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DISTRIBUTION, METABOLISM AND EXCRETION OF PERCUTANEOUSLY ADMINISTERED DITOPHAL ('ETISUL')

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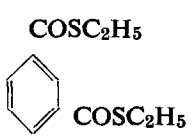
Abstract—When ^{35}S-labelled ditophal is rubbed into the skin it is rapidly distributed throughout the body via the circulating blood and radioactivity is concentrated in the skin, both at the site of application and elsewhere. In patients living in a tropical climate, ditophal is largely eliminated from the body in the sweat, together with volatile metabolic products. Urinary metabolites include small amounts of ethyl methyl sulphone and ethyl methyl sulphoxide and much larger amounts of inorganic sulphate and a product which yields sulphate on fairly vigorous acid hydrolysis; the urine probably also contains a little unchanged ditophal.

IN A previous paper[1] the absorption of ditophal (diethyl dithiolisophthalate, 'Etisul'‡) administered by rubbing into the skin was measured. Ditophal was labelled with



Ditophal

^{35}S and the excretion of radioactivity by various routes was determined. This paper extends these observations and gives evidence for the distribution of ditophal and its metabolites in blood and skin, and provides some indication of the metabolic fate of the drug.

METHODS

The preparation and administration of ^{35}S-labelled ditophal and the collection of urine and sweat have been described previously.[1] In these experiments the area used for the collection of sweat was increased from 280 to 325 cm^2.

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‡ 'Etisul' is a trade mark, the property of Imperial Chemical Industries Limited.

Collection and treatment of samples

Blood. Venous blood samples (2 to 5 ml) were diluted with water (2·5 ml) and extracted first with toluene (2 × 5 ml) and then with chloroform (3 × 15 ml). The extracted blood was oxidised by Benedict's method[2] and barium sulphate was precipitated for assay. The radioactivity of the extracts was determined separately but since the degree of extraction varied considerably the results were summed and presented as total radioactivity in the blood. Results were expressed in terms of the whole volume of body blood, which was taken to be one tenth of the body weight.

Biopsies. Specimens obtained by use of a punch were placed immediately in toluene (2 ml) and sealed in ampoules. Before assay each specimen was removed, measured and weighed (wet with toluene) and then transferred with the toluene to a conical Griffiths tube. The specimen was homogenised after addition of water (2 ml) and the mixture centrifuged. The toluene layer was removed and the material further extracted with chloroform (3 × 2 ml), the layers being separated by centrifugation. The residual tissue was resuspended in the aqueous extract, oxidised by Pirie's reagent[3] and the sulphate was precipitated with barium chloride. The total radioactivity of each sample was calculated by summation of the radioactivity of both the extracts and the oxidised residue.

Washings. The inuncted areas were washed with soap solution 1 hr after application, and the radioactivity of the washings determined.[1] Not more than 5 per cent of the administered radioactivity was recovered in the washings. The absorbed dose was calculated by subtracting radioactivity found in the washings from the amount applied to the inuncted area.

Radioactive counting. Blood and biopsy samples were counted as before.[1] Other measurements were made with a Tri-Carb Model 314 EX Liquid Scintillation Spectrometer (Packard Instrument Ltd., Wembley) in 20 ml vials of low potassium content. The counts were made at 0° with a 1045 volt setting and discriminator set at 10–100. Counting efficiency was determined by the addition of a solution of $C_6H_5{}^{14}COOH$ in toluene.

Aqueous barium sulphate suspensions and aqueous solutions from sweat, urines and washings were prepared for counting in the following manner. The aqueous solution or suspension (3 ml) was shaken vigorously with phosphor solution (17 ml) containing silica gel. This was prepared by adding Degussa 'Aerosil' (50 g) to 1 l. of a solution containing naphthalene (100·4 g), 2 : 5-diphenyloxazole (10 g) and 1 : 4-bis-2-(5-phenyloxazolyl)-benzene (0.25 g) in dioxane. The mixture formed a rigid gel. Maximum counting efficiency was 64 per cent; efficiencies as low as 12 per cent were obtained with the acidic supernatants from hydrolysed urine samples.

