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

Part 1. A Study Using Colorimetric Methods

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Introduction

1-(p-Dimethylaminophenyl)-3-(p-butoxyphenyl)-2-thiourea, known variously as SU 1906, Ciba 1906 and DPT, is one of a large number of substituted N : N′-diphenylthioureas shown to possess considerable *in vitro* and *in vivo* antitubercular activity (MAYER, EISMAN and KONOPKA, 1953). It supresses experimental tuberculosis in the mouse and guinea pig when given in the diet at concentrations of as little as 0.025% and 0.01% respectively (EISMAN, KONOPKA and MAYER, 1954; KONOPKA, EISMAN and MAYER, 1954) and was therefore given a trial, together with two other highly active diphenylthioureas, in human pulmonary tuberculosis.

At doses of up to 6 g. a day, given orally in three equal amounts after meals, DPT was considered to possess significant antitubercular activity in man (SCHWARTZ, OWENS and GIERSON, 1954; PHILLIPS, CHU and LYONS, 1955). JONES, JENKINS and YEN (1956) however failed to confirm this conclusion.

The first clinical trial of DPT in human leprosy was made by DAVEY and CURRIE (1956) who found that when given orally in daily doses of 1.5 g. to 3.0 g. it was non-toxic and displayed an activity against *Mycobacterium leprae* similar to that of DDS. Further reports from all over the world have confirmed these findings (ROSS INNES, SMITH and HARDEN-SMITH, 1957; ALONSO, 1958; DAVEY, 1958; GATE, ROUSSET and COUDERT, 1958; MUKHERJEE and GHOSH, 1958; GARROD, 1959). Unfortunately there is evidence that after about three years treatment, drug resistance develops leading to relapse if the patients are not transferred to another drug (DAVEY, 1960: Annual Report, East African Leprosy Research Centre, 1959–1960).

Among the many other disubstituted diphenylthioureas which possess considerable antitubercular activity, three have been shown to exhibit significant activity in controlling human leprosy (BUU-HOI, BA-KHUYEN and DAT-XUONG, 1957; HAYASHI, 1958) and another, known as thiocarbanidin, may be slightly active in supressing human pulmonary tuberculosis (LARKIN, 1960).

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Little has been reported concerning the pharmacology of the diphenylthioureas. SMITH and WILLIAMS (1959) have studied the fate in the rabbit of a number of phenyl and diphenylthioureas and have concluded that the relative freedom from toxicity of the diphenylthioureas, as compared with the monophenyl thioureas, is due to the relative resistance of the former compounds to metabolic desulphurization. DILL and GLAZKO (1958) devised a method for the estimation of 1-(p-α-pyridylphenyl)-3-(p-isobutoxyphenyl)-2-thiourea ("Thioban" tor thiocarbanidin) and showed that it was poorly absorbed in both man and the rat. In man between 94% and 98% of the oral dose is eliminated unchanged in the faeces and only about 0.3% of the drug is excreted unchanged in the urine.

Two studies have been made of the pharmacology of DPT, ROSS INNES, SMITH and HARDEN-SMITH (1957) reported that DPT reacts with ferric chloride to form what they believed to be a coloured coordination compound. By means of this reaction, they concluded that the drug was not well absorbed when given orally to man, since the great majority of the dose could be demonstrated in the faeces. They also concluded that the drug was not present in the urine unmodified but as a soluble derivative. Further unpublished work by SMITH (1957) led him to the conclusion that, when taken orally, about 10% of the drug is absorbed and then rapidly excreted in the urine as water-soluble metabolites and that about 70% is eliminated unchanged in the faeces.

SCHMID and TRIPOD (1959) studied the absorption and excretion of DPT in the rabbit and dog using radioisotopes. By dosing the animals with DPT labelled with ^{35}S or ^{14}C, they were able to estimate the blood concentrations and amounts excreted in the urine and faeces of unchanged DPT together with its metabolites. By extracting the body fluids with benzene and reacting the dried extracts dissolved in ethanol with bromine in carbon tetrachloride, they were able to estimate in parallel the unchanged drug. They showed, by these methods, that over 60% of the oral dose was absorbed by the rabbit and then rapidly and completely metabolized. After subcutaneous dosage some of the unchanged drug and/or its metabolites were secreted into the bile and intestine and then probably excreted in the faeces. In the dog absorption was less complete, only about 20% of the oral dose being excreted as metabolites in the urine. After oral dosage the maximum blood concentrations of DPT together with its metabolites in the rabbit was about 4 μg./ml., but measurable blood levels in the dog were not demonstrable by the techniques employed. They were unable to isolate in a pure form the metabolites of DPT from either the rabbit or dog.

In view of the extensive metabolism of DPT by these animals, SCHMID and TRIPOD (1959) cast doubt on the conclusions drawn by ROSS INNES, SMITH and HARDEN-SMITH (1957) who had employed

ferric chloride for the colorimetric estimation of DPT in the body fluids.

This paper describes our studies on the absorption, metabolism and excretion of DPT, which will, it is hoped, provide a basis for the rational treatment of leprosy with this drug. A brief summary of some of the results reported here has already been published (ELLARD, 1960a).

