THERAPEUTIC IMPLICATIONS OF DRUG METABOLISM

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Information on how drugs are metabolized is not only of importance in understanding their therapeutic action, but can furnish useful clues for the development of new drugs. The various aspects of drug metabolism studies which are considered in this paper are (i) Pathways of drug metabolism—conversion to active, inactive or toxic metabolites; (ii) Structural changes which determine a drug's metabolism and excretion; (iii) Species differences in drug metabolism; (iv) Enzyme induction as an important factor in drug safety evaluation.

PATHWAYS OF DRUG METABOLISM

Our knowledge of drug metabolism has increased rapidly in recent years. Brodie, Axelrod and coworkers[1,2] made the important observation that many clinically useful drugs are metabolized by enzymes in liver microsomes. These enzymes are quite versatile in that they can metabolize drugs by many reactions, including *N*-dealkylation, ether cleavage, hydroxylation, deamination and glucuronide formation. Examples of these reactions will be given here and, when possible, therapeutic implications will be pointed out.

Acetophenetidine

The major route for the drug's biotransformation involves ether cleavage to form *N*-acetyl-*p*-aminophenol (acetaminophen) which has been thought to be responsible for the analgesic activity of acetophenetidine[3] (*Figure 1*). However, this view has been questioned by recent experiments which show that acetophenetidine has analgesic activity in rats even when its metabolism to *N*-acetyl-*p*-aminophenol was blocked[4]. Acetophenetidine and acetaminophen are both converted to a slight extent to deacetylated products which are presumably responsible for the small amount of methemoglobinemia produced by these drugs in man. Recent studies have shown that about 1 per cent of a dose of acetophenetidine to man, dog and cat is excreted in urine as the glucuronide of 2-hydroxy-acetophenetidine[5]. Despite the considerable effort required, it is important to identify minor metabolites of a drug since they may be responsible for some of the drug's pharmacologic and toxicologic actions. In this case 2-hydroxy-acetophenetidine lacks analgesic activity and does not possess any unusual toxicity.

Phenylbutazone

Metabolic data have been of considerable importance in understanding the action of phenylbutazone[6]. The drug is metabolized slowly in man to two

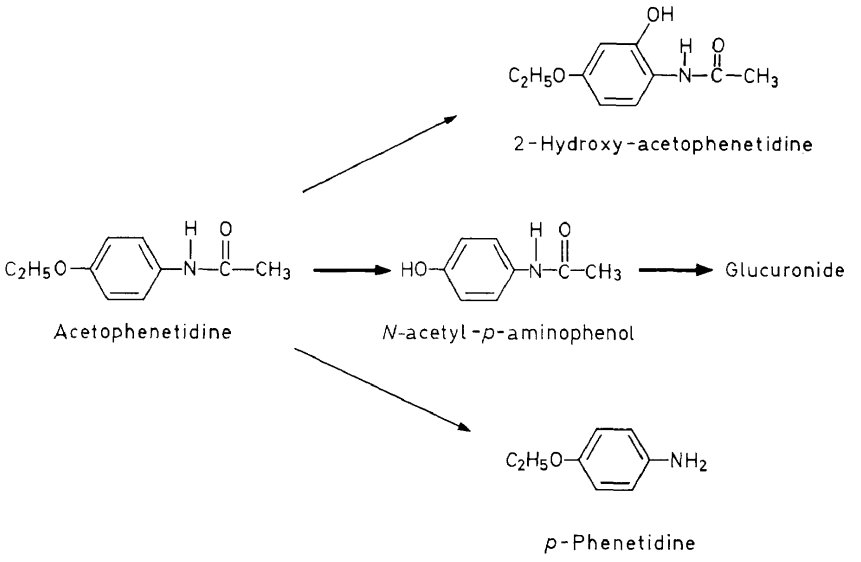


Figure 1. Metabolism of acetophenetidine.

