldine methyl methosulphate in these tissues gave values below 1 per cent of the administered dose and it was therefore evident that no significant quantity of the compound was present in these tissues at any time up to 60 min after injection.

(d) *Urine and faeces levels following intravenous dosage*

After determining the radioactivity injected into the two rats by quantitatively assaying aliquots of the injection solution, the percentage recoveries obtained for the urine and faeces samples collected are shown in Table 1.

(e) *Biliary excretion following oral dosage*

The recoveries of radioactivity up to 24 hr after administration were as shown in Table 2. In all the autoradiographs of chromatograms, the presence of multiple

radioactive spots was demonstrated and it was found that, even when an active area appeared to be a single spot on autoscanning, subsequent autoradiography often showed that the area contained two or even three compounds. Close examination of the autoradiographs showed that the bile may have contained as many as seven or eight labelled compounds.

TABLE 1. PERCENTAGE RECOVERY OF RADIOACTIVITY AFTER INTRAVENOUS INJECTION OF ^{14}C-POLDINE METHYL METHIODIDE

Time after injection (hr)	Rat A		Rat B	
	Urine	Faeces	Urine	Faeces
0– 24	22·9	25·5	36·8	40·4
24– 48	5·8	40·5	3·6	7·3
48–114	2·8	7·8	3·9	4·8
	31·5	73·8	44·3	52·5
Totals	105·3		96·8	

TABLE 2. PERCENTAGE RECOVERY OF RADIOACTIVITY IN BILE AFTER ORAL DOSING WITH ^{14}C-POLDINE METHYL METHIODIDE

Time after injection (hr)	Rat A	Rat B	Rat C
0–6	0·1	0·4	8·2
6–24	7·3	0·5	4·7
Totals	8·4	0·9	12·9

(f) *Gastric juice levels and biliary excretion following intraperitoneal dosage*

When 1·5–1·8 μc ^{14}C-poldine methyl methiodide was injected into each rat, less than 1 per cent of this was detected in 0–6 hr gastric juice samples from three of the rats, the fourth giving a 4·2 per cent recovery. This indicates that although poldine methyl methiodide inhibits gastric secretion, it is not itself excreted into the gastric juice in amounts greater than approximately 0·01 mg/ml in the rat.

Determinations carried out on the hourly bile samples obtained gave the results shown diagrammatically in Fig. 5. The total percentage recoveries over the 0–24-hr period were 36·3 and 21·8 per cent in the two animals examined. As expected, this was a much higher level of radioactivity than that obtained after oral dosing and reflects the poor absorption after oral dosing.

(g) *Examination of respired carbon dioxide following intraperitoneal dosage*

It was found that when samples were counted, the first thirteen samples gave a total of only 0·17 per cent recovery and that the counts on the remaining eleven samples

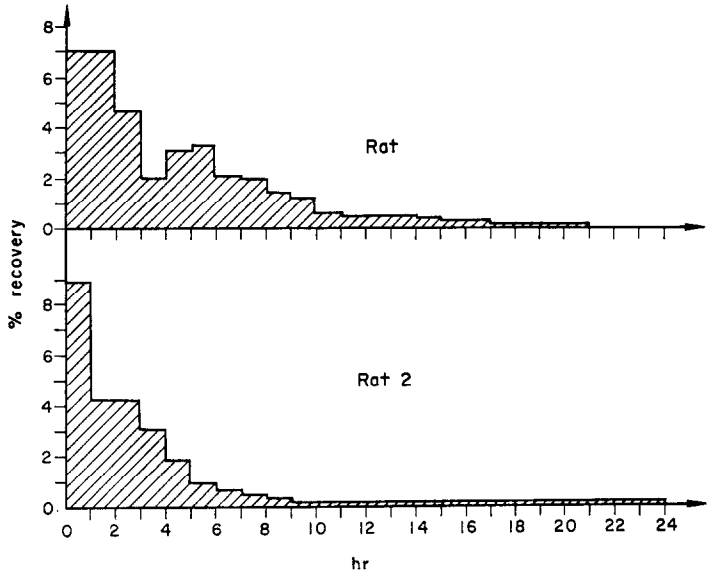

FIG. 5. Secretion of Radioactivity into the bile after intraperitoneal injection of ^{14}C poldine methyl methiodide.

were not significantly above background. It was concluded that no significant quantity of $^{14}CO_2$ was expired in the 24-hr following i.p. dosage of ^{14}C-poldine methyl methiodide.

DISCUSSION

Poldine methyl methosulphate, in common with other quaternary salts of amines, is only poorly absorbed from the gastrointestinal tract. The repeated oral dosage experiments show that nearly 70 per cent of the administered dose appears in the faeces. A proportion of this high level however is probably due to re-excretion via the bile, which has been shown to be a significant excretory route for this compound. When administered directly into the blood, the compound disappears from the circulation very rapidly, the half life in blood being measured in seconds rather than minutes. The build up in tissue appears to be a rapid and reversible process, since only trace amounts are detectable in the major organs 1 hr after intravenous dosage. Autoradiography of chromatograms of bile and urine extracts, after oral and intraperitoneal dosage of ^{14}C-poldine methyl methiodide, clearly indicated several radioactive zones other than unchanged compound and it is probable that some of these "metabolites" would not be determined by the spectrophotometric assay, for which the presence of a basic nitrogen atom is essential.

Excretion via the expired air, i.e. complete oxidation, was shown to be virtually absent.

Direct excretion of poldine methyl methiodide into gastric juice was found to be at a very low level, so there is no evidence from these studies of any localized concentration of this compound in gastric juice.

SUMMARY

(a) *Repeated daily oral dosing.* A high daily faecal excretion rate of nearly 70 per cent of the daily dose of poldine methyl methosulphate was evident by spectrophotometric assay, indicating poor absorption. ^{14}C-Poldine methyl methiodide experiments indicated that about 10 per cent of the administered radioactivity was excreted via the bile, after a single oral dose.

(b) *Intraperitoneal dosing.* About 11 per cent of a single dose of poldine methyl methosulphate was recovered from the urine, mainly within 12 hr. With ^{14}C-poldine methyl methiodide, 22–36 per cent of the radioactivity was excreted in the bile within 24 hr, but only a trace in the expired CO_2. No evidence for localized concentration of ^{14}C-poldine methyl methiodide in the gastric juice was found up to 6 hr after dosing.

(c) *Intravenous dosing.* A rapid fall in blood level occurred within seconds of dosing. Liver and kidney levels rose to peaks of 15 per cent and 10 per cent respectively at 3–5 min and nothing of significance was found in brain or spleen. With ^{14}C-poldine methyl methodide about 90 per cent of the radioactivity was recovered within 48 hr in the urine and faeces.

(d) Chromatographic/autoradiographic experiments suggested the excretion of several metabolites in urine and bile.

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