Methods

Introduction

The experimental methods described here have been developed from the work originally undertaken by SMITH (1957). Since DPT is metabolized by man, methods had to be devised to estimate quantitatively both unchanged DPT and its metabolites. The structure of these metabolites is still unknown and it will be realised that the quantitative estimation of unknown compounds is often unsatisfactory. These colorimetric studies have therefore been followed by isotopic studies (Part 2), which have confirmed the validity and accuracy of the colorimetric methods employed.

The reagents proposed by GROTE (1931) and CHESLEY (1944), for the estimation of thiourea and some of its simpler derivatives, failed to react with DPT or any of the other diphenylthioureas tested in these laboratories. DPT can however be detected and estimated by means of the blue colour it forms in the presence of oxidising agents such as ferric chloride (ROSS INNES, SMITH and HARDEN-SMITH, 1957), bromine (SCHMID and TRIPOD, 1959), acid permanganate and dichromate. In our hands the most satisfactory reagent has been found to be ferric chloride. The great advantage in using ferric chloride is that the reaction can be carried out in aqueous solution. The extraction of DPT or its metabolites from urine before reaction is therefore unnecessary. Extraction into an organic solvent is necessary when bromine is employed as the oxidising agent. This means in practice that only unchanged DPT can be estimated by bromine if the metabolites of DPT are highly water soluble. Ferric chloride is also a more sensitive reagent than bromine and the blue colour formed by DPT in its presence is more stable than that formed with bromine.

Nature and specificity of the colour reaction

It is likely that the blue compound formed by oxidation of DPT, under the conditions described in this paper, is its Wurster salt. DPT is a monosubstitution compound of N : N dimethyl-p-phenylene diamine (Wurster's Red) and might therefore be expected to be oxidised in a similar way to N : N-dimethyl-N′-methyl-p-phenylene diamine or N : N : N′ : N′-tetramethyl-p-phenylene

diamine (Wurster's Blue) (Michaelis, Schubert and Granick, 1939). Consequently the ferric chloride reaction will not be specific for DPT but should be given by all p-dimethylamino diphenylthioureas.

We have found that 1-(p-dimethylaminophenyl)-3-(p-isobutoxyphenyl)-2-thiourea, 1-(p-dimethylaminophenyl)-3-(p-2 : 3-dihydroxy-n-proxyphenyl)-2-thiourea and 1-(p-dimethylaminophenyl)-3-(p-3-carboxy-n-propoxyphenyl)-2-thiourea, react quantitatively on a molar basis, as DPT; whilst 1-(p-dimethylaminophenyl)-3-(p-n-propylphenyl)-2-thiourea and 1-(p-dimethylaminophenyl)-3-(p-n-butylphenyl)-2-thiourea react qualitatively in a similar fashion to DPT. 1 : 3-(Bis-p-dimethylaminophenyl)-2-thiourea forms a brown colour on reaction with ferric chloride which has been used here to estimate this compound quantitatively (unpublished work). 1 : 3-(Bis-p-aminophenyl)-2-thiourea, 1-(p-dimethylaminophenyl)-3-(p-ethoxyphenyl)-2-urea, 1-(p-dimethylaminophenyl)-3-phenyl-2-thiourea, 1-(p-aminophenyl)-3-phenyl-2-urea, 1-(p-dimethylaminophenyl)-3-cyclohexyl-2-thiourea, p-dimethylaminophenylthiourea, p-dimethylaminophenyl-isothiocyanate and 1 : 3-bis-p-butoxyphenyl)-2-thiourea all failed to react under the conditions employed for the estimation of DPT with ferric chloride.

It was therefore concluded that this method should estimate quantitatively, on a molar basis, both DPT and its metabolites provided that:

(a) the thiourea moiety is not metabolized,

(b) the phenyl groups are not reduced or destroyed,

(c) the dimethylamino group is left intact.

As mentioned previously, studies with isotopically labelled drug have shown that these conditions are fulfilled (Part 2).

Other constituents of the urine do give colours under certain conditions with ferric chloride. In DPT balance studies it has been essential to avoid concomitant dosage with salicylates, but it was not found necessary to take patients off DDS, thiosemicarbazone or diethyldithiol*iso*phthalate therapy. By taking these precautions false positives were never encountered during estimations of DPT by the methods described here.

Chemical and physical properties

DPT is a white, odourless crystalline compound with a slightly bitter taste. It is only slightly soluble in water (about 1 μg./ml.) but is more soluble in acid (about 250 μg./ml. in 10% (v/v) acetic acid), ethanol, n-propanol, n-butanol and benzene. It is very soluble in both acetone and chloroform.

DPT is unstable in ethanolic solution and consequently standard solutions of the drug should be prepared daily.

Reagents and standard solutions

0.5% (v/v) aqueous acetic acid (AnalaR).

20% (w/v) aqueous solution of ferric chloride, prepared each month by dilution of an (AnalaR) ferric chloride solution (60% w/v).

A standard solution of DPT containing 1 mg./ml. prepared daily by dissolving 100 mg. of DPT in 100 ml. ethanol.

