EXPERIMENTAL INVESTIGATION OF THE ABSORPTION AND EXCRETION OF CIBA-1906 (DPT) [1]

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Experimental and clinical studies with CIBA-1906, hereafter referred to as DPT, had suggested that a large proportion of the administered dose was excreted unchanged in the faeces, since in urine and blood no measurable concentration or only a fraction of the quantity administered could be demonstrated. These results were obtained by colorimetric methods of estimation. In our laboratories we used the colour produced when DPT in alcoholic solution is allowed to react with bromine in carbon tetrachloride. Other authors employed the colour produced by the reaction of DPT with ferric chloride[2].

In view of the uncertainty of the findings obtained with colorimetric estimations and in view of the practical importance of knowing the exact degree of absorption of DPT under various conditions it was decided to undertake studies with radio-active labelled material. Only in this way did it seem possible to obtain quantitative values for the absorption and distribution of the substance in the organism and also an indication of the metabolic pathways in various species of animal.

The following is an account of the results of our experiments with C^{14}—and S^{35}—labelled—DPT in the rabbit and dog. However, it must be remembered these are only preliminary findings which need to be verified by further work.

Method

(*a*) *Synthesis and Estimation of Labelled DPT*

In principle DPT may be labelled with C^{14} in various ways. In the method chosen by us, as indicated in Fig. 1, we used as starting material the readily available barium-C^{14}-carbonate as a source of radio-active carbon. The carbon-dioxide liberated from this carbonate was allowed to react with propyl magnesium bromide to produce butyric acid. The latter was esterified and after reduction the butyl alcohol obtained was converted into butyl bromide. Reaction of the butyl bromide with acetamino-phenol and subsequent hydrolysis gave p-butoxyaniline, which on reaction with p-dimethyl-aminophenyl-iso-thiocyanate gave DPT (overall yield with respect to barium carbonate: 12%, specific activity ca. 0.2μC/-mg). The labelled DPT thus obtained bears now in the butoxy side-chain a radio-active carbon atom.

SYNTHESIS OF C^{14}-DPT

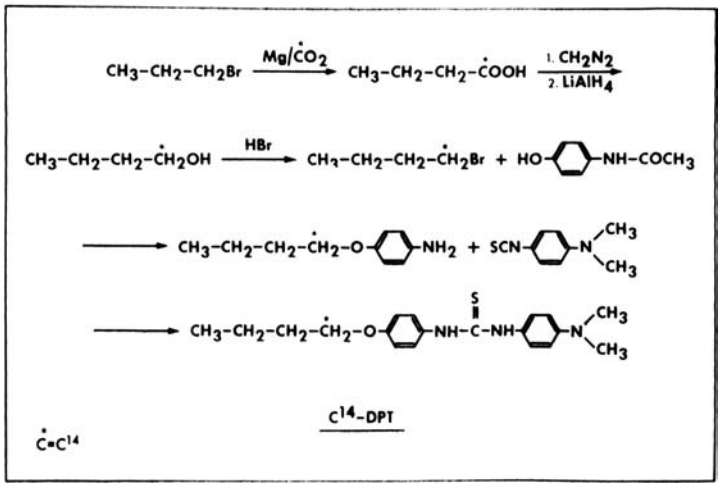

FIG. 1
Synthesis of C^{14}*-labelled DPT*

S^{35} labelled DPT was prepared as indicated in Fig. 2 according to the usual laboratory method, using radio-active carbon disulphide. S^{35}-carbon-disulphide was allowed to react with p-dimethylphenyl-aniline to give the corresponding isothiocyanate, which in turn reacts with p-butoxyaniline to give DPT in good yields (overall yield with respect to carbon disulphide: 78%, specific activity ca. 2.7 μC/mg).