Toluene and chloroform extracts were treated as before[1] and the dried toluene solution (15 ml) added to a phosphor solution (5 ml) containing 2 : 5-diphenyloxazole (16 g) and 1 : 4-bis-2-(5-phenyloxazolyl)-benzene (0·4 g) in toluene (1 l.). To avoid giving counting errors on individual results in all tables, the errors for selected activity levels in blood, biopsy, sweat and urine samples are shown in Table 1. All figures have been corrected for the natural decay of ^{35}S.

Examination of ^{35}S-labelled products in sweat

Combined toluene extracts (2·5 l. containing 450 mμC) from sweat pads from patients who had been inuncted with ^{35}S ditophal were mixed with 0·5 g unlabelled

ditophal. The solution was distilled through a Dufton column and distillate fractions (50 ml) collected in ice-cooled receivers. The first fraction contained 57 per cent of the radioactivity. Subsequent fractions contained very little radioactivity. To 25 ml of the first fraction, diethyl disulphide (0.1 ml) and ethyl mercaptan (0·15 ml) were added. The mixture was warmed to 50° and nitrogen was passed slowly through it for 25 hr. The issuing gas was bubbled through a 4 % aqueous solution of mercuric chloride (25 ml). The collecting solution was kept 2 days and then centrifuged. The precipitate was washed with cold water (2 × 10 ml) and dried over P_2O_5 to give a pale grey powder (0·30 g) which was assayed for radioactivity. Assuming that the mercury complex formed was $C_2H_5SHgCl.HgCl_2$ (Challenger[4]) recoveries were: by weight 16 per cent, radioactivity 17·5 per cent. The residue from the original distillation was further distilled at 15 mm from a water bath at 80°C. The residue (0·48 g) was dissolved in light petroleum (b.p. 40–60°C) (15 ml) and centrifuged to remove a little flocculent material. The solvent was evaporated and the residue crystallized repeatedly from light petroleum (b.p. 30–40°C) at low temperature to constant melting point (−1°C) and specific activity (0·40 mμC per mg).

TABLE 1. COUNTING ERRORS IN RADIOACTIVITY MEASUREMENTS

Figures show percentage errors on results to 95 per cent confidence limits for different levels of radioactivity in samples.

	Sample													
	Whole body blood (μC)				Biopsy Specimens (mμC/cm²)		Sweat (mμC/325cm²)					Urine Total excretion mμC		
Level of Radioactivity	2·0	1·5	0·7	0·02	8·5	4·5	224	117	20	10	2	400	250	100
% Error ±	6	8	10	30	4	5	2	4	5	7	10	5	7	9

Determination of the ^{35}S present in urine as inorganic sulphate and as total sulphate

Radioactive sulphate in the urine was precipitated as barium sulphate after the following treatments:

(a) A standard clinical method[2] for 'Inorganic sulphate' determination, in which the final acid concentration was 0·14 N and the solution was kept overnight at room temperature.

(b) A standard clinical method[2] for 'total sulphate' in which mild hydrolysis of the sulphate esters was carried out in a final concentration of 0·14 N acid by immersion in a boiling water bath for 20 min.

(c) A vigorous hydrolysis in 0·8 N acid by heating the solution for 4 hr in a boiling water bath or for 5 hr under reflux (in the method used before,[1] heating was for 2 hr in a boiling water bath).

In every case, inactive sulphate was added as a carrier before precipitation of barium sulphate for counting. After vigorous hydrolysis and precipitation of sulphate the radioactivity of the supernatant solution was also measured.