A dilute standard solution of DPT containing 100 μg./ml. prepared by ten-fold dilution with 0.5% acetic acid of the 1 mg./ml. standard solution.

Tubes marked at 10 ml. were used for all determinations.

The room temperature varied from 20° to 30°, but was usually about 25°.

Reaction and standard curve

Into a series of ten tubes were pipetted 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ml. respectively of the dilute standard solution of DPT and the tubes made to the mark with 0.5% acetic acid. To each tube was added 0.1 ml. of 20% ferric chloride solution. The contents of the tubes were mixed by inversion. Between one and two minutes after reaction, the densities of the blue colours were measured in an "EEL" colorimeter with No. 608 filter against a reagent blank (10 ml. 0.5% acetic acid and 0.1 ml. 20% ferric chloride). No serious errors arise if the colorimeter readings are made within two minutes of reaction since the blue colour fades exponentially with a half life of about 2 hours at 28°. A plot of colorimeter reading against final concentration of DPT was linear over the range 1.0 to 10.0 μg./ml.

Collection of specimens

Subjects for balance studies were picked at random from the group of East African Bantu and Nilotic patients receiving oral DPT in a clinical trial undertaken at this research centre (GARROD, 1959). An analysis of the data collected showed no dependence of the results on the clinical state, tribe, age or weight of the patients studied.

In view of the rapid excretion of the DPT and its metabolites in the urine after oral dosage, lengthy stabilization (for these urine balance studies) of patients on a given drug regimen was not considered necessary. In practice it was found satisfactory to give the test dose on two days only, namely that preceding and that of the urine collection.

Twenty-four hour urine collections were commenced at 7 a.m. If the volume or the DPT metabolite content of a urine collection was unusually low, the completeness of the collection was checked by a determination of its creatinine content by the Folin's alkaline

picrate method (King and Wooton, 1956). If this was also abnormally low, the collection was considered to be incomplete and the DPT results discounted. Another study undertaken in these laboratories has revealed the constancy of the daily creatinine excretion of the East African Bantu, Nilotic and Nilohamitic (unpublished results). In all 188 twenty-four hour urine collections were made from 46 patients. Most urine samples were analysed on the day that the collection was completed but when this was not possible they were stored overnight at 6° without the addition of any preservative, and analysed the following day.

Twenty-four hour collections of faeces were made from the patients receiving their normal daily dosage of DPT.

Estimation of unchanged DPT (together with any benzene-extractable metabolites) in the urine

These methods are based on the fact that DPT can be quantitatively extracted from alkaline aqueous media into organic solvents such as benzene. The compounds estimated by these procedures will be referred to as "unchanged DPT".

(a) By extraction of urine with benzene

Samples of urine for analysis (20 ml.) were made alkaline by addition of 0.1 ml. M-Na_2HPO_4 solution and extracted by shaking with 20 ml. benzene. The layers were separated by centrifugation and 15 ml. of the benzene layer pipetted into a 50 ml. flask and dried under reduced pressure. The residue in the flask was dissolved by shaking with 10 ml. 0.5% acetic acid and reacted with 0.1 ml. 20% ferric chloride solution. The colorimeter reading obtained was compared with that given by 10 ml. of a 10 μg./ml. solution of DPT in 0.5% acetic acid (made by diluting 1 ml. of the 100 μg./ml. dilute standard solution to 10 ml. with 0.5% acetic acid).

Hence the "unchanged DPT" content of the urine sample could be calculated.

(b) By extraction of dried urine with benzene

Samples of urine (20 ml.) were dried, first under reduced pressure and then overnight over calcium chloride in a vacuum desiccator. The dry residue was extracted three times with 10 ml. benzene and the extracts filtered through paper. The combined filtrate was dried under reduced pressure and the residue shaken with 10 ml. 0.5% acetic acid. The acetic acid extract was reacted with 0.1 ml. 20% ferric chloride and the DPT content of the original sample calculated from the colorimeter reading.

Estimation of DPT together with its metabolites in the urine

Since ferric chloride causes precipitation when added to undiluted urine, all urine samples must be diluted before reaction. By adding

known amounts of an ethanolic solution of DPT (1 mg./ml.) to normal urine diluted tenfold or more with 0.5% acetic acid and reacting with ferric chloride, it was found that compounds are present in the urine which inhibit the DPT-ferric chloride reaction. The estimation of other p-dimethylamino diphenylthioureas with ferric chloride is affected in an identical fashion. The inhibition is partly due to phosphates but other inhibitors are also present. No method was found whereby DPT and its metabolites could be quantitatively separated from these inhibitors.

The extent of the inhibition is closely related to the concentration of DPT, the specific gravity and the dilution of a given urine sample. Calibration curves were therefore constructed relating the extent of the inhibition of the ferric chloride reaction to these parameters, so that correction could be made for this inhibition in calculations of the DPT metabolite contents of urine samples.

Specimens of urine were collected from six patients not receiving DPT and their specific gravities adjusted with an urinometer to 1.010 g./ml. Equal volumes were pooled to give a representative blank urine of specific gravity 1.010 g./ml.