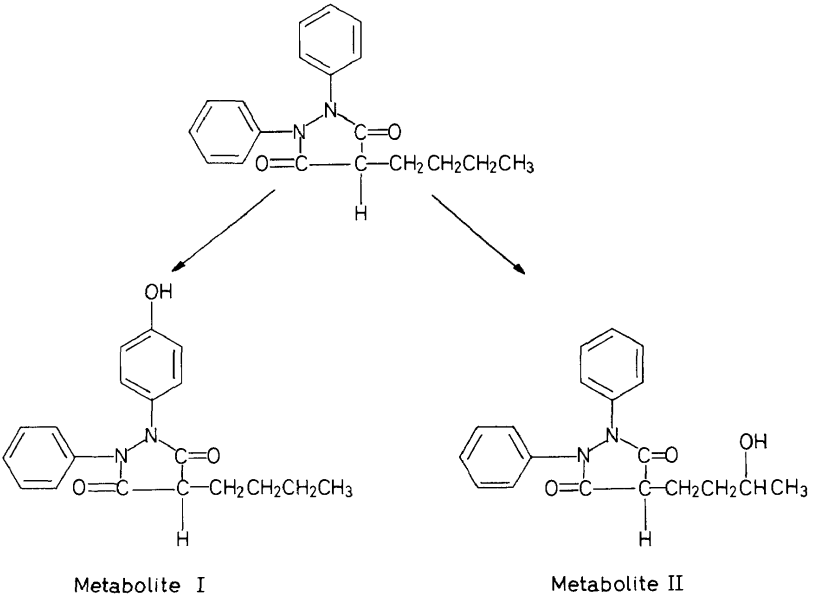


Figure 2. Metabolism of phenylbutazone in man.

metabolites which accounts for some of its pharmacologic activity (*Figure 2*). Metabolite I, which is formed by hydroxylation in the benzene ring, possesses the anti-inflammatory and sodium-retaining effect of the parent drug, whereas Metabolite II, which is formed by hydroxylation in the butyl side chain, accounts for the uricosuric effect of phenylbutazone. Metabolite I, now known as oxyphenbutazone, has been introduced as a new drug for the treatment of arthritis and gout. The observation that substitution in the side chain of phenylbutazone resulted in enhanced uricosuric activity led to the discovery of several other agents which have this action[6]. One of these drugs, sulphinpyrazone, is a potent uricosuric agent for the therapy of gout.

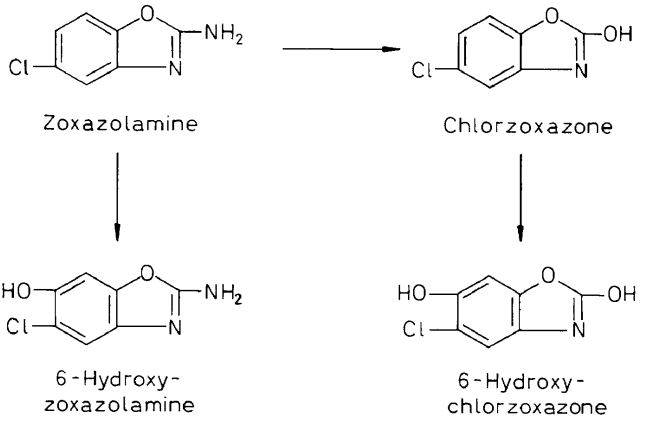


Figure 3. Metabolism of zoxazolamine and chlorzoxazone in man.

Zoxazolamine

The metabolism of the muscle-relaxant drug, zoxazolamine, was investigated in man[7,8]. Following the oral administration of 1·0 g doses of zoxazolamine, no detectable drug was found in the urines of these subjects. Evidence was found, however, for the urinary excretion of about 2·0 per cent of the dose as a metabolite in which the amino group of zoxazolamine was replaced by a hydroxyl group (*Figure 3*). This metabolite, identified as chlorzoxazone, possesses muscle-relaxant activity and was subsequently introduced as a new drug. The major route of metabolism of zoxazolamine and chlorzoxazone in man was by hydroxylation at the 6-position as indicated in *Figure 3*.