SYNTHESIS OF S^{35}-DPT

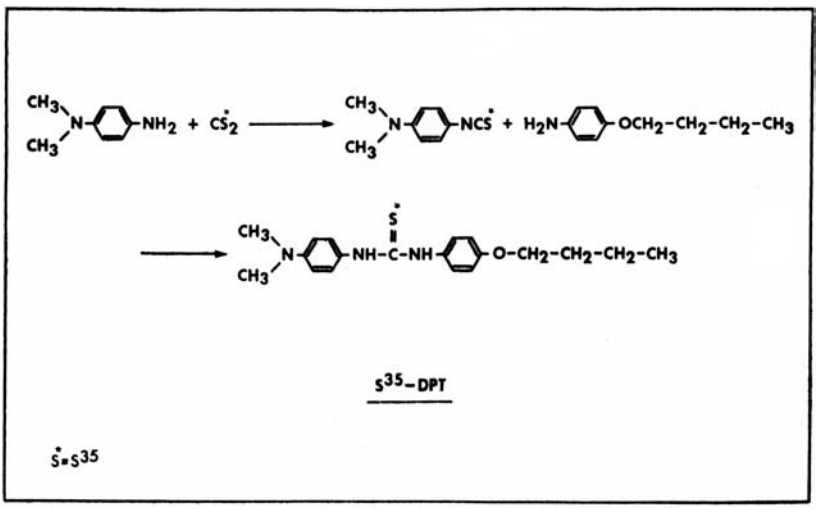

FIG. 2
Synthesis of S^{35}*-labelled DPT*

For the estimation of the radio-activity, known volumes of blood, urine, and bile or weighed portions of fresh faeces or tissue were first brought to dryness. In the case of the C^{14}-experiments the samples were converted to carbon dioxide by wet combustion, which after purification was measured in a gas counting tube. In the case of the S^{35}-experiments the samples were submitted to alkali fusion, and the sulphate obtained isolated as the barium-salt which when dried was used for counting in the solid state. These methods will be published in detail elsewhere. In the experiments described hereafter all values for DPT and its metabolites are expressed as DPT calculated on the basis of the measured radio-activity.

(*b*) *Studies in the Rabbit*

Rabbits of an average weight of 2 kilos received 0.03 g/kg DPT either by stomach tube in the form of a 1% suspension or intravenously as a 3% solution in polyethylene-glycol-400. For the determination of the blood concentration 1 ml. portions of blood were withdrawn at predetermined intervals from the ear vein. Urine and faeces were collected over 24 and 48 hour periods. Bile samples were collected through a cannula in the common bile duct simultaneously with the blood samples. In cases in which operative intervention was undertaken the rabbits were narcotised with urethane (0.8 g/kg s.c.). This dose of urethane is lower than that which is usually necessary to produce adequate anæsthesia (1.2 g/kg). This reduction in the dosage is possible because as a result of the intravenous injection of the solution of DPT in polyethyleneglycol-400, sedation and hypnosis is obtained which is evident even with a dose of 0.01 g/kg, and at a dose of 0.04-0.05 g/kg produces a state equivalent to narcosis. The solvent alone, at a dose of 1 ml/kg is without observable effect.

(*c*) *Studies in the Dog*

In these experiments DPT was administered orally at a dose of 0.03 g/kg as capsules of pure substance, or by the subcutaneous or intravenous route as a 3% solution in polyethyleneglycol-400. The amount of 2-3 ml. blood was taken for estimation at fixed intervals. Urine and faeces were collected during 24 and 48 hour periods. This dose of DPT (0.03 g/kg i.v.) produced in the dog only slight sedation of short duration, which was clearly less than that observed in the rabbit.

