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The distribution of chloroquine in man after fatal poisoning

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Detailed analysis of autopsy specimens from two fatal cases of chloroquine poisoning is reported. Post-mortem blood levels of 1·60 and 1·24 mg chloroquine/100 ml blood were found; results for liver blood, urine, stomach contents, liver, lungs and kidneys are given. The highest chloroquine concentration was in liver and the presence of a metabolite was demonstrated in the tissues.

Published information on the distribution of chloroquine [7-chloro-4-(4′-diethylamino-1′-methylbutylamino)quinoline] in the tissues and body fluids of man is incomplete, as it relates only to plasma concentrations of the drug after oral dosage, or to occasional reports of post-mortem concentrations in some tissues. We have examined the distribution of the drug in blood, urine, stomach contents, lung, liver, kidneys, heart and brain in two acute fatal overdose cases.*

Chloroquine is not a common suicidal agent (in the U.K.) but its use as an alleged abortifacient has resulted in fatalities (Kiel, 1964).

EXPERIMENTAL

Material

Post-mortem blood, liver blood, urine, stomach contents, lung, liver, kidneys, heart and brain was from a 33 year old female who had swallowed an estimated 12 tablets containing chloroquine at 5 am. She was taken unconscious to hospital at 6 am where she died 24 h later without regaining consciousness and despite treatment by dialysis. (Case No. 1).*

Ante-mortem blood, post-mortem blood, liver blood, urine, stomach contents, lung, liver and kidneys was from a 24 year old female who had taken an estimated 40 tablets containing chloroquine phosphate at about 4 am and later a few tablets of paracetamol and of aspirin. She collapsed while walking into hospital and died at 8.30 am after a cardiac arrest. (Case No. 2).

Direct extraction of body fluid specimens. The specimen (5 ml) was made alkaline with strong ammonia solution and extracted with ether (75 ml). Basic substances were recovered by extraction of the ether with dilute hydrochloric acid (5 ml, 0·1N).

Extraction of tissue. Minced tissue (10 g) was suspended in sodium hydroxide solution (5 ml, 20% w/v) and heated on a boiling water-bath for 90 min. The cooled hydrolysates were extracted with 100 ml of ether. The ether extract was washed free from alkali and the bases were extracted into 0·1N hydrochloric acid. A portion of blood (5 ml) was similarly treated for comparison.

Extraction of body fluids containing paracetamol and salicylates (*Case No.* 2). An aliquot (5 ml) of body fluid was extracted with 50 ml of chloroform. Salicylate was

* Details of case histories will appear in Medicine, Science and the Law, 1970.

recovered from the chloroform by extraction into 1% w/v sodium bicarbonate solution and assayed using Trinder reagent (Trinder, 1954). The chloroform extract was again extracted with sodium hydroxide solution (0·45N) to recover the paracetamol for spectrophotometric assay at 265 nm before and after the addition of acid. The chloroform was then washed free from alkali and the chloroquine recovered by extraction with 0·1N hydrochloric acid.

Determination of chloroquine. The absorption spectrum of each acid extract was recorded using a Unicam SP800 spectrophotometer over the range 220–360 nm and the chloroquine content was determined on the basis of the absorption at 343 nm.

Infrared absorption spectrum. The basic substances in the total stomach content specimen from Case No. 1 were extracted into ether and the solvent evaporated. The infrared absorption spectrum of the oily residue was examined as a liquid film in a Grubb Parsons spectrophotometer.

Thin-layer chromatography (TLC). Basic substances from the acid solutions used for assay were recovered into chloroform and the solvent evaporated. The residue was chromatographed on 250 μm silica gel plates with chloroform–ethanol–ammonia (80:20:1 by volume) as the solvent. Potassium iodoplatinate solution was used to detect basic substances on the chromatograms [chloroquine (Rf 0·75–0·80) and the metabolite (Rf 0·38) gave brown colour reaction].

Gas liquid chromatography (Case No. 1). The residues of the chloroquine extracts used for thin-layer chromatography were dissolved in about 100 μl volume of chloroform and aliquots were injected into an F & M model 400 gas chromatograph equipped with a 4 ft glass column packed with 3·8% SE 30 on 80–100 mesh Diatoport S. A flame ionization detector was used. The instrument was programmed to operate the oven isothermally at 195° and, after 10 min to increase the temperature by 5°/min up to 245°. Nitrogen was used as the carrier gas.

RESULTS

Chloroquine. The infrared spectrum from an extract of the stomach contents of the first case was comparable with that of authentic chloroquine similarly treated, and resembled the spectrum published by Kuroda (1962).