In our early metabolite isolation studies, it was found that samples of urine from zoxazolamine-treated human subjects contained crystals of a substance originally thought to be a hydroxyl metabolite of zoxazolamine. However, upon further investigation, this compound was identified as uric acid[9]. This serendipitous observation furnished the first clue to the potent uricosuric effect of zoxazolamine which led to its use in the treatment of chronic gout.

Chlorcyclizine

The antihistaminic chlorcyclizine is inactivated by demethylation to norchlorcyclizine[10] (*Figure 4*). This metabolic reaction is of interest since

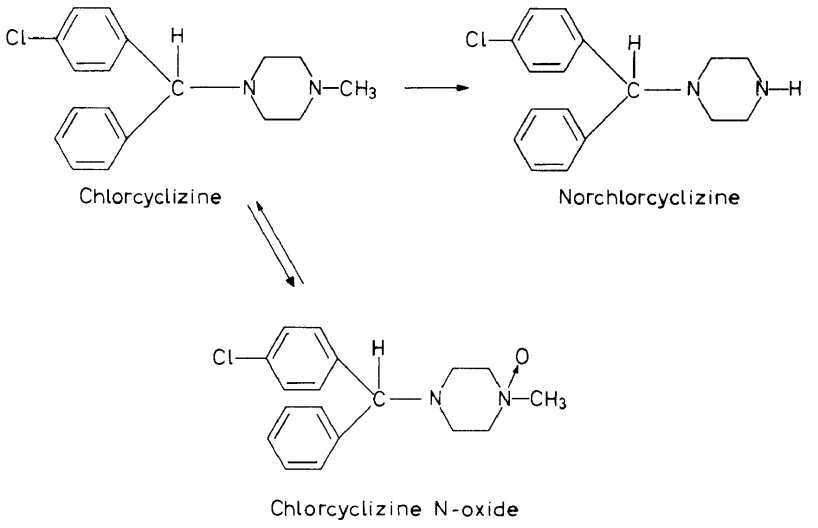


Figure 4. Metabolism of chlorcyclizine.

norchlorcyclizine is retained in humans for at least 20 days after termination of therapy[11]. During the course of studies on the metabolism of chlorcyclizine, another inactive metabolite was found in urine of rats and humans treated with the drug which was identified as the *N*-oxide[12] (*Figure 4*). *N*-oxides have been reported recently to be the metabolites of chlorpromazine and imipramine and they have aroused considerable interest as possible intermediates in dealkylation reactions[13,14]. When administered *in vivo*, chlorcyclizine *N*-oxide is converted to norchlorcyclizine through reduction of the *N*-oxide to chlorcyclizine and subsequent demethylation as shown in *Figure 4*.

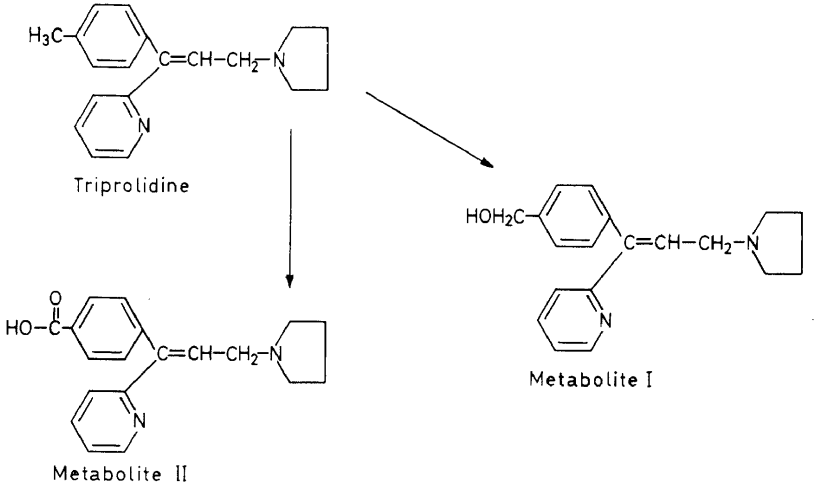


Figure 5. Metabolism of triprolidine.

