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PERCUTANEOUS ABSORPTION AND ROUTES OF EXCRETION OF DITOPHAL (ETISUL)

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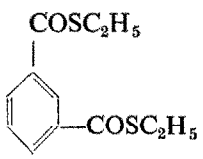
Abstract—When ^{35}S-labelled ditophal is rubbed into the skin about 90 per cent of the drug is absorbed in 1 hr. Excretion of ^{35}S occurs in urine, faeces and sweat and less so in breath. The greatest rate of excretion is at 6–24 hr after inunction, but excretion continues for several weeks. The principal forms of excretion are toluene-extractable material and sulphate.

DITOPHAL (diethyl dithiolisophthalate, Etisul†) is the most active member of a series of derivatives of ethyl mercaptan in protecting mice against acute experimental tuberculosis.[1]



Etisul

It is used effectively in leprosy[2–3] and Lupus vulgaris.[4] Since the usual routes of administration are unsatisfactory ditophal is rubbed into the skin, and has both a local and a general effect. Leprous lesions remote from the site of inunction respond satisfactorily to the treatment. In Lupus vulgaris some patients with bilateral lesions were inuncted on one side only; lesions cleared on both sides, although the inuncted side healed more quickly.

The fate of ditophal and some other esters of ethyl mercaptan has been studied in the mouse, guinea pig and rabbit.[5–6] The compounds were administered orally or by subcutaneous injection and were rapidly eliminated together with their metabolites. Most of the dose was accounted for, and the nature of the principal metabolites of ethyl mercaptan in these animals was elucidated.

Attempts to follow the metabolism of ditophal in man by chemical methods failed. The drug was therefore labelled with ^{35}S and radioactivity measurements were used to

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

follow its excretion. ^{35}S is an isotope with a half-life short enough to limit the radiation received even if it were retained in the body.

METHODS

Synthesis of ^{35}S-labelled ditophal

^{35}S-Thiourea (0·76 g, 11·1 mc) was refluxed for 1½ hr with ethyl iodide (1·5 ml) and dry acetone (15 ml). Volatile materials were then removed under vacuum, the resultant S-ethyl*iso*thiouronium iodide was dissolved in air-free water (4 ml) and the solution cooled to −70°. The vessel containing this cooled solution was connected to a second one also at −70 °C, and the whole apparatus was evacuated. Air-free sodium hydroxide solution (10 ml, 2·38 M) was added to the first vessel which was then warmed to 90 °C. Ethyl mercaptan and water distilled over into the second vessel and the transfer of the mercaptan was completed by further additions of water (2 × 5 ml) to the warmed residue in the first vessel. Aqueous potassium hydroxide (2·5 ml, 5 N) and isophthalic acid chloride (1·02 g) in toluene (10 ml) were added to the cooled receiver which was then allowed to warm. The liquified phases were vigorously stirred for 2 hr at 10 °C. The toluene phase was separated, dried and evaporated to give crude ditophal (1·00 g, 8·66 mc) m.p. −15 °C. Inactive ditophal (6·9 g) was added and the product was crystallized three times from light petroleum (30–40 °C) at −70 °C to constant m.p. (0 °C) and specific activity (0·90 mc/g). Yield 6·28 g. Labelled ditophal was suitably diluted with unlabelled drug and 4% of perfume (A 801, Lautier Fils) was added.

Doses

About 5 g, containing a known amount of radioactivity, was applied in 1 ml portions to the chest and rubbed in by the patient's hand until the liquid disappeared. With patient *H* the drug was applied by brush during 1 hr.

Collection of samples

Washings from inuncted area. One hour after inunction, the patients washed the inuncted area with cotton-wool swabs dipped in water containing non-ionic detergent (Triton X 100) (patients *A–D*) or soap (patients *E–H*). The water washings and swabs were repeatedly extracted with toluene; levels of the radioactivity in successive extracts demonstrated the effectiveness of the extraction procedure; ^{35}S sulphate was measured in the toluene-extracted washings from patients *E–H*.

Breath. An absorption column 25 × 1·6 cm packed with a mixture of broken porcelain and pumice was wetted with toluene from a tap funnel. A trap cooled to −70 °C at the outlet caught any material escaping absorption. The patient breathed through the column for five 1-min periods, with 1 min rest between each. The column and trap were finally washed out with toluene for radioactivity estimation. The whole apparatus was in glass and polythene.