Results

(*a*) *Single Administration in Rabbits*

After the intravenous injection of 0.03 g/kg C^{14}-DPT the maximum blood concentration is reached shortly after the end of the injection and falls steeply up to the fifth hour, after which it slowly returns to zero (Fig. 3). With S^{35}-DPT the values in the non-narcotised animals are somewhat higher in the first hour than in

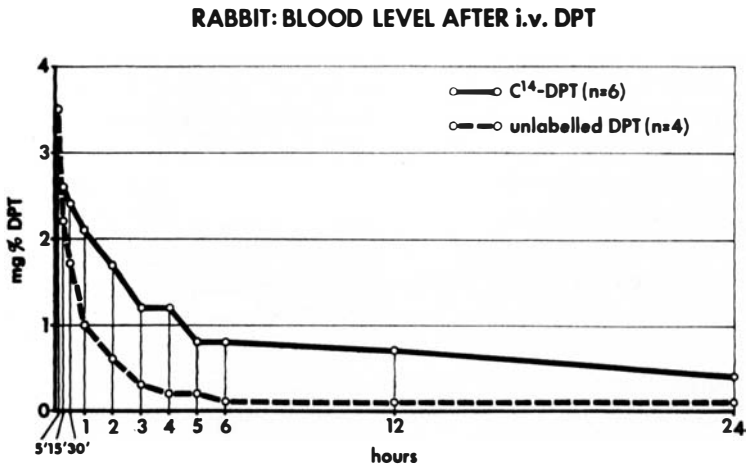

FIG. 3
Rabbit: Blood concentration after single i.v. dose (0.03 *g/kg*) *of DPT*

C^{14}-experiments after which time, however, the concentration curves approximate. With the exception of the first two, the values estimated colorimetrically are lower, the curve falling more steeply and reaching the baseline after six hours.

After oral administration of a suspension the blood concentration is considerably lower than after intravenous injection. The maximum reached is about 0.4 mg% and is only reached after about three to

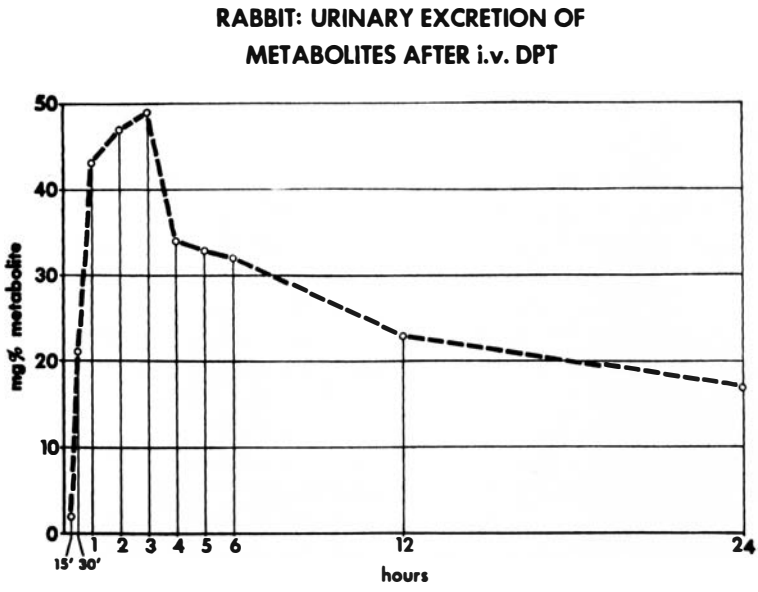

FIG. 4
Rabbit: Urinary excretion of DPT-metabolites after single i.v. dose (0.03 *g/kg of DPT* ($n = 4$).

six hours. After 12 to 24 hours the values have fallen below the detectable limits for the C^{14}-preparation employed (0.1 mg%).

After a single intravenous dose the maximum concentration in the urine is reached after three hours and up to this time about 20% of the administered radio-activity is demonstrable in the urine. Following this the urinary concentration slowly falls, but is still 17 mg% at 24 hours (Fig. 4).