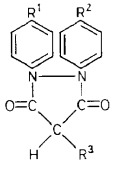
Triprolidine

Recent studies[15] have furnished evidence for two metabolites of the antihistamine triprolidine (*Figure 5*). Mass spectrometry and nuclear magnetic resonance studies carried out by Dr. P. Bommer and Dr. F. Vane of our laboratory, aided in elucidating the structure of these metabolites. The application of mass spectrometry to drug metabolism studies is most useful and this technique and other related ones were recently reviewed at a Conference on Applications of Newer Physical Techniques to the Study of Drug Metabolism which was organized by the National Academy of Sciences and held at the National Bureau of Standards, Washington, D.C., June 12–14, 1968.

STRUCTURAL CHANGES INFLUENCING DRUG METABOLISM

Relatively minor changes in chemical structure can markedly influence how long a drug stays in the body. This is well illustrated in studies[6] which showed marked difference in the biological half-life of various phenylbutazone analogues as determined by measuring the plasma levels after intravenous administration of 600 mg doses of the drug to human subjects (*Table 1*). It will be noted that the compounds disappear with a half-life varying from 3 days for phenylbutazone to 1 hour for G–32567. The striking difference in the rate of disappearance of two phenylbutazone analogues that

Table 1. Rate of disappearance of phenylbutazone analogues in man.



Compound	R^1	R^2	R^3	Half-life (h)
Phenylbutazone	H	H	$CH_2CH_2CH_2CH_2$	72
Metabolite I	OH	H	$CH_2CH_2CH_2CH_2$	72
G–15235	CH_2	CH_2	$CH_2CH_2CH_2CH_2$	24
G–28234	NO_2	H	$CH_2CH_2CH_2CH_2$ OH	20
Metabolite II	H	H	$CH_2CH_2CH_2CH_2$ O ‖	8
Sulphinpyrazone	H	H	CH_2CH_2—S—C_6H_5	2
G–32567	O ‖ S—CH_2 ‖ O	O ‖ S—CH_2 ‖ O	$CH_2CH_2CH_2CH_2$	1

possess similar chemical structures is shown by the data in *Figure 6*. The knowledge of how phenylbutazone and its analogues are metabolized has been of considerable importance in establishing proper dosage schedules for their evaluation in the treatment of arthritis and gout[6]. In general, slow metabolism of a drug is an advantage since it allows more even control of therapy. However, it has a disadvantage in that if a serious toxicity develops, the drug remains in the body for a considerable time.

Marked differences were observed in the rate of metabolism of chlorcyclizine and cyclizine, compounds which differ only by a chloro group in

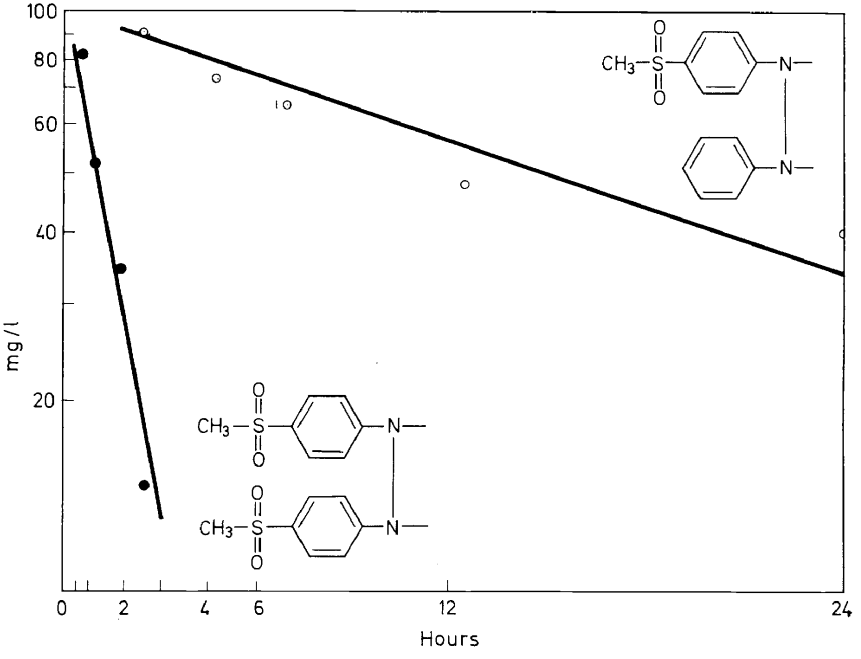


Figure 6. Metabolism of two closely related phenylbutazone analogues in man.

