SPECTROPHOTOFLUOROMETRIC DETERMINATION OF EMETINE IN ANIMAL TISSUES

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A simple rapid spectrophotofluorometric method for assaying emetine in animal tissues is described. Tissue emetine concentrations have been determined in rats at intervals up to 14 days after single doses of emetine hydrochloride or emetine bismuth iodide: the hydrochloride was administered orally or subcutaneously and the bismuth iodide complex orally. Subcutaneously administered emetine hydrochloride produced the highest concentrations. Given orally, emetine hydrochloride produced higher tissue levels than did emetine bismuth iodide.

Emetine is a valuable drug with a low therapeutic index. For 50 years it has found worldwide use in the treatment of amoebiasis, yet references to studies of tissue distribution are few (Gimble, Davison and Smith, 1948; Parmer and Cottrill, 1949; Radomski, Hagan, Fuyat and Nelson, 1952), probably because the assay methods available have been somewhat laborious. We are currently investigating the biological disposition of emetine and some derivatives; to facilitate these studies we have developed a simple spectrophotofluorometric assay. Details of the assay, and the results of some rat experiments in which it was used, are reported here.

Materials and Methods

The materials used in the experiments were emetine hydrochloride B.P., emetine bismuth iodide B.P., N sodium hydroxide, anaesthetic ether B.P. and a KCl/HCl solution of pH 2. Before using the last reagent, which is made by mixing 50 ml. M KCl with 53 ml. 0·2 N HCl and adjusting the volume to 1 litre with distilled water, it is essential to extract it with ether to remove extraneous fluorescent material.

The emetine contents were 70·1 per cent (B.P. assay) and 71·7 per cent (calculated on Kjeldahl nitrogen) for the hydrochloride, and 27·8 per cent (B.P. assay) and 28·6 per cent (calculated on Kjeldahl nitrogen) for the bismuth iodide complex. In calculating dosages it was assumed that the hydrochloride and bismuth iodide complex contained 71 and 28 per cent respectively of emetine base.

Fluorescence Characteristics of Emetine

A solution of emetine hydrochloride (0·05 μg./ml.) in KCl/HCl solution was scanned on a Farrand spectrophotofluorometer. Single peaks were present in the activating and analysing spectra at 290 mμ and 320 mμ, respectively (uncorrected values). At these wavelengths the emetine solution fluoresces three times as strongly as distilled water or the KCl/HCl solution itself; a full scale deflection is obtained on the most sensitive

scale of the microammeter at a concentration of 0·1 μg./ml. The intensity of fluorescence increases linearly over the concentration range 0·01–1·00 μg./ml.: it increases also with decreasing pH, being maximal and constant over the range 1–3. The intensity of fluorescence diminishes with rising temperature: over the range 15–30° each degree rise results in 0·5 per cent less intensity.

Assay of Emetine in Rat tissues

Rats were killed with coal-gas and their hearts, lungs, livers, kidneys and spleens were removed and weighed. Each organ was homogenised in a Townson and Mercer micro wet grinder, and the brei was diluted with distilled water to give a suspension containing 1 g. wet tissue per 10 ml. One ml. of suspension was shaken vigorously for 30 sec. in a shake-tube with 0·5 ml. distilled water, 0·5 ml. N NaOH and 10 ml. ether. In the recovery experiments standard emetine solutions were used in place of the distilled water. Of the ether phase (total volume after shaking, 9·7 ml.) 8 ml. was removed and shaken for 30 sec. with 10 ml. KCl/HCl solution. The ether layer was removed by aspiration and discarded. The fluorescence of the KCl/HCl solution was compared with that of a standard solution of emetine hydrochloride in KCl/HCl solution at the same temperature.

The mean percentage recovery of emetine hydrochloride added in amounts equivalent to 1–100 μg. emetine base/g. wet tissue was 99·3 ± S.E. 2·1 (30 observations). There were no differences in the recoveries from various tissues; as might be expected, there was greater variation at the lower emetine concentrations. Thus the mean percentage recovery at a concentration of 1 μg. emetine base/g. wet tissue was 104·3 ± 6·0 (10 observations), whereas that at 5–100 μg./g. was 96·9 ± 0·9 (20 observations). The fluorescence obtained by extracting tissues from undosed rats was not more than that given by the KCl/HCl solution itself.