Counter-current separation of urine extracts

Chloroform extracts from the urine of patients A–D were combined and concentrated to 80 ml at 35°C under nitrogen, and placed in the first and second tubes of a counter-current distribution machine. The remaining forty-eight tubes each received 40 ml chloroform saturated with water. Fifty-six transfers were carried out with water saturated with chloroform (40 ml per tube) as upper phase. The contents of every second or third tube were removed, and the chloroform evaporated off at 40–50°C under nitrogen in the presence of the aqueous phase. After measuring the volume of aqueous solution remaining, an aliquot (2·1 ml) was mixed with 'Diotol' phosphor solution (Herberg[5]) and counted by the liquid scintillation method (counting efficiency 38 per cent).

RESULTS

^{35}S in tissues

Blood. The data shown in Table 2 were obtained from the patients who were studied before.[1] Radioactive products appeared in the blood soon after inunction and

TABLE 2. TOTAL RADIOACTIVITY IN BLOOD FROM PATIENTS RECEIVING ^{35}S-LABELLED DITOPHAL

Figures show μC in whole body blood

Patient	A	B	C	D	E	F	G
Dose absorbed μC	61·4	62·3	61·8	34·9	39·7	44·8	43·0
Time after dose (hr)							
½	2·05	0·69	0·25	0·80			
2	0·96	2·16	2·12	1·23	1·70	0·57	0·69
4					0·86		0·36
6					0·38	0·51	0·70
8	0·60	1·16	0·79	0·57			
9					0·48	0·62	0·13
12					0·52	0·22	0·36
20					0·34	0·35	0·69
24	0·39	1·56	0·53	0·58			
28					0·17	0·47	0·47
36					0·46	0·58	
48					0·20	0·72	0·27
72	0·56	0·70	0·27	0·96			
89 days					0·02	0·02	
105 days							0·02

probably reached their highest level at a time varying from ½ to 2 hr. From 8 hr to 3 days after inunction the levels fluctuated, but showed little overall decline. Small, but measurable amounts of ^{35}S were found in the blood of three patients 12–15 weeks after inunction.

The results may be seen more clearly in Fig. 1 which shows the mean precentage of absorbed radioactivity in the blood at various times after dosing. The two curves differ in height, as might be expected in such small groups, but the general pattern is the same for both.

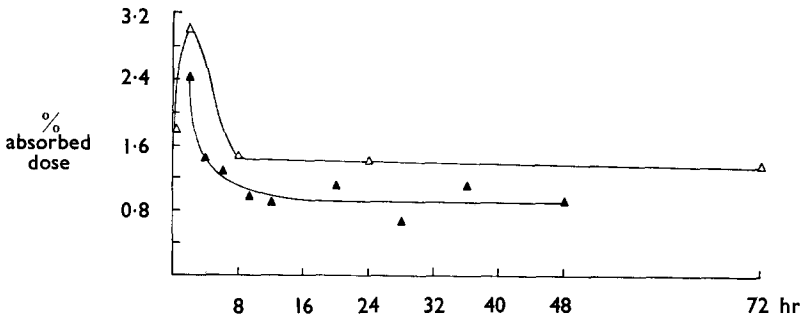

FIG. 1. Total radioactivity in blood from patients receiving ^{35}S-labelled ditophal plotted as per cent of absorbed dose. Curves show mean values for two sets of patients. (△, A-D; ▲, E-G.)

Skin. Results from a limited number of biopsy samples provided some indication of the amount of radioactivity present in the skin. Only a few patients were available and it would have been unreasonable to have taken serial biopsies from the same patient. The area and the thickness of the samples varied considerably and the results that have been obtained are therefore only very roughly quantitative. The level of radioactivity in the skin was high and showed no consistent change between 4 and 28 hr after dosage (Table 3); the level in biopsies from areas of skin to which ditophal had not been applied was only a little lower than that in biopsies from the inuncted areas of the same patient.

