THE ABSORPTION, METABOLISM AND
EXCRETION OF
1-(p-DIMETHYLAMINOPHENYL)-3-
(p-BUTOXYPHENYL)-2-THIOUREA IN MAN

Part 2. A Study Using ^{35}S -labelled Drug

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Introduction

Earlier work referred to in Part 1 of this paper has shown that 1-(p-dimethylaminophenyl)-3-(p-butoxyphenyl)-2-thiourea (DPT) is excreted in the urine of man largely in the form of benzene-insoluble metabolites. The colorimetric method of estimating DPT by use of ferric chloride was shown to be specific for diphenylthioureas containing a p-dimethylamino group. The results given in Part 1 therefore represent complete estimations of the excreted drug only if the water-soluble metabolites of DPT are diphenylthioureas containing the p-dimethylamino group. If any bond scission had taken place to destroy this unit the drug concentration estimated would be less than the true figure. To test this possibility by any chemical method would be difficult and use was therefore made of radioactive tracers to follow the absorption, excretion and metabolism of the drug.

The present paper describes a study of the pharmacology of DPT in man using the ^{35}S -labelled drug, which makes possible the estimation of DPT together with all its metabolites. The ferric chloride method was applied in parallel and in this way it was possible to check the accuracy of the colorimetric method.

SCHMID and TRIPOD (1959) studied the pharmacology of DPT in the rabbit and dog. They dosed these animals with ^{14}C - and with ^{35}S -labelled drug and so were able to estimate the blood levels and the excretion in the urine, bile and faeces of DPT together with its metabolites. They made parallel estimations of the unchanged drug by extracting samples with benzene and reacting the dried extracts in ethanol with bromine in tetrachloride. By these means they showed that DPT was excreted by the rabbit and dog almost entirely in the form of benzene-insoluble metabolites.

FIG. 4. THE ABSORPTION OF D.P.T. ON VARIOUS REGIMENS

Methods

Preparation of ^{35}S -labelled DPT

A sample of ^{35}S -labelled DPT was kindly supplied by Ciba Ltd. This has been prepared by the method described by SCHMID and TRIPOD (1959) and had a specific activity of 3.4μ curies/mg. on 29 April 1959. Preparations of DPT of various activities for oral ingestion were made by mixing chloroform solutions of radioactive and non-radioactive DPT and evaporating to dryness under reduced pressure. Five preparations were made for oral ingestion by five different subjects. These preparations were given on three different occasions.

The radioactive DPT was weighed into large hard gelatin capsules for oral dosage. Previous colorimetric studies with non-radioactive DPT had shown no significant difference in absorption of the drug when given in capsule or tablet form.

Subjects and their dosage with ^{35}S -labelled DPT

Details of the subjects studied and the ^{35}S -labelled DPT that they received are given in Tables 1 and 2. Subject 1 received DPT preparation 1 etc. All the subjects except number 5 had lepromatous leprosy but were otherwise apparently in normal health. No drug was given for at least two days before first administration of radioactive DPT; the drug-free period was extended to one month in experiment 3 in which faeces were collected.

Collection and storage of specimens.

Collections of urine and faeces were made at the times stated, zero time in all cases being 8 a.m. on Day 1. (See Table II). Colorimetric analyses were made on the day the collections were completed but samples were stored at 6° for periods of up to one month before analysis was made of their radioactivity.

Analysis of samples

(a) COLORIMETRIC

Creatinine contents of all urine samples were estimated by the alkaline picrate method (KING and WOOTON, 1956/) as a check on the completeness of collections. The DPT, together with its metabolites, in the urine and faeces was estimated by the method described in Part 1.

(b) RADIOACTIVE

All counting was carried out using an end-window counter (Mullard Tube M X 123, window thickness 1.9 mg./sq. cm.) with standard flat 1" aluminium planchettes situated 2 cm. from the window. Where samples and their respective standards were counted on different days, allowance was made for the natural decay of the activity of ^{35}S during the period in question. For most readings at least 2,000 counts were made but this figure was reduced to 1,000 in

TABLE I

<i>Subject</i>	<i>Sex</i>	<i>Weight kg.</i>	<i>Age yrs.</i>	<i>Tribe</i>	<i>Previous dosage with D.P.T.</i>
1	F	58	20-30	Luo (Nilotic)	2 years
2	M	60	40-50	Luo (Nilotic)	2 years
3	F	62	30-40	Luo (Nilotic)	2 years
4	M	58	30-40	Luhya (Bantu)	None
5	M	58	20-30	English (Caucasian)	3 occasions only