Tissue Emetine Concentrations in Dosed Rats

Two groups of female rats of the WAG strain (body weight range 130–170 g.) received single oral doses of either emetine hydrochloride (10 mg./kg. ≡ 7·1 mg. emetine base/kg.) or emetine bismuth iodide (30 mg./kg. ≡ 8·4 mg. emetine base/kg.). A third group received single subcutaneous injections of emetine hydrochloride (10 mg./kg.). The hydrochloride was administered in aqueous solution and the bismuth iodide complex as an aqueous suspension in gum tragacanth, 1 in 200. At various times, from 2 hr. to 14 days after dosing, 3 rats from each group were killed and their tissue emetine levels were determined. The organs of the rats dosed orally were assayed individually, but from those injected subcutaneously the corresponding organs from 3 rats were bulked before assay.

The fluorescent spectra of the tissue extracts were indistinguishable from those of emetine hydrochloride. Furthermore, when the fluorescent substance isolated from the tissues of rats dosed with emetine hydrochloride was examined by the chromatostrip method of Stahl (1958), one

spot only was obtained, and its R_F value agreed with that of emetine. The amoebicidal activity of the fluorescent substance was demonstrated in vitro using *Entamoeba histolytica.*

RESULTS

The concentrations and amounts of emetine found in the different organs at various times after dosing are given in Table I. The total amounts of emetine found in the organs at various times after dosing are shown in Fig. 1, the values being expressed as percentages of the doses administered.

TABLE I

TISSUE DISTRIBUTION OF EMETINE IN RATS AT VARIOUS TIMES AFTER SINGLE DOSES OF THE HYDROCHLORIDE OR BISMUTH IODIDE COMPLEX

Treatment and route	Time after dose	Emetine concentration (μg. base per g. wet tissue)*					Emetine content (per cent of dose)*					Total emetine content in tissues examined (per cent of dose)*
		Heart	Kidney	Liver	Lung	Spleen	Heart	Kidney	Liver	Lung	Spleen	
Emetine hydrochloride 10 mg./kg. (=7·1 mg. base/kg.) Subcutaneous	2 hr.	11·1	38·0	22·3	63·9	59·5	0·55	4·62	13·55	4·77	2·02	25·5
	4 ,,	9·0	49·5	38·2	98·0	99·3	0·43	5·68	22·20	8·53	2·90	39·7
	8 ,,	7·4	34·7	35·8	79·4	116·0	0·35	3·66	18·20	7·25	3·52	33·0
	1 day	7·5	33·2	30·9	47·4	94·0	0·37	3·23	15·60	5·23	3·78	28·2
	2 days	8·2	24·9	18·9	47·5	85·0	0·38	2·74	10·20	4·89	2·34	20·6
	3 ,,	8·4	20·8	20·8	44·4	33·5	0·38	2·13	9·70	3·56	1·76	17·5
	7 ,,	5·0	12·4	7·2	23·0	39·5	0·22	1·45	4·82	2·26	1·71	10·5
	14 ,,	1·2	2·6	2·1	7·1	12·6	0·06	0·36	1·38	0·74	0·66	3·2
Emetine hydrochloride 10 mg./kg. (=7·1 mg. base/kg.) Oral	4 hr.	2·3	8·5	19·4	11·1	8·3	0·12	0·90	10·70	1·06	0·29	13·0
	8 ,,	3·4	14·9	23·2	19·1	15·5	0·16	1·51	11·00	1·43	0·65	14·7
	1 day	3·3	12·4	15·9	25·1	24·1	0·16	1·40	7·95	2·03	1·09	12·6
	2 days	4·7	19·8	23·9	30·0	36·8	0·21	2·24	12·40	2·32	2·11	19·3
	3 ,,	5·9	20·2	17·4	30·7	50·8	0·26	2·26	8·03	2·30	2·17	14·9
	7 ,,	1·8	9·1	5·4	11·5	27·9	0·08	1·01	3·49	0·75	1·52	6·9
	14 ,,	1·0	3·7	2·3	5·5	17·4	0·05	0·44	1·35	0·38	0·82	3·0
Emetine bismuth iodide 30 mg./kg. (=8·4 mg. base/kg.) Oral	4 hr.	1·0	2·6	14·5	3·8	2·6	0·04	0·27	8·10	0·26	1·20	8·8
	8 ,,	3·3	13·1	21·3	20·5	13·6	0·13	1·14	9·01	1·55	0·49	12·3
	1 day	2·3	9·8	16·7	19·1	22·5	0·10	1·01	8·67	1·43	0·94	12·1
	2 days	4·6	16·6	21·2	29·5	42·6	0·18	1·65	10·31	2·02	1·66	15·8
	3 ,,	4·3	16·5	12·1	24·4	40·0	0·17	1·70	6·23	1·62	1·72	11·5
	7 ,,	2·0	10·3	4·7	13·6	25·6	0·08	1·07	2·54	1·02	1·12	5·8
	14 ,,	0·7	1·9	1·9	4·4	10·6	0·03	0·21	1·12	0·31	0·48	2·1