A standard curve relating colorimeter reading and concentration of DPT was prepared by pipetting 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml. of a 100 μg./ml. ethanolic solution of DPT into a series of ten tubes, making to the mark with 0.5% acetic acid and reacting with ferric chloride. These amounts of DPT were pipetted into a second set of tubes together with 0.5 ml. of the pooled blank urine, specific gravity 1.010 g./ml., representing a final urine dilution of 1 to 20. The tubes were made to the mark with 0.5% acetic acid and reacted with ferric chloride. The colorimeter readings obtained were compared with those from the first set and the ratio of the colorimeter reading in the presence of urine (2nd series) to that without it (1st series) calculated for each pair of tubes with the same concentration of DPT. These ratios were then plotted against the colorimeter readings in the second series. Similar graphs were prepared for final urine dilutions of 1 to 50 and 1 to 100 by reactions of two similar series of tubes containing 0.2 ml. and 0.1 ml. respectively of the pooled blank urine.

Urine samples were analysed in the following manner. The specific gravity of a sample was first adjusted to 1.010 g./ml. and the volume recorded. Twenty-, fifty-, and one hundred-fold dilutions were made by pipetting 0.5, 0.2 and 0.1 ml. respectively into tubes and making to the mark with 0.5% acetic acid. The tubes were reacted with ferric chloride and the colorimeter readings measured against a reagent blank. Urine samples from patients not receiving DPT gave negligible optical densities when reacted in this way. Allowance was then made for the inhibition of the urine at each of the three dilutions by reference to the graphs described above, and

by comparison with the colorimeter reading from a standard solution of DPT (10 μg./ml.) the mean concentration of DPT together with its metabolites, in the urine sample was calculated. The compounds estimated in this way will be referred to as "total diphenylthioureas".

When very high concentrations of DPT metabolites were encountered only the 1 to 50 and 1 to 100 dilutions were made. On the other hand when very low concentrations were encountered only twenty- and fifty-fold dilutions were employed. Urines with specific gravities of less than 1.010 g./ml. were first diluted to specific gravity 1.005 g./ml. and then further diluted ten-, twenty-five- and fifty-fold before reaction and the calculation modified accordingly.

EXAMPLE

One ml. of a 100 μg./ml. solution of DPT diluted to 10 ml. with 0.5% acetic acid and reacted with ferric chloride gave a colorimeter reading of 5.7.

Urine sample. Volume=150 ml. and specific gravity 1.015 g./ml. Fifty ml. diluted to 70 ml. had a specific gravity of 1.010. This diluted urine was then further diluted twenty-, fifty, and one hundred-fold and reacted with ferric chloride as described above.

The calculations are shown in Table I.

TABLE I

Dilution	*Colorimeter Reading*	*Ratio* $\frac{\text{Colorimeter reading with urine}}{\text{reading without urine at that reading and urine dilution}}$	*Corrected Reading*	*μg./ml. "total diphenylthioureas" in urine S.G. 1.010 g./ml.*
1 : 20	2.37	0.54	4.40	154
1 : 50	1.09	0.60	1.70	149
1 : 100	0.60	0.76	0.79	138

Hence the mean concentration of DPT together with its metabolites in the urine of specific gravity 1.010 g./ml.=147 μg./ml. "total diphenylthioureas" and the mean concentration of DPT together with its metabolites in the original urine=206 μg./ml. "total diphenylthioureas". The "total diphenylthioureas" content of the urine sample is therefore 30.9 mg. This represents 30.9 mg. of partially or completely metabolized DPT assuming that 1 mole. of each metabolite of DPT gives the same optical density as 1 mole. of DPT.

ACCURACY OF THE METHOD

Twenty-four hour urine collections were made from six patients each receiving three doses of 1.5 g. DPT at 7 a.m., 11 a.m., and 3 p.m. on that day. Samples from each collection were diluted to

specific gravity 1.010 g./ml. and their concentrations of DPT together with its metabolites estimated by the method described above. (The concentrations of "total diphenylthioureas" in these urines varied from 165 to 310 μg./ml.) A blank urine specimen was also obtained and its specific gravity adjusted to 1.010 g./ml. Each metabolite urine was then diluted 1 in 4/3, 1 in 2, 1 in 4 and 1 in 10 respectively with the blank urine giving urines of specific gravity 1.010 g./ml. which contained 75%, 50%, 25% and 10% respectively of their initial concentrations of DPT and its metabolites. These concentrations were then measured by the above method and compared with the theoretical values. In this way it was concluded that the ferric chloride method described here to estimate DPT together with its metabolites in the urine, is accurate to better than $\pm 5\%$ at concentrations of greater than 50 μg./ml. and accurate to better than $\pm 10\%$ in the concentration range 20–50 μg./ml. Concentrations of less than 20 μg./ml. cannot however be estimated satisfactorily by this method. These conclusions have been confirmed by the isotopic studies reported in Part 2.