TABLE II

<i>Experiment No.</i>	<i>Drug Preparation No.</i>	<i>Dose Administered Time* hours</i>	<i>Date</i>	<i>Dose given g.</i>	<i>Dilution of ³⁵S- DPT with inactive drug</i>	<i>Radioactivity of preparation μc./mg.</i>	<i>Radioactive Dose μc.</i>
1	1	0	27.7.59	0.50	1: 51	0.0386	19.3
1	2	0	27.7.59	1.00	1: 102	0.0193	19.3
1	3	0	27.7.59	1.50	1: 153	0.0129	19.3
2	4	0, 4, 8	15.2.60	3 × 1.50	1: 85	0.00469	21.1
3	5	0, 4, 8	27.5.60	9 × 1.50	1: 200	0.00069	9.3
		0, 4, 8	28.5.60				
		0, 4, 8	29.5.60				

* Zero indicates time of administration of 1st dose of radioactive DPT.

cases of very low activity. Samples were prepared for counting by one of two methods.

(i) *Direct counting of dried urine samples*

A graph was prepared showing the relationship between the observed and true counts for different weights of urine solids. Standard solutions of labelled drug in urine were prepared by addition of an ethanolic solution of the drug to diluted urine. One millilitre lots were then dried on planchettes, using an infra-red lamp, and counted, the theoretical count being obtained by extrapolation to zero weight of urine solids. The data obtained agreed closely with those calculated by reference to the nomogram given by FAIRES and PARKES, (1957) assuming an effective area of urine solids on the planchettes of 4.5 sq. cm.

One or two millilitre samples of urine for analysis were then dried on weighed planchettes. The planchettes were allowed to cool and then to equilibrate with the atmosphere for half an hour before reweighing and counting. When a sample was counted for more than an hour it was weighed both before and after counting and the mean weight calculated. The counts were then corrected for self-absorption by reference to the graph described previously and expressed as counts per minute per millilitre. A standard solution of the DPT given to the patient was prepared in chloroform and a small known volume was allowed to dry on a planchette. This was counted in the end-window counter and the count corrected for self-absorption by reference to the nomogram previously mentioned, the area of the solid being taken as 4.5 sq. cm. Comparison of the count of a urine sample with that of the appropriate DPT standard gave a value for the total weight of DPT and its metabolites which will be referred to as "total diphenylthioureas".

(ii) *Counting after conversion to barium sulphate*

The activity of many of the urine samples from the third experiment was too low to be accurately measured by the direct method, which was applied only to those samples with concentrations of over 140 $\mu\text{g./ml.}$ of DPT metabolites (as estimated by the ferric chloride method). Attempts to use ethanolic extracts of faeces proved unsatisfactory mainly because of creeping of the solution during drying.

All urine samples and ethanolic extracts of faeces from the third experiment were therefore converted to barium sulphate for counting. Samples of urine (8 ml.) and ethanolic extracts of faeces (10–40 ml.) were oxidised by Benedict's method for total sulphur (HAWK, 1954). Barium chloride was then added and the resultant precipitate of barium sulphate centrifuged, washed with water, recentrifuged and pipetted on to weighed planchettes. The precipitate was then dried for one hour at 80–100° and allowed to cool for one hour over

calcium chloride in a desiccator. The planchettes were then weighed and counted, the counts being corrected as before for self-absorption. Standard ethanolic solutions of the preparations of DPT were oxidised and counted in the same way. Previous gravimetric and radioactive studies had shown that the sulphur of DPT was quantitatively converted to sulphate by the procedures employed.

Results

The excretion of DPT, together with its metabolites, on the various regimens as estimated by both methods is shown in Tables III-VI and illustrated in Figures 1-4.

TABLE III
Experiment 1. Subject 1. Preparation 1. 0.50 g. DPT

<i>Time</i> <i>hours</i>	<i>Creatinine</i> <i>mg./hr.</i>	<i>"Total diphenylthiureas"</i> <i>FeCl₃ method</i> <i>mg.</i>	<i>Radioactive</i> <i>method</i> <i>mg.</i>
0-2*			
2-4	55	13.4	15.3
4-6	none	—	—
6-9	50	34.0	50.8
9-12	58	19.7	22.5
12-24	50	26.5	27.6
24-36	44	9.7	11.2
36-48	52	13.2	6.0
48-60	48	—	2.0
60-72	50	—	1.7

* Sample lost.