TABLE 3. RADIOACTIVITY IN BIOPSY SPECIMENS FROM PATIENTS RECEIVING ^{35}S-LABELLED DITOPHAL

Time after inunction (hr)	Patient	Inuncted area				Uninuncted area			
		Biopsy		^{35}S		Biopsy		^{35}S	
		Wt. (mg)	Area (mm^2)	Total (mμC)	mμC per cm^2	Wt. (mg)	Area (mm^2)	Total (mμC)	mμC per cm^2
4	E	32	12	0·89	7·4	22	12	0·69	5·7
8	F	10	6	0·45	7·5	26	15	0·67	4·5
28	G	30	6	0·52	8·7	10	4	0·34	8·5

^{35}S *Excretion*

Sweat. The previous study[1] of the excretion of radioactivity in sweat has now been extended to include nine other patients and samples have been collected over longer periods (Table 4). Large amounts of radioactivity can be excreted in sweat during the first day. Most of this excretion probably occurs within the first 8 hr and the peak may sometimes come in the first 4 hr. In some patients the maximum output may have occurred before collection began.

Urine. In the latest series of patients only the total output of radioactivity over the first 24 or 48 hr was measured (Table 5). This was much lower than for an earlier group of patients[1] whose urinary excretion was studied in more detail.

Nature of the excretion products

In sweat. In the present experiments most of the radioactivity found in sweat was extractable into toluene. More than half was easily volatile and after the addition of carriers could be trapped as a mercury complex. Although the recovery of radioactivity in the complex was low, it tallied with the weight recovery, and the results were consistent with the volatile fraction being ethyl mercaptan or diethyl disulphide or a mixture of both.

TABLE 4. RADIOACTIVITY OF SWEAT FROM PATIENTS RECEIVING ^{35}S-LABELLED DITOPHAL

Figures show total radioactivity in mμC from 325 cm^2 skin.*

Time of collection	4–8 hr (a)	8–26 hr (b)	4–26 hr (a + b)	1¾–24 hr	1–2 days
Patient					
J	16	17	33		10
K	127	11	138		5·7
L	8·6	23	32		4·1
M				83	3·7
N				150	7·3
O				216	24
P				117	2·8
Q				224	5·7
R				203	4·7

	Mean values 1st day	2nd day
% ^{35}S in sweat extractable with toluene	90	77
% ^{35}S in sweat extractable with $CHCl_3$	2	3
% ^{35}S in sweat not extractable	8	20

* Area of pad increased from 260 cm^2 used in studies described in the first paper

TABLE 5. RADIOACTIVITY IN URINE FROM PATIENTS RECEIVING ^{35}S-LABELLED DITOPHAL

Figures show total excretion during the period of collection.

Period of collection (hr)										
24	Patient	S	T	U	V	W				
	Absorbed dose μC	49·4	49·7	48·9	48·5	49·1				
	^{35}S in urine mμC	153	308	191	141	329				
48	Patient	J	K	L	M	N	O	P	Q	R
	Absorbed dose μC	47·8	47·8	46·3	52·3	52·2	52·4	52·0	52·0	52·2
	^{35}S in urine mμC	340	413	174	132	393	224	256	110	405

The relatively non-volatile material in sweat was shown to be mainly ditophal by addition of inactive carrier, crystallization, and measurement of specific activity.

Negligible amounts of radioactive materials extractable with chloroform but not extractable with toluene were excreted in sweat.

In urine. Total urine samples collected during the first 1 or 2 days after dosing with labelled ditophal were extracted with toluene and with chloroform and the radioactivity present as inorganic sulphate was measured; it averaged 52 per cent of the

radioactivity present in the extracted solution (Table 6). Hydrolysis under conditions commonly used to release sulphate from 'etheral sulphate' only slightly increased the proportion of radioactivity precipitable as barium sulphate. More vigorous hydrolysis however liberated all the radioactivity as precipitable sulphate.

TABLE 6. RADIOACTIVITY AS SULPHATE IN THE URINE OF PATIENTS RECEIVING ^{35}S-LABELLED DITOPHAL

Figures show radioactivity as per cent of the total extractable ^{35}S, figures in parentheses show errors (±) to 95 per cent confidence limits.