Estimation of DPT (and its metabolites) in the faeces

Faeces for analysis were homogenized with a convenient amount of water in a Waring Blender. The volume of the homogenate was made to 1 litre with water and the mixture well shaken. Immediately after shaking, duplicate 5 ml. amounts were pipetted into 100 ml. flasks using a wide-tipped pipette and 50 ml. lots of ethanol added. The flasks were shaken for five minutes in a "Microid" shaker and the ethanolic extracts separated by filtration. The ethanolic extract was diluted twenty-, fifty- and one hundred-fold with 0.5% acetic acid and reacted with ferric chloride. By comparing the colorimeter readings obtained (read against a reagent blank) with that from a standard solution of DPT (10 μg./ml.), the DPT (and/or metabolite) content of the faeces was calculated.

When this was less than 200 mg. the ethanolic extract was only diluted five-fold before reaction and the colorimeter reading compared with that obtained from a standard solution of DPT (10 μg./ml.) containing the same final percentage of ethanol (20%). This is necessary because high concentrations of ethanol raise the optical densities obtained by reaction of DPT with ferric chloride in dilute acid solution.

The presence of substances which inhibit the DPT-ferric chloride reaction in ethanolic extracts of faeces could not be demonstrated. Measurable colours were not obtained when these procedures were applied to faeces from subjects not receiving DPT.

Paper chromatography

The direct chromatography of urine from patients receiving DPT was not considered satisfactory due to the large amount of

urine solids which were unavoidably applied to the paper at the same time as the metabolites of DPT. These metabolites were therefore extracted from the urine in the following manner and the extracts thereby obtained used for chromatography. Samples of urine (100 ml.) were acidified with glacial acetic acid (1 ml.) and shaken with ammonium sulphate (50 g.), benzene (10 ml.) and ethyl acetate (10 ml.). The benzene-ethyl acetate extract was then separated by centrifugation.

Between 20 and 100 μg. of DPT and/or its metabolites was applied to each spot and the papers (Whatman No. 4) developed by ascending chromatography. After drying they were exposed to bromine vapour and the blue spots which formed marked immediately. Alternatively the papers were sprayed with a solution of 0.2% (w/v) ferric chloride in 0.5% (v/v) acetic acid. This reagent is more sensitive than bromine vapour but less convenient to use.

Results

(a) Excretion of "unchanged DPT" in the urine

(1) *By extraction of urine with benzene*

Twenty-four hour urine collections were made from six patients, each of whom was receiving 3 g. DPT daily in a single dose. The mean daily excretion of unchanged DPT was 0.5 mg. or 0.02% of the dose.

(2) *By extraction of dried urine with benzene*

Twenty-four hour urine collections were made from seven patients receiving 4.5 g. daily of DPT in three doses (1.5 g. at 7 a.m., 12 noon and 4 p.m.). The mean extraction of unchanged DPT was 3.6 mg. or 0.08% of the dose. (The concomitant excretion of DPT together with its metabolites totalled 365 mg. "total diphenylthioureas" or 8% of the dose.)

(b) Excretion of DPT together with its metabolites

(1) *As a function of time*

The hourly excretion of DPT together with its metabolites in the urine after daily dosage (7 a.m.) is shown in Fig. 1 and after twice daily dosage (7 a.m. and 2 p.m.) in Fig. 3. In order to study the amount of DPT metabolites excreted between 24 and 48 hours after a single dose (e.g. of 1.5 g.), more sensitive methods of estimation were developed which could estimate concentrations of DPT metabolites of as little as 1 μg./ml. These methods (to be published) showed that after a single dose of DPT only about 15% of the total excretion of DPT metabolites occurs during the 24–48 hour period. This figure, together with the data plotted in Fig. 1, was used in calculating the data for Fig. 2, which illustrates the exponential excretion of DPT metabolites during the period 1–15 hours after dosage. Thereafter excretion is less rapid.

TABLE II

Dosage	*No. tablets*	*Weight drug given g.*	*No. subjects*	*No.* determinations*	*Mean excretion "total diphenyl-thioureas" ± S.D. mg.*	*Range mg.*	*% Dose excreted in the urine*
Once daily (7 a.m.)	1	0.5	12	14	54± 25	22–110	10.8
	2	1.0	12	13	87± 54	49–220	8.7
	3	1.5	12	18	122± 38	57–191	8.1
	4	2.0	6	10	129± 42	76–181	6.5
	5	2.5	6	7	123± 36	67–175	4.9
	6	3.0	8	8	116± 36	65–176	3.9
	12	6.0	8	11	148± 30	60–173	2.5
Twice daily (7 a.m. and 2 p.m.)	2×1	1.0	7	8	135± 81	40–298	13.5
	2×2	2.0	7	11	207± 93	86–374	10.4
	2×3	3.0	17	21	266± 88	126–412	8.8
Thrice daily (7 a.m., 12 noon and 4 p.m.)	3×1	1.5	1	13	161	—	10.7
	3×3	4.5	13	29	363± 87	179–491	8.1
Schedule A†	13	6.5	9	9	375±111	218–550	5.8
Schedule B	16	8.0	10	10	401±186	169–760	5.0
Schedule C	18	9.0	6	6	360±148	182–594	4.0

* When more than one study was made on a single patient at a given dosage, the mean of the results obtained was used for the calculation of the mean excretion of the group on that dosage.