TABLE IV
Experiment 1. Subject 2. Preparation 2. 1.00 g. DPT

<i>Time</i> <i>hours</i>	<i>Creatinine</i> <i>mg./hr.</i>	<i>"Total diphenylthiureas"</i> <i>FeCl₃ method</i> <i>mg.</i>	<i>Radioactive</i> <i>method</i> <i>mg.</i>
0-2	52	10.9	11.6
2-4	65	16.6	19.4
4-6	54	7.6	11.8
6-9	83	6.7	11.6
9-12	75	3.7	4.8
12-24	57	10.2	5.0
24-36	68	15	6.8
36-48	61	—	1.1
48-60	57	—	1.0
60-72	51	—	—

TABLE V
Experiment 1. Subject 3. Preparation 3. 1.50 g. DPT

Time hours	Creatinine mg./hr.	"Total diphenylthioureas"	
		FeCl ₃ method mg.	Radioactive method mg.
0-2	76	5.7	1.4
2-4	46	12.5	14.6
4-6	64	14.6	14.2
6-9	50	10.9	11.4
9-12	43	6.0	4.4
12-24	55	16.7	11.4
24-36	51	15.4	12.1
36-48	46	16	10.7
48-60	52	19	12.7
60-72	48	12	8.4

TABLE VI
Experiment 2. Subject 4. Preparation 4. 4.5 g. DPT
(1.5 g. administered at 0, 4 and 8 hours)

Time hours	Creatinine mg./hr.	"Total diphenylthioureas"	
		FeCl ₃ method mg.	Radioactive method mg.
0-2	52	4.1	4.4
2-4	43	8.5	11.3
4-6	48	3.5	4.2
6-9	48	11.3	12.0
9-12	54	4.1	2.9
12-24	56	11.4	11.8
24-48	48	65	65
48-72	61	—	21.3

As a check on accuracy of counting a representative sample was made by pooling 10% of each urine sample over either a one or three day series; the radioactive content of this sample was compared with that obtained by summation of the individual results. Agreement was normally within 10%.

Figure 5 shows the correlation of the concentrations of DPT together with its metabolites, as estimated by the colorimetric and radioactive (direct) methods.

The results from Experiment 3, in which the more sensitive barium sulphate method was used to determine the radioactivity of the urine samples, are enumerated in Table VII and illustrated in Figure 6.

TABLE VII
Experiment 3. Preparation 5. 13.5 g. DPT
 (1.5 g. administered at 0, 4, 8, 24, 28, 32, 48, 52, and 56 hours)

<i>Time</i> <i>hours</i>	<i>Creatinine</i> <i>mg./hr.</i>	<i>"Total diphenylthiureas"</i> <i>FeCl₃ method</i> <i>mg.</i>	<i>Radioactive</i> <i>method</i> <i>mg.</i>
0-2	58	5.1	5.6
2-4	58	10.1	9.4
4-6	66	5.6	6.3
6-8	65	7.8	9.3
8-10	65	13.6	12.8
10-12	61	23.8	11.6
12-24	53	559	328*
24-26	58	48.1	46.0
26-28	57	40.5	40.0
28-30	58	33.5	32.8
30-32	61	38.5	41.2
32-34	57	47.5	49.5
34-36	56	74.7	79.0
36-48	59	196	192
48-50	62	21.1	21.6
50-52	65	33.7	33.5
52-54	51	36.4	32.5
54-56	62	27.8	Lost
56-58	63	36.2	38.0
58-60	56	64.0	70.8
60-72	55	345	360
70-74	58	27.6	26.3
74-76	54	18.9	18.4
76-78	55	16.6	16.1
78-80	60	14.0	14.4
80-84	58	22.1	24.7
84-96	56	50	48.5
96-108	76	—	30.3
108-120	59	—	11.3

* A check on the DPT content of pooled samples (by both methods) indicated that the value obtained by the radioactive method for the 12-24 hour urine collection was probably low; otherwise there was good agreement ($\pm 10\%$).

The correlation of the concentrations of DPT together with its metabolites, as estimated by the colorimetric and radioactive (barium sulphate) methods is shown in Figure 7.

The colorimetric and radioactive estimations on the ethanolic extracts of faeces collected in the third experiment are shown in Table IX.

A grand summary of the amounts of DPT, together with its metabolites, excreted in the urine, as estimated by colorimetric and radioactive methods, is given in Table VIII. For comparison, the corresponding data for the groups of patients reported in Part I are also given.

TABLE VIII

<i>Experiment</i>	<i>Subject</i>	<i>Dose DPT</i> <i>g.</i>	<i>Total diphenylthioureas excreted in the urine</i>		<i>Diphenylthioureas excreted in urine expressed as percentage of the dose</i>		<i>Comparative average figure for thioureas excreted in urine (taken from FeCl₃ figures for whole group)</i>	
			<i>FeCl₃ method mg.</i>	<i>Radioactive method mg.</i>	<i>FeCl₃ method %</i>	<i>Radioactive method %</i>	<i>%</i>	<i>Standard Deviation</i>
1	1	1 × 0.50	117	137	23.4	27.4	10.8	5.0
1	2	1 × 1.00	71	73	7.1	7.3	8.7	5.4
1	3	1 × 1.50	129	101	8.6	6.7	8.1	2.5
2	4	3 × 1.50	211	239	4.7	5.3	8.1	1.9
3	5	9 × 1.50	1807	1867*	13.4	13.8	8.1	1.9

*As a radioactive determination on the 54–56 hr. sample was not possible and as the radioactive result for the 12–24 hr. sample was shown to be erroneous, the colorimetric data for these two samples were used in the calculation for this total.