Faeces. Complete stool collections were made from patients *A*, *B* and *D* for days 0–5 and 8–9, although not all patients were able to pass a stool each day. Each daily collection was homogenized with water to a total volume of 1·5 l. Aliquots (5 or 10 ml) were extracted with toluene (2 × 5 ml) and then with chloroform (3 × 15 ml). The extracted homogenates were centrifuged and the supernatant liquors oxidized

by Benedict's method.[7] Sulphate was precipitated as barium sulphate after adding a little inactive carrier. An aliquot of each homogenate was also dried to constant weight.

Urine. Patients passed urine immediately before inunction. Thereafter complete urine collections were made at intervals during 3–14 days. Twenty-four-hour urine collections were obtained from some patients on isolated days as long as 5 weeks after inunction. The completeness of these collections was checked by the estimation of creatinine.[8] An aliquot (5 or 10 ml) of each sample was extracted with toluene (2 × 5 ml) and then with chloroform (3 × 15 ml). The extracted urine was oxidized by Benedict's method[7] (patients *A–D*) or with Pirie's reagent[9] (patients *E–H*), and sulphate precipitated as barium sulphate. A second aliquot (5–10 ml) was mixed with half its volume of hydrochloric acid (2 N), heated for 2 hr at 100 °C and the sulphate precipitated.

Sweat. Weighed pads of "Kleenex" paper tissues, area 260 cm^2 were covered with polythene sheets and strapped to the backs of the patients. After varying time intervals they were removed, quickly weighed and sealed into polythene bags. The bags were later opened at one corner, and thoroughly wetted with a mixture of toluene and water. The pads were cut into small pieces and extracted by shaking with toluene; the mixture was separated centrifugally. After three further extractions with twice its volume of chloroform, portions of the aqueous layer were oxidized or hydrolysed and sulphate precipitated.

Radioactive counting

Most measurements were made on the Ecko liquid scintillation counter; a few were made with an end-window Geiger–Müller tube. Toluene extracts were dried overnight over sodium sulphate; the volume was made up to 20 ml with toluene containing *p*-terphenyl and 1:4-bis-2-(5-phenyloxazolyl)-benzene as phosphors;[10] the counting efficiency was 48 per cent for ^{35}S. Chloroform extracts were carefully evaporated to dryness at 30–35 °C in a slow stream of nitrogen. The residue was dissolved in toluene, and the solution dried and assayed as before. Internal standards of ^{14}C were occasionally included to check the counting efficiency of the colourless extracts. Coloured extracts (mainly those from faeces) were always counted with and without internal standards and corrections applied for the reduction in counting efficiency. Barium sulphate precipitates were washed, dried and homogenized in a Griffiths tube with the phosphor solution containing 'Thixcin' (Baker Castor Oil Co., New York). The stable thixotropic suspension was assayed with a counting efficiency of 40 per cent.[11] Counting times were fixed according to the type of material being counted and the level of activity. To avoid giving counting errors on individual results in later tables the errors for selected activity levels with different extracts are shown in Table 1. All figures presented in the tables have been corrected for the natural decay of ^{35}S.

RESULTS

Absorption

The results (Table 2) show that for seven patients absorption varied from 79 to 95 per cent. Patient *H* showed a rather poorer absorption and some breakdown products of the drug were found in the wash water.

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	Level of radioactivity in sample	% Errors	
		Toluene and chloroform extracts	$BaSO_4$ precipitates
Washings	(μc)		
	10	2	
	4	3	
	3·5		1·5
Faeces	(mμc)		
	4000	3	
	1000	4	
	400	2	
	100	12	16
	50	20	30
	20		60
Urine	(mμc)		
	400	5	7
	100	10	13
	10	25	35
	3	50	65
Sweat	(mμc)		
	5	2	3
	1	8	10
	0·1	30	40

For breath the sampling errors much outweighed counting errors.