The quantitative results from the ultraviolet absorption analysis are in Table 1. There was no significant difference between results obtained by direct extraction of

Table 1. *Tissue levels of chloroquine*

Specimen	Chloroquine base mg/100 ml or /100 g	
	Case 1	Case 2
Ante-mortem blood	—	0·86
Post-mortem blood	1·60	1·24
Liver blood	9·00	4·40
Urine	2·00	6·84
Stomach contents*	1·80	55·20
Lung	3·80	9·80
Liver	17·50	34·40
Kidneys	7·00	30·00
Heart	5·70	—
Brain	1·60	—

* Volume of stomach contents of Case No. 2—125 ml.

the body fluids or by extraction after alkaline hydrolysis. Recovery experiments in which a known amount of chloroquine was added to tissue and taken through the entire procedure showed that the recovery of the chloroquine was better than 95%.

Results were calculated as total chloroquine base; this probably included a metabolite of chloroquine corresponding with that identified as a mono-des-ethylated compound (McChesney, McAuliff & others, 1954; Kuroda, 1962).

There was less metabolite than unchanged drug present in specimens of post-mortem blood and urine. The stomach contents from the second case contained mostly unchanged drug and a much smaller quantity of the metabolite the unexpected presence of which might be the result of gastric secretion. The absence of the metabolite from the ante-mortem blood sample only of all the specimens analysed, may reflect the time interval before the formation of detectable amounts of metabolite.

In the first case the metabolite was present only in the liver, kidney and lung. A qualitative examination of the TLC residues from this case by gas-liquid chromatography gave a major peak for each extract which had retention times in the range of 19–23 min and corresponded with chloroquine. In addition to the major peak, the tissue extracts produced between 4–7 minor peaks, while extracts from the body fluids produced four minor peaks. Most of these peaks may be attributed to tissue constituents.

DISCUSSION

Absorption of chloroquine in therapeutic dosage is almost complete from the gastrointestinal tract (Goodman & Gilman, 1965) but our findings for stomach contents and liver blood, compared with peripheral blood, indicated that absorption of the drug was incomplete at death in both cases. The post-mortem blood concentrations of paracetamol and salicylates did not suggest that excessive amounts of these drugs had been taken.

A chloroquine metabolite, the properties of which corresponded with 7-chloro-4-(4′-ethylamino-1′-methylbutylamino)quinoline, was present in most extracts, indicating that the detoxifying enzymes were still functional in both cases. Several chromatographic systems failed to demonstrate more than the one metabolite.

The post-mortem blood concentrations in both cases and the ante-mortem concentration in the second case greatly exceeded the peak concentrations reported after therapeutic dosage with the drug. Thus Alving, Eichelberger & others (1948) reported that peak plasma concentrations 6 h after one 500 mg dose never exceed 3·5 μg of chloroquine/100 ml, while Berliner, Earle & others (1948) found peak plasma concentrations ranging from 2·2 μg/100 ml after 50 mg to 21·7 μg/100 ml after 400 mg dose of the drug. Hoole (1966) however, reported the chloroquine blood concentration of a 45 year old male found dead as 9·9 mg/100 ml.

The tissue concentrations in the second case were also higher than the ranges reported by Prouty & Kuroda (1958) for eight non-suicide cases. Present data are compared with those published by others in Table 2. Apart from the post-mortem blood chloroquine concentration reported by Hoole (1966), there are no reported data for body fluids and tissues other than liver, kidney, heart and brain.

During therapeutic dosage, chloroquine is excreted partly unchanged in the urine (50–70% of the dose) and partly as the mono-des-ethylated metabolite (25–50% of the dose) according to McChesney, Conway & others (1966). An earlier study

Table 2. *Published reported tissue levels of chloroquine in man compared with levels found in the same tissues of the two cases reported in the present text*

	Prouty & Kuroda (1958)			Kiel (1964)	Hoole (1966)	This paper†	
	8 Non Suicides	2 Suicides		13 Suicides*	1 Overdose	Case 1	Case 2
	mg/100 g	mg/100 g		mg/100 g	mg/100 g	mg/100 g	
Liver	0·43–4·8	—	90·0	0·23–75·0	88·3	17·5	34·4
Kidney	0·06–0·58	—	47·0	11·0–64·0	18·8	7·00	30·0
Heart	0·41–2·0	8·4	—	4·0	—	5·70	—
Brain	0·07–0·73	1·0	1·10	0·04–5·0	—	1·60	—

* In only one case are all of the tissue levels reported.
† Data for other specimens are given in Table 1.

(McChesney & others, 1954) based on solvent soluble dye complex assays showed that 8% of the daily oral dose was excreted in the faeces and 14% in the urine (range 10–25%): the urinary excretion would be expected to be increased or decreased by administration of acid or alkali. Chloroquine was present in the urine of both cases at post-mortem and significant concentrations of the drug, accompanied by the metabolite, were found in the kidneys (Table 2).

The ratio of kidney: liver levels probably reflects the combination of the time interval and treatment between ingestion of the drug and death: for the first case the kidney/liver ratio is 0·4 and the survival time was 24 h; for the second case the ratio is 0·87 and the time interval was 6 h but renal damage might have prevented excretion of the drug. The highest concentration of the drug was in the liver, which is consistent with previous reports, including those where overdosage was not the immediate cause of death.

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