Using the colorimetric method the maximum concentration found in the urine was 0.1 mg%, whereas using the radio-active method concentrations up to 50 mg% were demonstrated. *It is therefore clear that DPT is excreted in the urine substantially in the form of metabolites.*

The distribution of DPT in various organs was determined 4 and 24 hours after intravenous and 24 hours after oral administration (Table 1). It is remarkable that after intravenous injection, *radio-active material was demonstrable in the wall and also the lumen of the gastro-intestinal tract, so that it must be concluded that a part*

RABBIT: DISTRIBUTION OF DPT AND METABOLITES IN THE ORGANS AFTER SINGLE ADMINISTRATION (0,03g/kg)

	mg per organ		
	i. v.		p. o.
	4 hrs.	24 hrs.	24 hrs.
stomach cont.	5,0	5,5	7,1
" wall	1,1	0,1	0,04
small intest. cont.	2,2	3,8	0,6
" " wall	2,8	0,3	0,2
bile	0,03	0,02	0,01
large intest. cont.	3,8	11,3	11,0
" " wall	2,3	0,1	0,02
spleen	0,01	0,04	0,0
kidney	1,1	0,8	0,1
adrenal	0,0	0,0	0,0
liver	1,8	0,6	0,5
lung	0,02	0,09	0,04
heart	0,1	0,04	0,01
bones	18,1	1,3	0,0
muscle	13,3	2,1	0,4
skin	1,8	0,7	0,0
subcut. fat	0,3	0,0	0,0
brain	0,2	0,01	0,0

TABLE 1

Rabbit: Distribution of DPT and DPT-metabolites in the organs after single administration (0.03 *g/kg*) *of DPT.*

of the administered drug is excreted through the intestinal wall. Furthermore with the exception of the bones which show a relatively high concentration after four hours no especially marked concentration of radio-active material was observed in any of the other organs or tissues examined, in fact the distribution was approximately the same as is found with other drugs. Especially interesting with regard to leprosy is the fact that neither in the skin nor subcutaneous fat could any specially marked concentration of radio-active material be found. After 24 hours only traces of radio-active material were detectable in the tissues since at this time the majority of the administered dose has already been excreted in the urine.

Comparison of the concentration in the urine with that in the faeces confirms the supposition already made on the basis of the tissue analyses, that after intravenous administration radio-active material is excreted through the intestine (Table 2).

RABBIT: EXCRETION OF DPT AND METABOLITES AFTER i. v. AND p. o. DPT

	24 hrs. excretion in % of administered dose		
	i. v.		p. o.
	C^{14} (n=1)	S^{35} (n=3)	C^{14} (n=3)
stomach cont.	7,1	12,1	12,6
small intest. cont.	5,0	0,9	0,8
large intest. cont.	14,7	11,1	17,5
	26,8	24,1	30,9
urine	48,0	54,1	54,9
faeces	—	7,8	6,6

TABLE 2
Rabbit: Excretion of DPT and DPT-metabolites during 24 hours after single i.v. and p.o. administration (0.03 g/kg) of DPT.

The quantity demonstrable in the faeces after 24 hours is approximately the same when DPT is given by either route of administration. Similarly the excretion in the urine after intravenous injection is comparable with that obtained after oral administration and amounts to over 50% of the quantity given. Therefore it can be concluded that in the rabbit, DPT is well absorbed after oral administration and that no essential difference exists between the routes of excretion following the two methods of administration.

In order to clarify further the mode of excretion of DPT into the gastro-intestinal tract, the concentration of the substance in the bile was estimated (Fig. 5).

RABBIT: BILIARY EXCRETION OF METABOLITES AFTER i.v. DPT

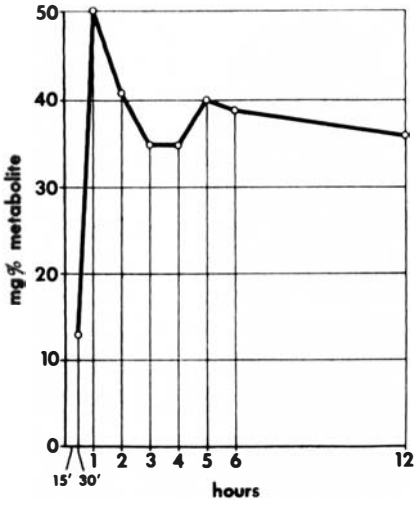

FIG. 5
Rabbit: Excretion of DPT-metabolites in the bile after single i.v. administration (0.03*g*/*kg*) *of DPT* ($n = 2$).

