REVIEW ARTICLE

PATHWAYS OF DRUG METABOLISM*†

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THE action of a drug would probably last a lifetime if the body did not have ways of limiting its duration. In this lecture I shall discuss the nature of a number of these mechanisms. At one time the kidney was considered the most important organ in enabling the body to dispose of drugs. But it is becoming more and more evident that the kidney usually excretes only a small proportion of a drug in an unchanged form and the bulk as inactive derivatives. Of course there are notable exceptions: tolazoline, an adrenergic blocking agent, is excreted almost entirely unchanged; a number of sulphonamides are only in part metabolised; and a considerable fraction of penicillin is found unchanged in urine. But, by and large, the action of most drugs is terminated by their biotransformation. Although the biotransformation of a vast number of drugs in the whole organism has been studied, we have known very little, until recently, of the nature of enzymatic mechanisms concerned in these processes of "detoxication."

The fate of drugs in the body has interested our laboratory for a number of reasons. At one time we considered it possible that biotransformation mechanisms might explain how certain drugs exert their pharmacological action; that is, drugs in being metabolised might become enmeshed in mechanisms involved in the normal economy of the body and thus interfere with normal function. As we shall see later, this viewpoint is difficult to entertain since most drugs are not measurably metabolised in the organ where they act. Another reason for our interest in drug metabolism was the possibility that this might be a backdoor approach to general biochemistry, with the drug being used as a bait to induce unknown enzymes to disclose themselves. Finally, it is thought that a detailed knowledge of enzymes involved in drug "detoxication" might help the medicinal chemist to develop compounds of either high or low stability in the body, whichever would be more desirable in gaining a desired therapeutic result.

THE INHIBITORY ACTION OF SKF 525-A

Although certain aspects of this programme had been going on in our laboratories for some time, it was a phone call from Dr. Glenn Ullyot

* This article is based on the first of two University of London special university lectures in pharmacology on "Biochemical and Clinical Implications of Studies in Drug Metabolism," given at Guy's Hospital Medical School, May 17, 1954. A second article will appear later.

† The studies presented here include the work of Drs. J. Axelrod, J. Cooper, J. Fouts, J. Gillette, B. N. La Du, C. Mitoma, G. Quinn, and S. Udenfriend.

of Smith, Kline and French Laboratories in Philadelphia that really "sparked" our studies. He told us that their laboratory had developed a strange compound (SKF 525–A), a congener of Trasentin that lacked its spasmolytic activity. Thinking that it might have sedative action, they determined the effect of the compound in prolonging the duration of hypnosis induced by hexobarbitone and other barbiturates. For example, rats were given a dose of hexobarbitone which ordinarily resulted in their sleeping for about 40 minutes. However, when pretreated with a 100 mg. per kg. dose of SKF 525–A they now slept for several hundred minutes. Obviously, this was more than an additive effect. In fact, SKF 525–A by itself was subsequently shown to exhibit no sedative effect whatever, or any other obvious effect except at very large doses. We were also told that SKF 525–A prolonged the action of the narcotic analgesics—pethidine, codeine, and morphine—and of mephenesin, a centrally-acting muscle relaxant. But all the drugs thus far mentioned depressed the central nervous system and it seemed possible to us that SKF 525–A might affect some central component common to them all. But on hearing that

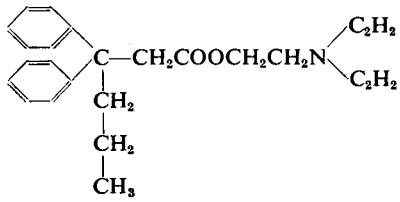


TABLE I

EFFECT OF SKF 525–A ON METABOLISM OF HEXOBARBITONE IN RATS

Rats received 100 mg./kg. hexobarbitone intraperitoneally (control). One week later experiments repeated except that 15 mg./kg. SKF 525–A was given before the hexobarbitone injection.