† Schedule A—3 tablets at 7 a.m. and 2 p.m. 1 tablet at 8, 9, 10, 11 a.m., 12 noon, 3 and 4 p.m.
B—3 tablets at 7 a.m. and 2 p.m. 1 tablet at 8, 8.30, 9, 9.30, 10, 10.30, 11, 11.30, 12 noon and 4 p.m.
C—3 tablets at 7 a.m. and 2 p.m. 2 tablets at 8, 9, 10, 11 a.m., 12 noon and 4 p.m.

(2) *As a function of dosage*

The results are presented in Table II and illustrated in Fig. 4.

(3) *Daily variation in the excretion of DPT metabolites in the urine by a single patient on two drug regimens*

The daily excretion of DPT metabolites in urine by one patient receiving three tablets once daily (7 a.m.) for seven consecutive days and then one tablet thrice daily (7 a.m., 2 and 6 p.m.) for thirteen consecutive days was studied. The mean excretions of "total diphenylthioureas" for these regimens were 57 $\pm$16 mg. (range 29–73 mg.) and 161 $\pm$ 32 mg. (range 106–208 mg.) respectively.

(c) Excretion of DPT (and/or its metabolites) in the faeces

Twenty-four, 24 hour collections of faeces were analysed for DPT (and its metabolites) from patients receiving their normal daily dosage of DPT and the mean percentage of the dose found in the faeces was 75. In a subsidiary study one subject, who had not received any DPT before, was given 4 g. DPT orally and the excretion of DPT in the faeces followed as function of time. The major part of the excretion occurred within three days. DPT could not be demonstrated in the faeces after the fifth day.

(d) Nature of the substances estimated

(1) *As DPT together with its metabolites in the urine*

The results reported in sections (a) and (b) show that less than 1% of the compounds reacting like DPT in the urine of patients receiving oral DPT are extractable into benzene. It must therefore be concluded that after absorption DPT is almost completely metabolized by man.

Further evidence is provided by paper chromatographic studies. DPT fails to move in water or 10% aqueous ammonia, has an R_F of 0.72 in 10% (v/v) acetic acid and moves at the solvent front in ethanol, n propanol, n butanol and benzene. In contrast two metabolites of DPT, obtained by the extraction procedure described above, fail to move in n butanol or benzene, have convenient R_{Fs} in 10% (v/v) acetic acid (0.77 and 0.49 respectively), water and 10% aqueous ammonia, and move at the solvent front in ethanol.

(2) *As DPT (and/or its metabolites) in the faeces*

Paper chromatography of the ethanolic extracts of faeces revealed the presence of only one substance which reacted with bromine or ferric chloride. This moved in an identical fashion to DPT in the eight solvent systems tested. (The most suitable solvents were 5%, 10% and 20% (v/v) acetic acid.) Dried faeces on extraction with benzene gave substances reacting like DPT which chromatographed in the same way. Aqueous extracts of faeces however did not give any colour on reaction with ferric chloride.

It was therefore concluded that metabolites of DPT are not present in the faeces of patients receiving the drug orally, and that the results show the amounts of the unchanged drug voided by this route.

Discussion

Details have been given of methods for the estimation of 1-(p-dimethylaminophenyl)-3-(p-butoxyphenyl)-2-thiourea (DPT) and other p-dimethylamino diphenylthioureas. Methods are described for the estimation of DPT and its metabolites which are accurate to within $\pm 10\%$ at urine concentrations of down to 20 μg./ml. and for daily excretions in the faeces of down to 50 mg.

These methods have been used to study the absorption, metabolism and excretion of DPT in man. There was a considerable daily variation in the amount of DPT metabolites excreted in the urine by a patient on a given dosage and an even greater variation between different subjects.

Since there is no justification for assuming that the metabolites of DPT cannot be extracted to a certain extent into benzene, it must be concluded that the amount of unchanged DPT excreted in the urine each day by patients receiving a therapeutic dose of DPT (1.5 g. or more once daily) is less than 1 mg. This raises the possibility that the antileprotic activity of DPT may be due to its metabolites rather than to the unchanged compound itself. It is also possible that there may be other diphenylthioureas, as yet untested, possessing considerable antitubercular and antileprotic activity.

When DPT was given to man by injection, metabolites of DPT were readily demonstrated in the urine but no DPT could be detected in the faeces (unpublished results). It was therefore concluded that biliary excretion of DPT (an important route in the rabbit, SCHMID and TRIPOD, 1959) does not occur to any significant extent in man. Consequently the unchanged drug which is eliminated in the faeces and which totals about 75% of the dose, is that part of the drug which had not been absorbed. Hence the absorption of DPT by man (only about 10% of an oral dose) is considerably inferior to that in the rabbit or dog (SCHMID and TRIPOD, 1959).

These findings emphasise the difficulty in choosing compounds for clinical trials in human leprosy (ELLARD, 1960b).

Not only may there be differences in the absorption, metabolism and excretion of a drug between the experimental animal and man, but differences are also to be expected in the susceptibility of *M. leprae*, as compared to *M. tuberculosis*, to a given drug or its metabolite. It is therefore extremely unlikely that the most active member of a group of compounds in suppressing experimental tuberculosis

will also prove to be the most useful compound in the treatment of human leprosy.