the para position of one of the benzene rings[11]. For instance, norchlorcyclizine is metabolized in man with a biological half-life of about 6 days, whereas norcyclizine has a half-life of less than 1 day. The slower rate of metabolism of norchlorcyclizine appears to result from its more marked affinity for plasma and tissue proteins than is observed for norcyclizine (*Table 2*). As has been pointed out earlier in this paper, norchlorcyclizine is retained in humans for a considerable period after therapy is terminated.

SPECIES DIFFERENCE IN DRUG METABOLISM

Knowledge of species difference in drug metabolism helps in the extrapolation of pharmacological and toxicological data obtained in experimental animals to man. Studies with the antirheumatic agent, phenylbutazone, have pointed out in a striking way the importance of species difference in

Table 2. Binding† of norchlorcyclizine and norcyclizine by rat plasma and tissue homogenates.

Tissue	*Norchlorcyclizine* (% bound)	*Norcyclizine* (% bound)
Brain	82	38
Heart	75	36
Kidney	72	34
Liver	81	44
Lung	80	31
Plasma	88	59

† Binding of metabolites was determined by equilibrium dialysis. Undiluted rat plasma and 2·5% tissue homogenates were used in these experiments.

drug metabolism. Phenylbutazone is metabolized in man at a very slow rate with a half-life averaging 3 days[6]. However, in the monkey, dog, rabbit, rat and guinea pig the drug is metabolized at a very rapid rate (*Table 3*). Information on the marked species difference in the metabolism of phenylbutazone has made it possible to demonstrate its anti-inflammatory and sodium-retaining effects in experimental animals.

Phenylbutazone exerts an anti-inflammatory effect in patients with rheumatoid arthritis comparable to cortisone and, like the steroid, is capable of blocking the inflammatory effect produced by glycerin injected into the anterior chamber of the rabbit eye. However, in order to show this effect in the rabbit, it was necessary to take into account the fact that the drug is metabolized much more rapidly in this species than in man (*Table 3*). When phenylbutazone was administered intramuscularly to the rabbit in a dose of 100 mg/kg every 8 hours, plasma levels ranged from 100 to 175 mg/l. which are comparable to those obtained in humans receiving a total daily dose of about 10 mg/kg. Similarly, in order to demonstrate sodium retention with phenylbutazone in the rat it was also necessary to administer the drug at a high dosage (150 mg/kg orally) so as to achieve plasma levels of the same order as those which gave sodium retention in man.

There are unusual differences in the manner in which animal species metabolize the anticoagulant drug, ethyl biscoumacetate[16]. Man and rabbit both metabolize the drug rapidly with a biological half-life of about

Table 3. Species differences in the metabolism of phenylbutazone.

Species	*Biological half-life* (h)
Man	72
Monkey	8
Dog	6
Rabbit	3
Rat	6
Guinea pig	5

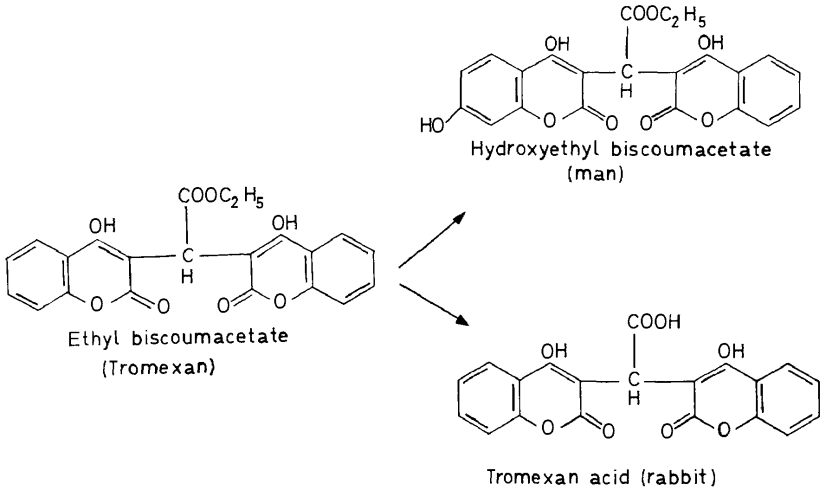


Figure 7. Metabolism of ethyl biscoumacetate.