Rat No.	Duration of action*		Hexobarbitone half-life		Plasma level on awakening*	
	Control min.	SKF 525–A treated min.	Control min.	SKF 525–A treated min.	Control mg./l	SKF 525–A treated mg./l
1	21	89	24	69	48	38
2	27	90	24	60	42	34
3	34	86	32	71	40	41
4	23	68	23	49	27	33
5	28	68	27	50	46	38

* Return of righting reflex.

SKF 525–A also prolonged the action of (+)-amphetamine, a central nervous system stimulant[1–5], we realised this compound might be extremely useful in the study of drug action. We are grateful to Smith, Kline and French for giving us the opportunity of investigating their compound, for it has proved a particularly rewarding tool in providing a uniform picture of the biotransformation process of a variety of drugs.

We first studied the effect of SKF 525–A on barbiturate drugs *in vivo*[6]. The sleeping times of rats injected with the new compound and hexobarbitone were compared with those of rats injected with hexobarbitone alone. Table I shows that rats pretreated with 15 mg. per kg. of SKF 525–A slept three times as long as the same animals given barbiturate alone. The rate of metabolism of the barbiturate was affected, the

biological half-life (the time required for disappearance of half the drug from the body) being prolonged about threefold. With larger doses of SKF 525–A this action was more pronounced; for example, with 50 mg. per kg. the biological half-life of hexobarbitone increased about fivefold. Of particular interest was the finding that plasma concentrations of hexobarbitone when the rats awoke were roughly the same whether or not the animals had received SKF 525–A (Table I), indicating that the potentiator did not act by sensitising the site of action of the barbiturate. It may be concluded that the increased duration of action resulted only from inhibition of the metabolic transformation of the barbiturate. An inhibitory effect on the metabolism of hexobarbitone was also demonstrable in other animal species. As shown in Table II, SKF 525–A decreased the rate of biotransformation and increased the duration of action of the hexobarbitone in dogs and these effects were markedly enhanced with an increase in dosage of the former. The metabolism of other barbiturates such as pentobarbitone and quinalbarbitone was also depressed. Since barbiturates are metabolised mainly through oxidations in the sidechain[7,8,9] it seemed apparent that these oxidations were antagonised by the compound *in vivo*.

TABLE II

EFFECT OF SKF–A ON METABOLISM OF HEXOBARBITONE IN DOGS

Dogs received 30 mg./kg. hexobarbitone intraperitoneally. One week later experiment was repeated except that SKF 525–A was administered before the hexobarbitone injection.

Dog No.	SKF 525–A administered mg./kg.	Hexobarbitone half-life min.
1	0	90
	15	480
2	0	120
	15	720
	50	1200
3	0	810
	15	390

Previous work has demonstrated that one of the pathways of metabolism of the analgesic pethidine is through demethylation to yield norpethidine[10,11]. The effect of SKF 525–A on the metabolic transformation of pethidine was studied in mice. Table III shows that the total amount of the analgesic was higher in SKF 525–A treated than in untreated mice. Conversely, there was about five times as much of the

TABLE III

EFFECT OF SKF 525–A ON METABOLISM OF PETHIDINE IN MICE

Ten mice received 1·5 mg. of pethidine intraperitoneally. Five of these also received 50 mg./kg. SKF 525–A before the injection of pethidine. Animals were killed 60 minutes later and analysed for pethidine and norpethidine.

Without SKF 525–A		With SKF 525–A	
Pethidine mg./mouse	Norpethidine mg./mouse	Pethidine mg./mouse	Norpethidine mg./mouse
0·26	0·52	0·35	0·14
0·25	0·53	0·53	0·08
0·34	0·60	0·40	0·06
0·27	0·53	0·48	0·13
0·20	0·49	0·42	0·16
Average 0·26	0·53	0·44	0·11

demethylated product, norpethidine, in the untreated animals. These results point to a blocking of the demethylation of pethidine. Similarly, in the dog, the metabolism of amidopyrine, which is known to be demethylated to yield 4-aminoantipyrine *in vivo*[12], was antagonised by first giving the new compound (Fig. 1). From these and a number of similar experiments with other drugs it may be concluded that potentiating effects result from its inhibitory effects on the metabolism of a variety of drugs.