This demonstrated that after intravenous injection about 20% of the dose was excreted in the bile. The maximum concentration reached 50 mg% one hour after the injection and remained at approximately the same value during the following hours. Even after 12 hours 30 mg% was still present.

However, the biliary excretion does not represent the only source of the material demonstrable in the intestine after intravenous injection, since even with an occluded bile duct 20% of the total dose appeared in the intestinal wall and contents (Table 3). It may therefore be concluded that a proportion of the radio-active material is excreted directly through the intestinal mucosa.

RABBIT — BILIARY FISTULA OR OCCLUDED BILE DUCT: EXCRETION OF DPT AND METABOLITES AFTER i. v. DPT

	12 hrs. excretion in % of administered dose	
	biliary fistula (n=3)	occluded bile duct (n=1)
stomach cont.	16,1	9,9
small intest. cont.	1,7	5,0
large intest. cont.	4,5	5,7
	22,3	20,6
bile	20,1	—
urine	43,7	47,8
faeces	—	—

TABLE 3
Rabbit: Excretion of DPT and metabolites during 12 *hours by animals with biliary fistula or occluded bile duct after single i.v. administration* (0.03 *g*/*kg*) *of DPT.*

Furthermore, it is clear that excretion through the stomach wall also occurs since in the intact animal and also in the animal with a biliary fistula or occluded bile duct about 7-16% of the administered dose is found in the stomach contents (see Table 2).

After subcutaneous injection different excretion patterns occur depending on whether the preparation is given as a solution in polyethyleneglycol-400 or as a suspension. Neither in blood nor faeces did a measureable concentration appear, probably as a result of the slow absorption from the depot at the site of injection. On the other hand material appeared in the *urine* after subcutaneous injection of both forms. The higher concentration which was excreted after the injection of DPT in solution shows that absorption, as would be expected, is more rapid than after the injection of the suspension (Fig. 6).

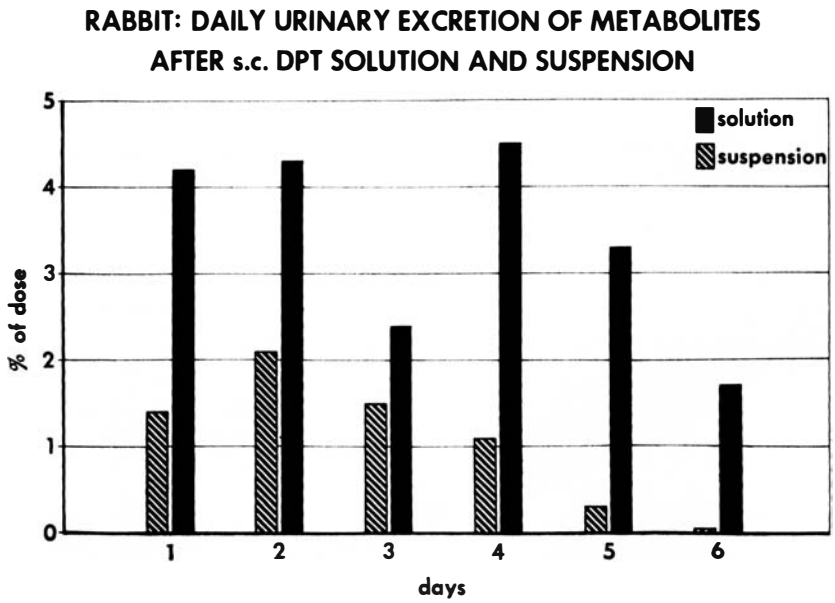

FIG. 6
Rabbit: Daily excretion of DPT and metabolites in the urine after single s.c. injection (0.03 *g/kg*) *of DPT in solution and suspension* ($n = 2$).