2 hours and in both species it exerts a brief anticoagulant effect. The dog, on the other hand, metabolizes ethyl biscoumacetate extremely slowly, so slowly in fact that pharmacological studies of the drug in the dog would have given little indication of its short action in man. Although man and rabbit metabolize ethyl biscoumacetate at about the same rapid rate, they do so by different mechanisms (*Figure 7*). The rabbit metabolizes the drug by hydrolysis of the ester group to form an inactive acid derivative. In contrast, man inactivates ethyl biscoumacetate by introduction of a phenolic group into the benzene ring. Thus it is possible to have qualitative as well as quantitative differences in drug metabolism among animal species.

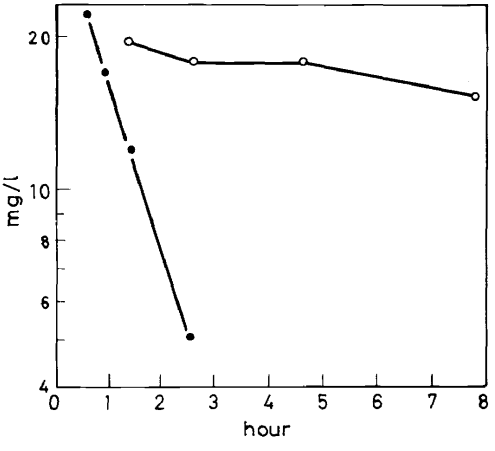


Figure 8. Metabolism of an experimental barbiturate in man and dog. [○—○, plasma levels in man; ●—●, plasma levels in the dog.]

A striking species difference was noted in the metabolism of the oxybarbiturate derivative, 5-allyl-5(2-bromo-2-cyclohexenyl)-2-barbituric acid[17]. In the dog this drug disappeared at a very rapid rate (half-life less than 1 hour) and had a fleeting duration of action. However, when the drug was given to man it disappeared at an extremely slow rate and its anesthetic effects persisted for many hours (*Figure 8*).

Marked species differences have also been observed in the metabolic fate of the analgesic drug, *N*-acetyl-*p*-aminophenol (APAP)[18]. In man and dog the drug is mainly excreted in the urine as a glucuronide conjugate (*Table 4*). However, no detectable glucuronide of the drug was found in the urine of cats receiving the drug. The nature of conjugates formed by the cat is not known. The failure of this species to form glucuronides can be explained by

Table 4. Metabolism of *N*-acetyl-*p*-aminophenol (APAP) in different animal species.

	Per cent of APAP *dose in urine*		
Species	*Total conjugates by acid hydrolysis*	APAP *glucuronide*	APAP *sulphate*
Man	90	58	4·4
Dog	78	54	4·1
Cat	78	<3	<2

the absence of the necessary liver enzyme required for the conjugation reaction. It is possible that the altered metabolism of *N*-acetyl-*p*-aminophenol in the cat explains the sensitivity of this species to the drug.

ENZYME INDUCTION AS AN IMPORTANT FACTOR IN DRUG SAFETY EVALUATION

The chronic toxicity test is an important part of safety evaluation studies with a new drug. The drug is administered on a fixed daily dosage schedule to several animal species for periods ranging from a few weeks to a year or more. The observation has often been made by the toxicologist that the most meaningful toxic effects are observed in the early period of a chronic test, rather than after prolonged exposure to the drug. Studies in recent years have furnished an explanation of these observations. It appears that the repeated administration of many drugs stimulates their own metabolism by inducing the synthesis of drug-metabolizing enzymes in liver microsomes[19,20]. For instance, the chronic administration of phenylbutazone to dogs in doses of 100 mg/kg/day leads to a marked increase in the drug's metabolism. During the first week when plasma levels of the drug are high, various side effects were observed. However, following repeated administration of the drug, the plasma levels fell off markedly and the side effects of the drug disappeared.