Urine treatment: Concentration of HCl (N)	0·14	0·14	0·8	0·8	
Urine treatment: Temperature	20°	96–100°	96–100°	Reflux	
Urine treatment: Time	18 hr	20 min	4 hr	5 hr	
	Inorganic sulphate	'Total Sulphate'			Radioactivity not precipitable as sulphate after treatment c or d
Patient	a	b	c	d	
J	33 (5)		99 (3)		0 (4)
K	42 (5)		84 (3)		17 (12)
L	42 (5)		78 (5)		21 (15)
M	61 (6)		90 (5)		10 (7)
N	62 (6)		85 (5)		14 (10)
O	62 (6)		86 (5)		14 (10)
P	48 (5)		100 (3)		0 (4)
Q	51 (5)		100 (3)		0 (4)
R	43 (5)		85 (5)		13 (10)
S to W	56 (2)	62 (3)		100 (4)	0 (5)

The small fraction of urinary radioactive metabolites extractable into chloroform after a preliminary extraction with toluene was examined by distribution between chloroform and water in a counter-current extraction apparatus, using pooled material from many urine samples. Radioactivity was found in three peaks, with K values corresponding to ditophal, ethyl methyl sulphone and ethyl methyl sulphoxide in the ratios of 1 : 0·8 : 0·3 by weight (Fig. 2).

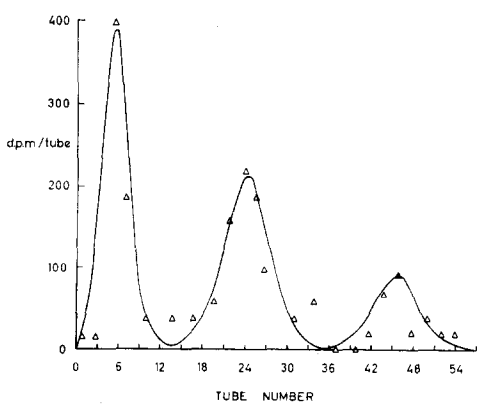

FIG. 2. Counter-current distribution of radio active products from chloroform-extractable fraction of urine from patients receiving ^{35}S-labelled ditophal. Points (Δ) show measured radioactivity. The curve shows theoretical distribution for ditophal K, 0·082, ethyl methyl sulphone K, 0·75 and ethyl methyl sulphoxide K, 4·9 in the ratio 1 : 0·8 : 0·3 by weight. Solvent system $CHCl_3$–water.

DISCUSSION

The rapid absorption of ditophal after inunction shown by measurements on washings from the inuncted site[1] is confirmed; it is also demonstrated by measurements in blood where radioactivity from the drug probably reaches its highest level in ½–2 hr. The subsequent maintenance of a lower, fairly steady level for several days agrees with the continuing and only slowly declining excretion in breath and urine over the same period, and the long persistence of radioactivity previously found in sweat and urine is also seen in the blood.

The results on biopsy specimens, although scanty, show that the whole skin is an important reservoir for the labelled drug and its metabolites. Radioactivity in uninuncted areas quickly reaches a similar concentration to that found in areas where the drug was applied. Jamison and Palmer[6] in autoradiographic studies on biopsies from patients who had received ^{35}S-labelled ditophal found considerable penetration below the site of inunction at three hours, the shortest time investigated. He suggested that some of the compound enters the lower layers of the skin by passing down around the hair shafts. He found considerable radioactivity in the skin at 25 hr, especially in the cells and lumen of the sweat gland ducts. One biopsy specimen was taken from an uninuncted arm 48 hours after dosage with ^{35}S-ditophal. This tissue also showed radioactivity mainly in cells surrounding the neurovascular bundles. Jamison's results underline the importance of the skin as a site of storage of ditophal or its breakdown products.

In our present experiments sweat losses were higher than those previously reported.[1] The highest excretion occurred soon after administration of the drug. Most of the ditophal administered to patients in a hot and humid environment may be excreted through the skin either in the sweat or as volatile metabolic products which are difficult to trap effectively.