TABLE IX

*Subject 5. Preparation 5. 13.5 g. DPT
1.5 g. administered at 0, 4, 8, 24, 28, 32, 48, 52 and 56 hours)*

Day	DPT content of faeces	
	<i>FeCl₃ method</i> g.	<i>Radioactive method</i> g.
1 and 2	1.85	1.16
3	2.60	2.34
4	4.33	3.68
5	0.35	0.26
Total	9.13	7.44
% recovery in 5 days	68	55

Discussion

The great advantage in employing isotopically labelled compounds in the study of the absorption, excretion and metabolism of a drug is that the results are unaffected by any molecular changes which may occur. Chemical methods for the estimation of DPT are valid for its metabolites only if these metabolites retain both the diphenylthiourea and the p-dimethylamino groupings. Moreover with a colorimetric method of estimation there is always the possibility of quantitative variation with molecular change even when the method itself is still applicable. To employ isotopically labelled DPT for an extensive study of the human pharmacology of the drug is obviously impracticable. A limited number of experiments with radioactive drug were therefore undertaken in which both radioactive and colorimetric methods were employed. It was considered that if these methods yielded substantially identical results, then the colorimetric method could with confidence be used alone in more extensive studies.

In the present experiments the degree of correlation between the results obtained by the ferric chloride and radioactive methods, while not perfect, has been good (Figures 6 and 7).

Below a concentration of 20 $\mu\text{g./ml.}$ however the colorimetric method was not satisfactory, but above that level the method gave a reliable value for the concentrations of DPT together with its metabolites. It is therefore apparent that the ferric chloride method employed in these and earlier studies is more accurate than suggested by SCHMID and TRIPOD (1959).

The present study has thrown further light on the fate of DPT in the body although more work is required before any firm conclusions can be drawn. It has been shown that less than 1% of the radioactive compounds excreted in the urine after giving ^{35}S -labelled DPT to

man were extractable into benzene and that an equally small amount was converted to sulphate. Thus although DPT is almost completely metabolized to benzene-insoluble compounds very little metabolic desulphurization takes place.

These findings show certain similarities to those reported by SMITH and WILLIAMS (1959). These workers showed that in the rabbit little desulphurization occurred in the diphenylthioureas in contrast to the monophenylthioureas. They therefore suggested that the relative toxicity of the latter compounds was due to the liberation of hydrogen sulphide *in vivo*. The lack of toxicity of DPT in man noted by many workers may therefore depend on its resistance to metabolic desulphurization in man.

These radioactive studies have confirmed the low degree of absorption of DPT of man, originally reported by ROSS INNES, SMITH and HARDEN-SMITH (1957) and confirmed in Part I.

Summary

1. DPT and its metabolites have been estimated in urine and faeces by radioactive (using ^{35}S -labelled drug) and colorimetric methods in parallel.
2. Between concentrations of 20 $\mu\text{g./ml.}$ and 500 $\mu\text{g./ml.}$ good correlation was obtained between the two methods. The radioactive method could be used to detect drug concentrations down to 2 $\mu\text{g./ml.}$
3. These studies have confirmed that after oral administration of DPT to man only 10% of the drug is absorbed and that it is metabolised to benzene-insoluble compounds which are rapidly excreted in the urine.

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EXPERIMENT I. SUBJECT I. DOSE 0.50 g. PREPARATION I

FIG. I.

EXPERIMENT 1 SUBJECT 2 DOSE 1.00g. PREPARATION 2.

FIG. 2.

EXPERIMENT I SUBJECT 3 DOSE 1.50g. PREPARATION 3.

FIG. 3

EXPERIMENT 2. SUBJECT 4. DOSE 3x1.50g PREPARATION 4

FIG. 4.

EXPERIMENT 3. SUBJECT 5. DOSE 9 X 1.50g. PREPARATION 5.

FIG. 5.

$\mu\text{g./ml.}$

THE MEASURED CONCENTRATIONS
OF DPT TOGETHER WITH ITS METABOLITES
(TOTAL DIPHENYL THIOUREAS)

FIG 6 CORRELATION OF RADIOACTIVE & COLORIMETRIC METHODS

$\mu\text{g./ml.}$

THE MEASURED CONCENTRATIONS
OF DPT TOGETHER WITH ITS METABOLITES
(TOTAL DIPHENYL THIOUREAS)

DRUG PREPARATION 5

FIG 7 CORRELATION OF COLORIMETRIC & RADIOACTIVE METHODS