The incomplete absorption following subcutaneous injection is also manifest by the persistence of material at the site of injection after six days, which also occurs even when the preparation is injected in solution. It is probable that, as is also the case with other drugs in solution, the absorption of the solvent and the dissolved substance does not proceed at equal speed so that precipitation of the compound occurs at the site of injection. Thus it is clear that a depot effect must obtain after subcutaneous injection of DPT.

(*b*) *Single Administration in Dogs*

In order to clarify the specificity of the excretion pattern for

various animal species experiments analogous to those carried out in rabbits were also undertaken in the dog. Intravenous injection gave, in general, blood concentrations comparable to those found in the rabbit (Fig. 7). For technical reasons the C^{14} values could only be estimated up to two hours after the injection, whereas with S^{35} labelled material observations were carried out for 48 hours.

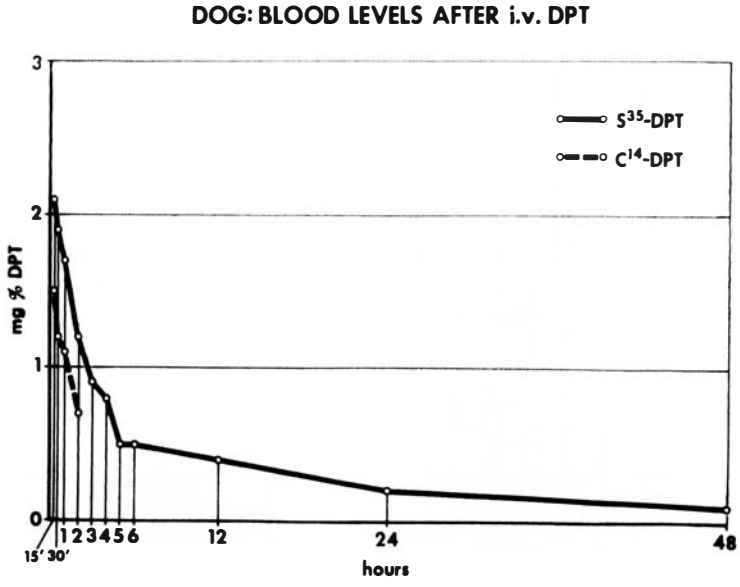

FIG. 7

Dog: Blood concentration after single i.v. injection (0.03 *g/kg*) *of DPT* ($n = 2$).

The tissue concentrations in the dog were not measured. After subcutaneous and oral administration no measurable blood concentration was detected.

The urinary excretion after oral S^{35}-labelled substance and especially after C^{14}-material was much less than in the rabbit over the first 48 hours (Table 4). Similarly the faecal levels were higher. Clearly the absorption after oral administration is less complete in the dog than in the rabbit.

After subcutaneous injection of DPT dissolved in polyethyleneglycol-400, 39.2% was excreted in the urine and 36.9% in the faeces. The ratio is about the same as after intravenous injection but with the difference that after subcutaneous administration the excretion was more prolonged (Fig. 8). In this respect the findings are similar to those in the rabbit experiments, the urinary excretion being significantly delayed, probably as a result of the retarded absorption of the subcutaneous depot. The quantity of material eliminated in the urine, however, is less in the dog than in the rabbit which is also the case after intravenous injection. The cause of this species difference is not known.