TABLE 2. DESCRIPTION OF PATIENTS, AND RADIOACTIVITY OF WASHINGS FROM SITE OF INUNCTION WITH ^{35}S-LABELLED DITOPHAL

Patient			Labelled ditophal administered		Radioactivity in washings		
Reference	Weight (kg)	Duration of ditophal treatment (months)	Weight (g)	Activity (μc)	Toluene-extractable (μc)	As sulphate (μc)	Total as % dose given
A	73	>12	5·0	65·3	3·8	Not estimated	6
B	68	>12	5·0	65·3	3·0	Not estimated	5
C	51	2	5·0	65·3	3·5	Not estimated	5
D	63	>12	5·0	37·6	2·7	Not estimated	7
E	61	1	5·2	50	10·3	0	21
F	65	6	5·2	50	5·2	0	10
G	68	5	5·2	50	7·0	0	14
H	66	12	9·1	66	11·4*	7·5*	29*

* Drug applied with brush. Recovery includes radioactive material wiped off with dry cotton wool.

Excretion

Breath. The patterns of excretion of three of the four patients examined (*A*, *C* and *D*) were very similar (Table 3). The maximum rate of excretion was seen in the 8¼-hr collection. By 24 hr the rate had declined but thereafter the decline was slower. The results from patient *B* were rather erratic. The cumulative excretion of compounds in the breath during the first 8 days after inunction was calculated to be between 0·8 and 2·3 per cent of the absorbed dose.

TABLE 3. RADIOACTIVITY IN BREATH OF PATIENTS RECEIVING ^{35}S-LABELLED DITOPHAL

(Figures show mμc excreted per hr.)

Time after inunction (hr)	Patient Dose absorbed (μc)	*A* 61·4	*B* 62·3	*C* 61·8	*D* 34·9
¾		2·1	2·8	2·5	1·0
2¼		6·8	3·4	3·3	1·0
4¼		11	0·5	Lost	8·0
8¼		14	2·8	8·5	17
24¼		7·1	0·7	4·1	Lost
72		7·0	3·7	4·0	4·2
192		5·3	2·0	2·3	1·5

Faeces. Most of the ^{35}S detected in the faeces was extractable into chloroform (Table 4). For two of the three patients the faecal excretion was highest in the first stools passed after the day of inunction and fell to a low level by the ninth day, but for patient *B* there was a lower but fairly steady excretion showing only a slight decline on day 9. With all three patients the decline in the excretion of chloroform-extractable and non-extractable ^{35}S was quite small between days 2 and 9. The output of ^{35}S in the faeces for days 5–8 was interpolated assuming a steady decline during this time, and from these figures the total faecal excretion over 8 days was estimated to range from 6–19 per cent of the absorbed dose.

Urine. Table 5 shows results from two groups of patients. In patients *A–D* the excretion of ^{35}S rose to a maximum rate some time between 6 and 24 hr. Thereafter the rate declined to about a third of the peak value by the third day. Patients *E–H* were studied for a longer period. Excretion was continuing at the eighteenth day; the cumulated total urinary excretions of ^{35}S in Table 5 are expressed as per cent of the absorbed dose; the figures are calculated assuming a steady decline between any days for which no sample was examined. Since the ratio of the cumulative excretions at 8 days to those at 3 days was similar for patients *E* to *H* (mean value 1·5) it was reasonable to apply the same ratio to calculate the 8-day excretions of patients *A–D*.

The toluene- and chloroform-extractable fractions accounted for about 3 and 4 per cent, respectively, of the total urinary radioactivity, although for patients *F* and *H* they represented larger fractions of the total as time went on. The ^{35}S sulphate (inorganic and esterified) measured in the urine from patients *A* and *B* suggested there was some non-extractable ^{35}S other than sulphate. Measurement of both non-extractable ^{35}S and ^{35}S sulphate was therefore made for all samples from patients of

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

Results in mμc,(*T* = toluene extract, *C* = chloroform extract, *R* = residue after extraction, W/t = dry weight of faeces)

Time after inunction (days)	Patient and absorbed dose											
	A 61·4 μc				*B* 62·3 μc				*D* 34·9 μc			
	Wt. (g)	*T*	*C*	*R*	Wt. (g)	*T*	*C*	*R*	Wt. (g)	*T*	*C*	*R*
0–1	35·0	0	Lost	14	33·7	0	0	24	11·9	0	0	0
2–3	25·4	4000	80	100	67·6	450	100	110	12·3	2450	150	68
3–4	30·5	1850	250	16	67·8	300	200	20	46·6	1150	100	26
4–5	39·8	1050	220	32	89·4	500	200	17				
8–9	39·3	0	200	36	62·2	350	50	34	123·5	400	100	76

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

Results in mμc, *T* = toluene extract, *C* = chloroform extract, *R* = residue after extraction, *S* = sulphate, *L* = lost sample.