A good example[18] of the importance of enzyme induction for carrying out chronic toxicity tests in rats is given in *Figure 9*. Treatment of rats with 74 mg/kg intraperitoneally of phenylbutazone twice daily for 14 days resulted in a sharp increase in the drug's own metabolism. This is shown by the reduced plasma levels of the drug which were observed at 14 days,

compared to the high levels found initially. When phenylbutazone was given to rats in a dosage of 150 mg/kg intraperitoneally, 65 per cent of the rats developed gastrointestinal ulceration within 24 hours. However, no ulcers were observed when the same dosage was given to rats which were treated chronically with phenylbutazone or with phenobarbital, which also stimulates the metabolism of phenylbutazone.

Similar to phenylbutazone, enhanced metabolism of the following drugs has also been observed after repeated dosage: tolbutamide, hexabarbital, probenecid, chlorcyclizine and diphenylhydramine. The significance of enzyme induction to the overall design of toxicity studies will have to await

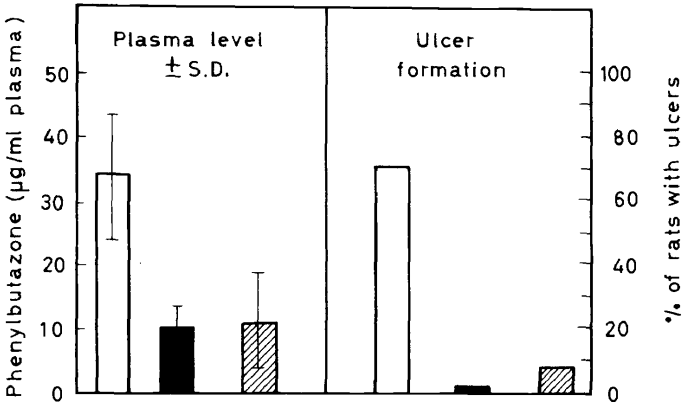


Figure 9. Effect of drug pretreatment in rats on gastric ulcer formation induced by phenylbutazone. The procedure described by Domenjoz[21] was used to induce ulcers with phenylbutazone [□, control; ■, pretreatment—phenylbutazone (150 mg/kg daily for 14 days); ▨, pretreatment—phenobarbital (75 mg/kg daily for 14 days].

further experiences that correlate drug metabolism with drug toxicity. However, it is possible that such metabolic data may furnish a rational basis for establishing the duration of the chronic toxicity test. The importance of enzyme induction in drug safety evaluation was discussed recently by a WHO Scientific Group[22].

SUMMARY

Information on the metabolism of drugs can be of considerable importance in understanding their therapeutic action. For instance, the antirheumatic drug phenylbutazone owes most of its pharmacological action through conversion to metabolic products. Metabolic studies given here show that at least some of the pharmacological effects of acetophenetidine can be explained through formation of metabolites of the drug. The importance of protein and tissue binding in determining the retention of the demethylated metabolites of chlorcyclizine and cyclizine was pointed out. A serendipitous observation made in the course of metabolite isolation studies with zoxazolamine furnished the first clue for the drug's uricosuric action in gout.

Results were presented which indicate that relatively small changes in chemical structure can markedly influence how long a drug is retained in man. Studies with phenylbutazone well illustrate this point since the drug is very slowly metabolized compared to some of its analogues which have an extremely short stay in the body.

Knowledge of species differences in drug metabolism aids in extrapolating pharmacological and toxicological data from experimental animals to man. Examples of such species differences have been given in this paper from studies with antirheumatic, anticoagulant, analgesic and hypnotic drugs.

The ability of a drug to stimulate its own metabolism on repeated administration was pointed out. This effect results from induction of drug metabolizing enzymes in liver microsomes and appears to have considerable importance in the interpretation of chronic toxicity tests.

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