DOG: EXCRETION OF DPT AND METABOLITES AFTER i. v. AND p. o. DPT

	48 hrs. excretion in % of administered dose			
	i. v.		p. o.	
	C^{14} (n=2)	S^{35} (n=2)	C^{14} (n=1)	S^{35} (n=3)
urine	31,5	38,0	11,4	27,2
faeces	39,1	28,2	57,1	57,0

TABLE 4
Dog: Excretion of DPT and metabolites in urine and faeces after single i.v. and p.o. administration (0.03 *g/kg*) *of DPT.*

DOG: DAILY EXCRETION OF DPT AND METABOLITES IN FAECES AND URINE AFTER s. c. DPT

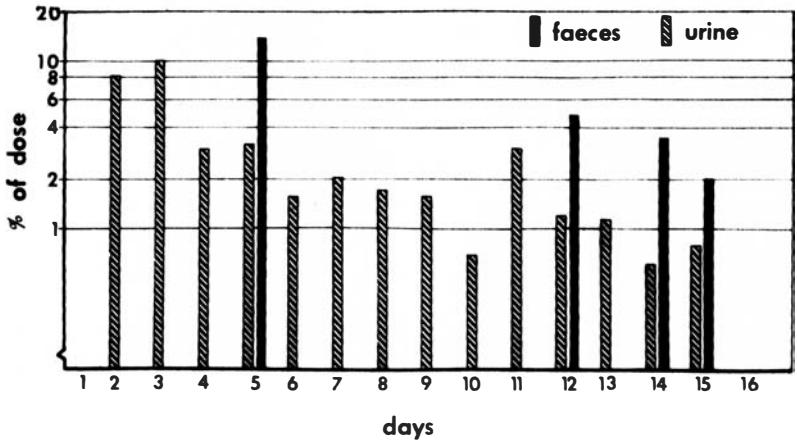

FIG. 8
Dog: Daily excretion of DPT and metabolites in urine and faeces after single s.c. administration (0.03 *g/kg*) *of DPT* ($n = 1$).

(*c*) *Repeated Administration in the Rabbit*

In order to determine whether accumulation of DPT occurs in particular organs 0.03 g/kg labelled-DPT was administered to rabbits daily for one week. One day after the last dose the animals were

killed and the radio-activity in various tissues measured. As after the single oral administration the blood concentration, estimated once daily remained below 0.1 mg% during repeated daily dosage. In the tissues studied, higher concentrations were not obtained than 24 hours after a single dose (see Table 1). There is therefore no evidence of a cumulative effect in the tissues. In contrast the majority of the daily intake was excreted in the urine and faeces within a short time (Fig. 9). Of the total dose given over seven days 61 % was found in the urine, 14% in the faeces and 14% in the intestinal tract of the sacrificed animals.

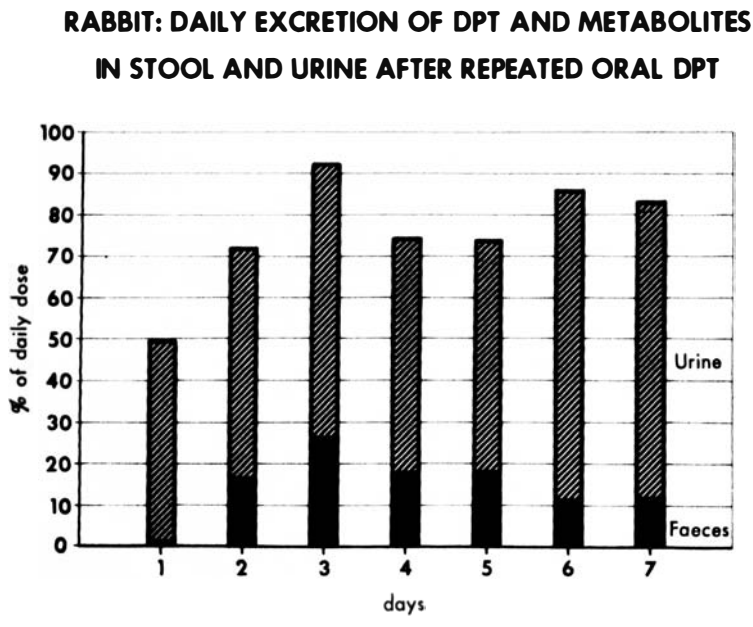

FIG. 9
Rabbit: Daily excretion of DPT and metabolites in urine and faeces after repeated p.o. administration (0.03 *g/kg*) *of DPT* ($n = 2$).