Time after inunction	Patient and absorbed dose															
	A, 61·4 μc				*B*, 62·3 μc				*C*, 61·8 μc				*D*, 34·9 μc			
	T	*C*	*R*	*S*	*T*	*C*	*R*	*S*	*T*	*C*	*R*	*S*	*T*	*C*	*R*	*S*
0–2	14	33	70	17*	6	13	45	3*	0	4	60		4	14	200	
2–4	2	5	32	100	8	8	34	11	9	11	30		3	2	38	
4–6	8	16	420	60	3	6	32	15	3	4	46		6	10	60	
6–9	5	4	140	60	7	4	110	50	11	10	160		3	6	210	
9–12	29	5	230	120	9	4	90	60	24	33	420		32	21	810	
12–24	70	28	1300	530	31	30	570	500								
24–36	21	*L*	750	410	18	16	210	130	22	23	310		12	19	230	

TABLE 5—*continued*

36–48	7	20	400		2	13	200	100	1	9	370		5	2	160	
48–72	60	1	820	310	22	17	1030	220	34	10	410		17	53	290	
(days)		*E*, 39·7 μc				*F*, 44·8 μc				*G*, 43·0 μc				*H*, 47·1 μc		
0–½	2	1	170	260	0	12	240	250	24	29	350	60	9	8	280	270
½–1	0	2	410	160	8	16	240	290	14	23	400	43				
1–2	4	14	450	100					13	32	590	0	6	10	290	270
2–3	3	8	440	40	6	20	160	110	11	61	490	160	6	5	140	150
3–4	9	27	90	25	8	20	130	150	4	46	250	150	7	11	80	90
4–5	5	*L*	*L*	*L*	10	12	140	50	14	52	220	190	2	13	60	48
5–6	0	0	120	160	3	6	110	150	2	28	220	150	4	12	60	60
6–7	3	6	*L*	45	9	16	90	100	8	22	80	280	4	19	31	55
7–8	4	1	160	80	5	11	*L*	*L*	3	14	130	180	8	13	19	35
8–9	3	1	27	140	7	4	35	33	2	10	130	200	5	10	11	17
9–10	0	1	170	21	7	7	60	0					7	7	9	26
10–11					4	9	41	0					7	7	0	23
11–12	2	5	80	18	6	12	90	0					4	7	4	13
12–13													2	11	6	19
13–14													3	5	10	18
14–15	0	0			0	10	14	12	0	4	26	90				
17–18	1	0	70	80	11	0	12	37	3	0	28	25				

* Sulphate from patients *A* and *B* counted on planchettes with an end-window counter.

Total estimated cumulative urinary excretions as % absorbed dose.

Patient	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*
3 days	7·3	4·1	3·2	6·3	3·8	2·2	4·7	1·6
8 days	(11·0)	(6·1)	(4·8)	(9·5)	5·5	3·7	7·3	2·3
18 days					7·6	4·9	8·6	2·6 (14 days)

Figures in parentheses are extrapolated.

the second group (*E–H*). Fig. 1 shows the pattern of cumulative excretion for patients *F* and *G* who showed the greatest differences. The cumulative sulphate and non-extractable ^{35}S excretions of patient *F* were similar, whilst with patient *G* there was a wide discrepancy during the first 3 days. By the ninth day all the ^{35}S not extractable into solvents was recoverable as sulphate.