The reason for the much smaller urinary excretion of radioactivity than previously found[1] is not certain, but may be correlated with the greater sweat losses. The distribution of the drug can evidently vary markedly from one occasion to another, and this may have an important bearing on its therapeutic effect.

Ditophal in man is probably absorbed unchanged and is carried partly in the same form in the blood, where it contributes to the early high levels of radioactivity observed with the labelled drug. From the blood it passes preferentially to the skin and is excreted, still unchanged, in the sweat at parts of the body remote from the site of application. The drug is also metabolized, probably initially by hydrolysis of the thiolester groups to ethyl mercaptan; indeed this change is the presumed basis of its therapeutic action. Such a break-down is indicated by the appearance in the breath[1] and sweat of volatile compounds, probably ethyl mercaptan and/or diethyl disulphide. In urine the small amount of radioactivity extractable by toluene may represent unchanged drug or volatile active metabolites, but the main metabolic products are therapeutically inert. One important urinary metabolite in man as in animals is inorganic sulphate. A second, varying in amount from one patient to another, is a product which liberates sulphate after vigorous acid hydrolysis. It seems to be a direct metabolite of the administered ditophal, and quite distinct from the 'ethereal sulphate' normally found in urine. Some labelling of the ethereal sulphate fraction would be expected, but its radioactivity should not exceed 15 per cent of that of the inorganic sulphate, whereas the amount of labelled sulphate liberated on vigorous hydrolysis is

sometimes more than the labelled inorganic sulphate present in the same sample. Moreover, the normal ethereal sulphates are hydrolysed by quite mild acid treatment whilst the metabolic product requires considerably more vigorous treatment. This product is responsible for the earlier report[1] of the presence of a non-extractable radioactive urinary metabolic fraction other than sulphate; this could evidently have been converted to sulphate if the hydrolysis had been more prolonged. Figure 1 of that paper shows that the firmly bound sulphate can in some patients form the main urinary excretion product in the first few days after dosing, but that at later times (8–9 days) the excretion is mostly as inorganic or easily hydrolysable sulphate. About 3 per cent of the total radioactivity in the urine is extractable into chloroform after a preliminary extraction with toluene.[1] This extract has been examined by counter-current distribution. Although partition coefficients of the separated components do not establish their nature unequivocally, they strongly suggest that one component is unchanged ditophal which escaped extraction with toluene, and that the other labelled metabolites are ethyl methyl sulphone and ethyl methyl sulphoxide. Both are known metabolites of ethyl mercaptan in mouse and guinea pig[7, 8] though in these animals the sulphone is a relatively important urinary metabolite and the sulphoxide is a minor component. Extracts from human urine do not show the unknown metabolite ($K = 2{\cdot}70$ between chloroform and water) which has been found in the animal urines. Some labelled sulphate was found in sweat, especially at times after the first day.

The tendency of ditophal and its metabolites to concentrate in the skin may well be of significance in relation to its therapeutic effect in leprosy, and Jamison[6] has shown that labelled material from the drug accumulates preferentially in and around the adventitial cells characteristic of leprosy in the skin. Losses of drug through the skin may thus have an important bearing on the effectiveness of its use in the treatment of leprosy. If the patients could be kept in an environment sufficiently cool to avoid sweating, the drug might be retained longer and at higher concentrations in the body, and this should enhance its effectiveness.

The information collected in these experiments unfortunately does not permit any firm conclusions to be reached on the optimum frequency of dosing of ditophal. The highest levels of drug probably occur in a surge during the first few hours after dosing. After the first 12 hr the level of radioactive products derived from the labelled drug appears to be fairly steady for at least three days as judged by blood levels and the rate of excretion in breath and urine. However the therapeutic effectiveness of the drug over this period will depend on the extent of its metabolic breakdown and possibly on the location of the drug and its products in the body; these factors are as yet undetermined.

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