* Group mean values (3 rats per group.)

The concentrations of emetine in the tissues were higher after subcutaneous injection than after oral administration. Only small differences were seen between the two oral preparations, but the total tissue content of emetine was consistently lower after dosing with the bismuth iodide complex than with the hydrochloride.

DISCUSSION

The assay method described is simple and rapid; it gives reproducible results provided that precautions are taken to eliminate extraneous fluorescence.

The method can also be used for determining emetine in muscle and brain (in which we found only traces) and in urine and blood. With blood, the aqueous and ether phases do not separate as rapidly as with

tissue extracts. Because they produce high blank values, it is essential that droplets of diluted blood are not removed with the ether extract. Preliminary experiments indicated that emetine disappeared rapidly from the blood stream in rats, being almost undetectable 10 min. after a single intravenous dose of emetine hydrochloride (10 mg./kg.).

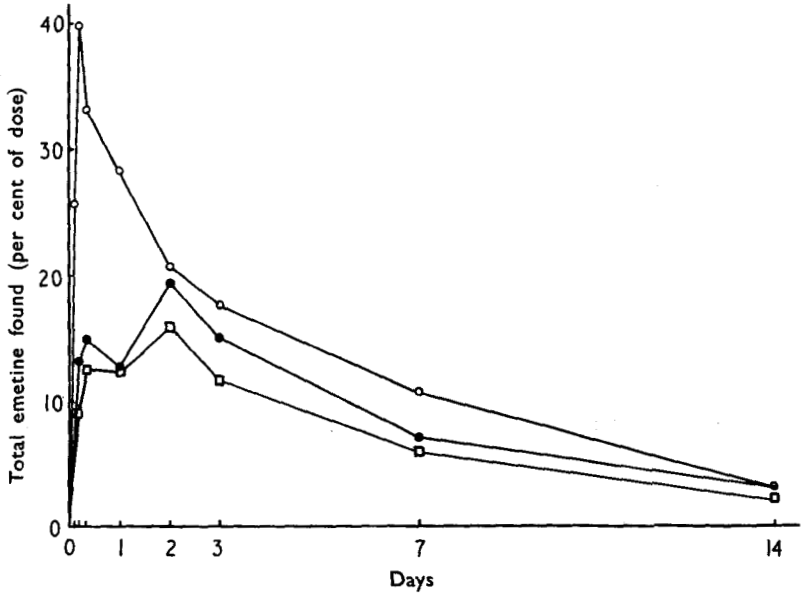

FIG. 1. Total amounts of emetine found in the heart, kidneys, liver, lungs and spleen of female rats at various times after single doses of the hydrochloride or bismuth iodide complex of emetine.

—○— emetine hydrochloride (10 mg ≡ 7·1 mg base/kg.)—subcutaneous
—●— emetine hydrochloride (10 mg. ≡ 7·1 mg. base/kg.)—oral
—□— emetine bismuth iodide (30 mg. ≡ 8·4 mg. base/kg.)—oral
(values are means for groups of 3 rats)

The results reported here agree well with those of Parmer and Cottrill (1948) and are in general agreement with those of Gimble, Davison and Smith (1948). When differences exist, they probably derive from the use of different species of animal and different routes of administration: Parmer and Cottrill used rabbits and intramuscular injection; Gimble, Davison and Smith used rats and the intraperitoneal route.

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