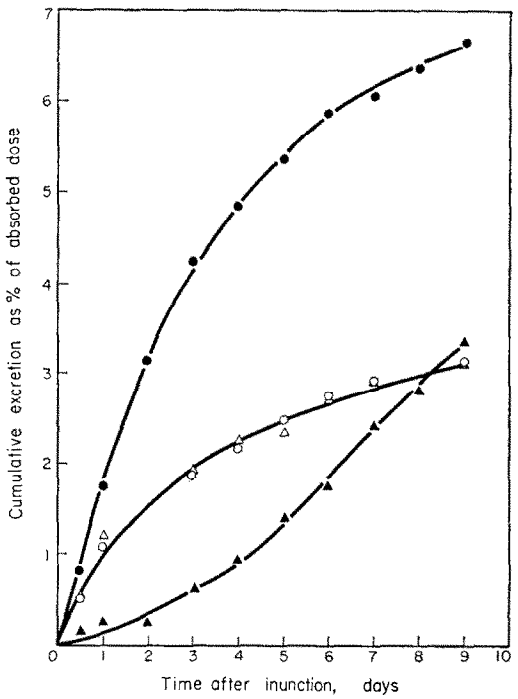

FIG. 1. Cumulative urinary excretion of radioactivity from two patients receiving ^{35}S-labelled ditophal. Non-extractable ^{35}S: patient *F* ○——○; *G* ●——●, Sulphate ^{35}S: *F*, △——△; *G*, ▲——▲

Total excretion in the breath, faeces and urine. The excretion of ^{35}S by patients *A*, *B* and *D* by these routes during the first 8 days has already been discussed in the previous sections. The totals for all three routes vary from 13 to 31 per cent of the absorbed dose, with a mean of 24 per cent. Although in no patient was excretion complete at 8 days, it seemed unlikely that further excretion by these routes would be sufficient to account for the rest of the dose administered.

Sweat. The results for patients *E–H* are presented in Table 6. The only regular collections of sweat made over a prolonged period were from patient *H* whose urinary excretion of ^{35}S compounds was only about a third of that of the other patients. The biggest losses occurred during the first day, and for two patients very high figures were recorded in the early collections. The few samples collected overnight indicated that the excretion then was much slower than during the day, although the ^{35}S content was not proportional to the weight of sweat collected. ^{35}S was still detectable after three months; though the levels were low most were statistically significant at 95 per cent confidence limits. Samples from patient *H* showed that the rate of excretion of ^{35}S fell approximately exponentially over 13 days. The cumulative amount of ^{35}S excreted over

the area of the pad during this time was calculated at about 46 mμc. Assumptions were made that excretion was fairly uniform during the 12 day-time hours and during the 12 night hours was a sixth that of the previous day. Sweat collections from the site of application showed an understandably high figure for the first hour, but thereafter did not differ greatly from those collected elsewhere. The ^{35}S of the sweat was partly toluene-extractable, but there were also substantial amounts of sulphate. No chloroform-extractable material could be demonstrated.

TABLE 6. RADIOACTIVITY IN SWEAT FROM PATIENTS RECEIVING ^{35}S-LABELLED DITOPHAL

(Results in mμc, from 260 cm² skin; *T* = toluene extractable, *S* = as sulphate.)

Patient	Time after inunction when collection began	Duration of collection (hr)	Uninuncted skin			Inuncted skin		
			Sweat wt. (g)	*T*	*S*	Sweat wt. (g)	*T*	*S*
E	1 hr	5½	1·7	1·5	0·04			
	6½ hr	3½	1·2	1·8	0·5			
	10 hr	7½	2·5	2·9	1·5			
	90 days	5½		0·1	0·3			
F	1 hr	6½	0·8	0·97	Lost			
	7½ hr	2½	1·2	1·9	0·25			
	10 hr	7½	4·4	3·3	3·4			
	90 days	5½		0·38	0·04			
G	1 hr	7	0·3	41	5·0			
	8 hr	2	4·4	3·2	3·3			
	10 hr	7½	3·5	2·9	1·4			
	106 days	5½		0·24	0·65			
H	1 hr	1	0·03	0·81	1·8	0·2	29	41
	2 hr	2	0·3	16·5	1·9	0·5	10	10
	4 hr	2	6·4	0·52	4·7	1·5	0·36	3·0
	6 hr	3	1·5	0·29	0·98	1·0	Lost	1·3
	9 hr	3	0·4	0·50	0·33	0·6	1·1	0·85
	12 hr	12	0·1	0·35	0·28	1·1	0·93	1·0
	24 hr	5	7·1	0·22	0·96			
	2 days	5½	5·0	0·20	1·7			
	4 days	10½	7·2	0·01	0·85			
	6 days	7	1·5	0·25	0·30			
	8 days	9½	7·9	0·02	0·13			
	11 days	8	8·3	0·24	0·08			
	13 days	7½	13·1	0·25	0·07			