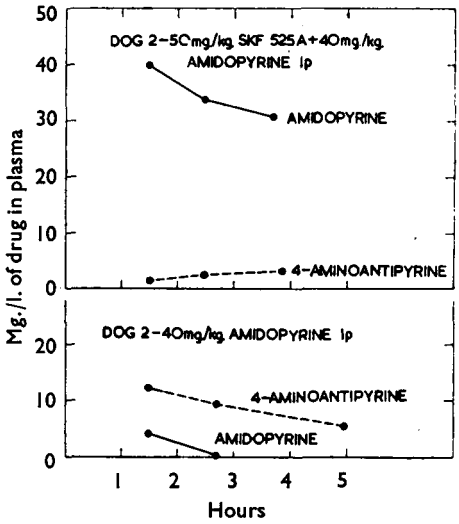


FIG. 1. Effect of SKF 525–A on the demethylation of amidopyrine in the dog.

At this point it might be desirable to clarify just what we mean by the term "potentiating" agent. SKF 525–A might better be called a prolonging agent since it acts only by interfering with the rate of metabolism of drugs. In contrast, the tranquillising agents, chlorpromazine and reserpine, which potentiate the action of hypnotics such as barbiturates and ethanol, are true potentiators since they do not affect the metabolism of these drugs but act by increasing the sensitivity of the central nervous system[13]. The difference between the actions of SKF 525–A and chlorpromazine was shown rather dramatically in the following way. When the former was given intravenously to mice and dogs which had just recovered from hypnosis induced by hexobarbitone, the animals were not visibly affected, since the compound could only slow down the biotransformation of the non-hypnotic amount of barbiturate remaining in the body. In contrast, if chlorpromazine were given to the awakening animals they reverted almost immediately to a deep hypnosis.

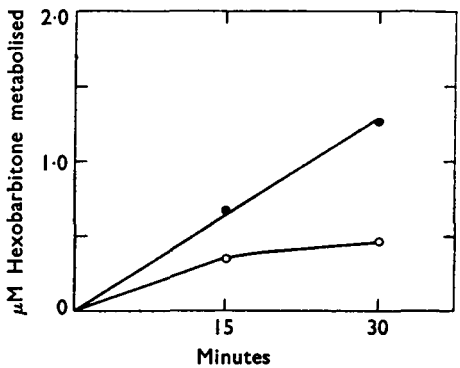


FIG. 2. Comparison of hexobarbitone metabolism in liver slices of control rats and rats pretreated with 25 mg./kg. SKF 525–A.
●——● Control.
○——○ SKF 525–A—treated.

To see how SKF 525–A affected a wide variety of drug metabolic pathways and to determine its mechanism of action at a tissue level we

turned to studies employing tissue slices and homogenates[14]. Liver slices of rats that had previously been injected with it were incubated with hexobarbitone. It is evident from the typical result shown in Figure 2 that hexobarbitone was metabolised considerably less rapidly in liver slices from these rats than in those of control animals. Since hexobarbitone is metabolised only by the liver[15] it is evident that SKF 525–A acts on this organ and this led us to examine its effects when added directly to liver slices.

SKF 525–A (2×10^{-4} M) added directly to liver slices was found to markedly inhibit the metabolism of hexobarbitone (Fig. 3) and other barbiturates such as pentobarbitone and quinalbarbitone. It also affected the biotransformation of a number of alkylamines including amidopyrine and pethidine (Fig. 4). Other drug metabolic pathways that the compound was shown to antagonise *in vitro* included deamination of amphetamine and splitting of the ether linkage in codeine to form morphine.