Eisman, Konopka and Mayer (1957) have already concluded that another diphenylthiourea, 1-(p-ethoxy-phenyl)-3-(p-isobutoxy-phenyl)-2-thiourea, which is highly active in the treatment of tuberculosis in the mouse and guinea pig, fails to control human pulmonary tuberculosis due to insufficient absorption.

The rather disappointing results obtained when DPT was used in the treatment of human tuberculosis may also be due to its poor absorption by man. The marked activity of DPT in treating human leprosy might therefore be due to *M. leprae* being more susceptible to DPT or its metabolites than *M. tuberculosis*.

Once DPT has been absorbed from the gut it is rapidly excreted in the urine in the form of benzene-insoluble metabolites. It has a biological half-life in man of about six or seven hours. These findings explain the need for daily dosage previously noted by Davey and Currie (1956).

The demonstration that maximal absorption of DPT occurs after a dose of 1.5 g. is of great importance. This accounts for the fact that higher doses than this are not toxic and do not give improved therapeutic effect. (Alonso, 1958; Gate *et al.*, 1958; Mukherjee and Ghosh, 1958.) Davey (1958), had concluded from his clinical observations that optimal therapeutic effect was achieved with a daily dose of 2 g. These studies have shown that the amount of DPT absorbed can be greatly increased by giving 1.5 g. of the drug several times each day. Thus 1.5 g. given twice daily (at 7 a.m. and 4 p.m.) doubles the amount of drug absorbed each day. The amount absorbed can be trebled by giving 1.5 g. DPT thrice daily (at 7 a.m., 12 noon and 4 p.m.). Figure 4 illustrates the finding that further increase and subdivision of the daily dose does not result in any further increase in the amount of drug absorbed. It will be apparent that poisoning by DPT is virtually impossible.

A group of seven patients received DPT twice daily for two years during a clinical trial at this research centre (Garrod, 1959). No toxic effects were observed. Their bacillary improvement was slightly superior to that of the parallel single dosage group but in view of the small number of cases studied, this was not significant (Garrod, personal communication). The appearance of *M. leprae* resistant to DPT was however significantly retarded in the group who received DPT twice daily (Annual Report, East African Leprosy Research Centre, 1960). This suggests that increased daily absorption of DPT may result in improved therapeutic effect.

At this Centre patients have been treated for up to six months with the triple dosage without evidence of significant toxicity. Whether or not this regime is superior in therapeutic effect to that employing twice daily dosage remains to be investigated.

Summary

1. Details are given of a colorimetric method for the estimation of 1-(p-dimethylaminophenyl)-3-(p-butoxyphenyl)-2-thiourea (DPT) and other p-dimethylamino diphenylthioureas, and its application to the measurement of DPT and its metabolites in the urine and faeces of patients receiving the drug orally.

2. Only about 10% of an oral dose of 1.5 g., or less, is absorbed and about 75% is eliminated unchanged in the faeces. Over 99% of that part of the dose which has been absorbed is rapidly excreted in the urine in the form of benzene-insoluble metabolites.

3. Maximal absorption of DPT occurs after a dose of 1.5 g. Further increase in dosage does not result in a significant increase in the amount of drug absorbed. The amount of DPT absorbed each day can however be increased by giving 1.5 g. of the drug twice or thrice daily. Further increase or subdivision of the daily dose does not result in any further significant increase in the amount of drug absorbed.

4. The clinical importance of these findings is considered and the literature concerning the treatment of human leprosy with this drug interpreted in the light of these findings. The implications of the extensive metabolism of DPT by man are also discussed.

5. It is suggested that for optimal therapeutic effect 1.5 g. of DPT should be given thrice daily.

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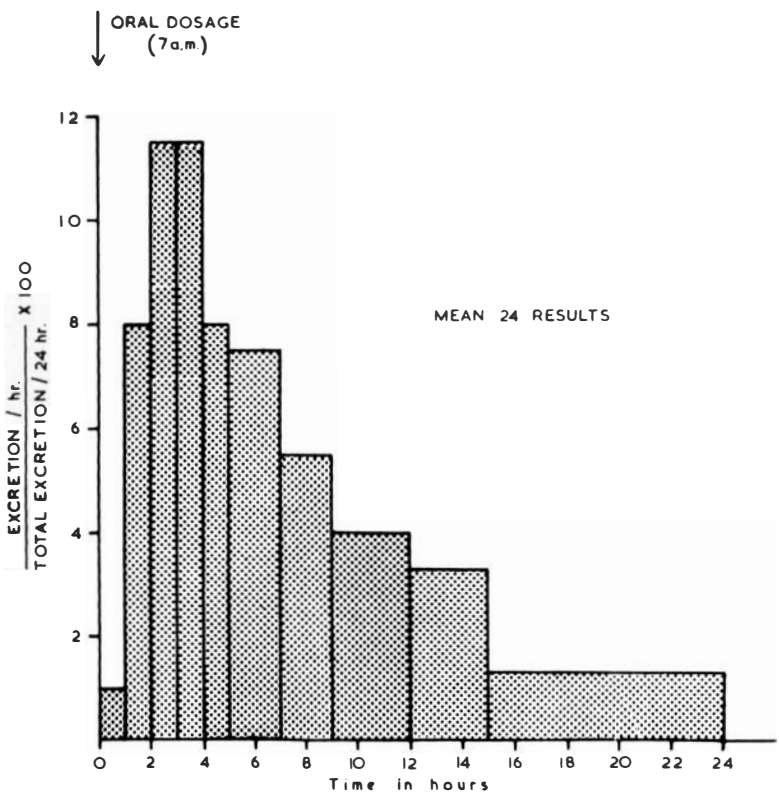