DISCUSSION

When ditophal was rubbed on to the chest it was absorbed; at least 80 per cent and often more of the drug disappeared into the skin within 1 hr. The excretion which followed showed that the drug rapidly passed into general circulation. Four routes of excretion were examined and substantial amounts of ^{35}S from the labelled drug were eliminated by each route. Earlier animal experiments on ditophal and some other derivatives of ethyl mercaptan[5–6] had demonstrated the elimination of volatile ^{35}S compounds in the breath, and of sulphate together with small amounts of ethyl methyl sulphone and related compounds in the urine. The solvent extractions used in

the present experiments were intended to provide a rough separation of sulphate, sulphones and toluene- extractable material which could include ditophal, ethyl mercaptan and diethyl disulphide.

Faecal excretion of ^{35}S was substantial during the first 8 days and was continuing at the end of that time. The large amount of toluene-extractable ^{35}S suggests that the drug itself may be excreted in the bile. In considering the results on faeces the possibility of metabolism of the product by bacterial action in the intestine or in the faecal homogenate should be kept in mind. Urinary output of ^{35}S was comparable with the faecal output during the first 8 days after dosing; by far the greater part was in a form not extractable by toluene or by chloroform. In some patients this fraction was almost entirely sulphate, the main metabolite found in animal experiments. However, during the first few days other patients excreted a large amount of some other compound not extractable into these two solvents. The results on urinary excretion of ^{35}S explain why the preliminary experiments intended to detect likely excretion products of unlabelled ditophal were unsuccessful. The smallness of the chloroform-extractable ^{35}S fraction confirmed that ethyl methyl sulphone, the most characteristic ethyl mercaptan metabolite in guinea pigs and mice, could hardly have been detected by chemical means in urine from patients treated with unlabelled ditophal. The amount of urinary sulphate derived from the drug would also be too small to be detected in the presence of the normal variable output of sulphate.

Excretion of ^{35}S in sweat was considered possible, both because of the hot environment of the patients (shade temperature 20–30 °C) and because sulphate, a proved metabolite, is known to be excreted in sweat. We found labelled sulphate and toluene-extractable ^{35}S in comparable amounts in the sweat. The loss by this route could not be measured, but considering that the area of the absorbing pads was about one-seventy-fifth of the body surface area, the total output could have equalled or exceeded that in urine and faeces.

The whole pattern of ditophal elimination is unusual. The rate of excretion is slow and is divided between at least four routes. The collection of compounds eliminated in breath and sweat excretion presented difficulties which could hardly be overcome under the most favourable circumstances. It is possible that some volatile materials derived from the drug escape undetected through the skin. It is thus impossible to draw up a strict balance sheet of input and output although certain general conclusions can be drawn.

When ^{35}S labelled ditophal is given by inunction, excretion of ^{35}S begins almost at once. Apart from a possible transient burst of excretion in urine and sweat during the first hour, the rate of output rises steadily to a maximum between 6 and 24 hr after dosing. The rate of excretion can be effectively measured only in breath and urine, though sweat excretion may well follow the same course. Logarithmic plots of the urinary ^{35}S from patients *E* to *H* agree with an exponential decline in excretion between days 3 and 18. The half-excretion times were about 9 days for patients *E* and *F* and about 3 days for patients *G* and *H*. Measurable levels of ^{35}S have been found in the urine of some patients at 1 month and in sweat at 3 months.

Our patients had received unlabelled ditophal daily for 1 month or more before the labelled drug was given, and this daily administration was continued during the period of collection of samples. These conditions were chosen so that the results should represent the behaviour of the drug in the middle of a prolonged course of treatment.

By then the patients were presumed to have reached a dynamic equilibrium with ditophal and its metabolites, so that the reservoir of sulphur compounds derived from the drug was at a maximum and intake and output were in balance. The excretion pattern of ^{35}S following a single dose of labelled ditophal might then be influenced by the rate and extent to which the ^{35}S labelled material mixed with a pre-existing reservoir of unlabelled compounds. Extensive mixing with a large reservoir would delay the excretion of ^{35}S compounds by the dilution of the isotopic label. Each subsequent entry of fresh unlabelled material into the system would accentuate the delay. The nature of the metabolites of ditophal and their concentrations in the blood and skin will be discussed in a later paper.

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