(*d*) *Experiments on the Isolation and Identification of Metabolites of DPT in Urine*

The studies on the isolation and identification of degradation products of DPT and the determination of their biological activity are incomplete and do not yet allow any definite conclusions regarding the metabolism of the drug to be drawn.

In one preliminary experiment about 100 ml urine was collected from one rabbit which had received intravenous C^{14}-DPT. On the basis of its radio-activity this urine contained about 20 mg DPT-metabolites from which by extraction with benzol-butanol, 4 mg of extract, containing 75-80% of the radio-active components, was isolated. The *in vitro* tuberculostatic activity of this fraction was found to be 100 times less than that of DPT[3]. This activity can either

be attributed to traces of unchanged DPT or to metabolites with weaker tuberculostatic activity than the parent compound. Which of these two possibilities is valid will be decided in further experiments.

Using different methods of extraction, the pooled urine of several rabbits yielded only about 50% of the available radio-active metabolites. Only after hydrolysis of the urine by boiling with acid was it possible to extract further quantities of radio-active material. This finding shows that part of the excretion products are in bound form. Paper chromatographic analysis showed that the extract is composed of at least two different metabolites. In further purification experiments these have been found to be rather unstable, so that up to now it has not been possible to isolate either of them in pure form, and therefore their nature can only be guessed at. However, all the indications are that they are probably carboxylated or hydroxylated derivatives of DPT, which are excreted partly in the free and partly in the conjugated form.

Discussion

The most important conclusions to be drawn from the experiments which have been described are the clear demonstration that absorption does occur after oral administration in the dog and the rabbit and secondly the fact that DPT is excreted unchanged in the urine only to a very small degree. For these reasons all the earlier results obtained using the characteristic color reactions for DPT were misleading.

It is not yet possible to deduce where and how the degradation of DPT is accomplished in the organism, since definite metabolites have not yet been identified in urine, bile or blood. For the same reasons also it is not possible to give any information regarding the tuberculostatic or anti-leprotic activity of these metabolites. Furthermore it is not yet possible to ascertain whether the degradation pathways in various animal species and in man are the same nor whether the resulting intermediate and end products are identical.

An important new finding is the demonstration of the excretion of DPT-metabolites in the bile and through the intestinal wall, so that the liver must be regarded as being involved in metabolism and excretion. Whether a true "enterohepatic circulation" exists so that the material excreted in the bile is again re-absorbed through the intestinal wall is not yet verified. However, it seems probable that the greater part of the substance secreted in the bile is also eliminated in the faeces. Therefore all the material demonstrable in the faeces has not failed to be absorbed but in contrast some of it has passed through the liver suffered degradation or modification and returned to the intestine. In a few preliminary experiments in the dog in which the faeces were extracted after oral administration, unchanged DPT was identified, so that it must be concluded that in this species a

proportion of the oral dose is not absorbed but passes through the intestinal tract unchanged.

On the basis of the failure to demonstrate DPT in the blood or urine of patients after oral administration using colorimetric methods it was concluded that absorption from the gastro-intestinal tract was minimal. Assuming that the mode of absorption in man is not entirely different from that in the dog and rabbit, the above conclusion is not tenable. On the contrary it seems more likely that even after oral administration the majority of the drug enters the organism. However, the question whether DPT itself possess tuberculostatic or anti-leprotic activity or if the effects are due to a metabolite cannot yet be definitely answered. *What is clear is that DPT is rapidly and completely metabolized after absorption*, and whether the products of this degradation possess antibacterial activity or not must be clarified by further experiments.

References

1. Presented in part at the 7th International Congress on Leprology in Tokyo (November 12th—19th November 1958).
2. J. ROSS INNES, M. SMITH and W. HARDEN SMITH, East African Medical J. **34**, 395 (1957).
3. F. KRADOLFER, private communication.