FIG. I. GRAPH SHOWING THE HOURLY EXCRETION OF D.P.T TOGETHER WITH ITS METABOLITES IN THE URINE AFTER DAILY DOSAGE

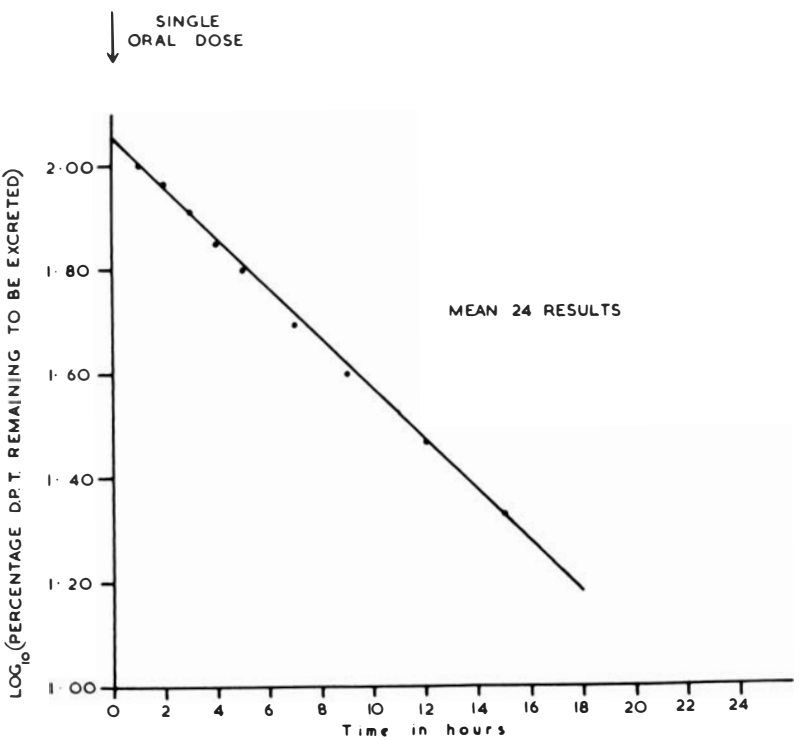

FIG. 2. GRAPH SHOWING THE EXPONENTIAL URINARY EXCRETION OF D.P.T. TOGETHER WITH ITS METABOLITES

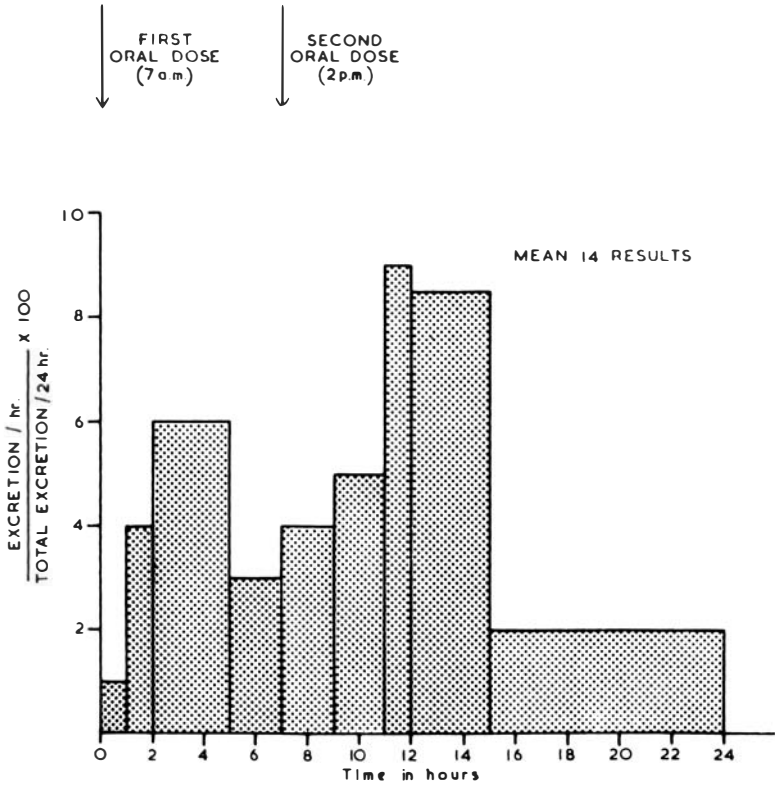

FIG. 3. GRAPH SHOWING THE HOURLY EXCRETION OF D.P.T. TOGETHER WITH ITS METABOLITES IN THE URINE AFTER TWICE DAILY DOSAGE