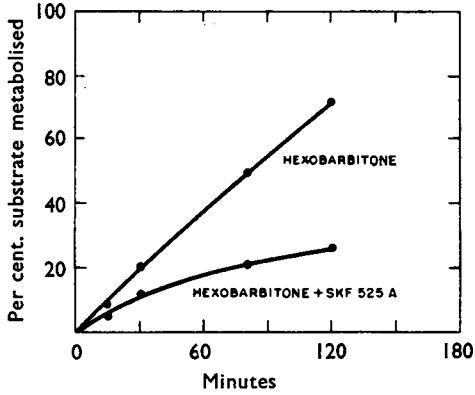


FIG. 3. Inhibition of hexobarbitone metabolism by SKF 525–A (2×10^{-4} M) added directly to rabbit liver slices.

The effect of varying the concentration of SKF 525–A was determined. The influence of the inhibitor on hexobarbitone metabolism was evident at concentrations as low as 1×10^{-4} M and as the concentration of the

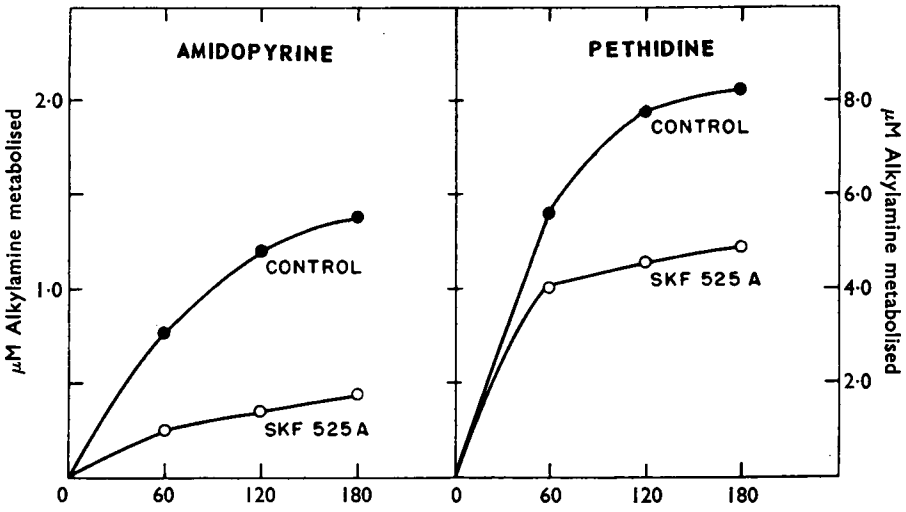


FIG. 4. Inhibition of amidopyrine and pethidine metabolism by SKF 525–A (2×10^{-4} M) added directly to rat liver slices.

former was increased there was a progressive increase in the degree of inhibition (Fig. 5). It is of interest that the simple acid component of SKF 525–A, diphenylpropylacetic acid (SKF acid), had inhibitory effects *in vitro* equal to those of the compound itself yet showed little effect *in vivo*, perhaps because of its rapid excretion or metabolic transformation.

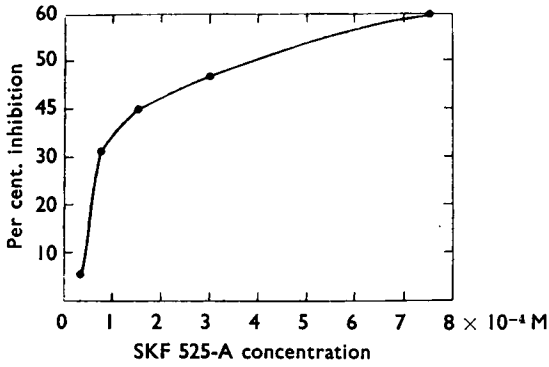


FIG. 5. Effect of various concentrations of SKF 525–A on hexobarbitone metabolism in rabbit liver slices.

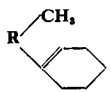
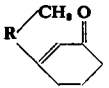
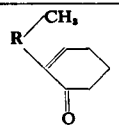
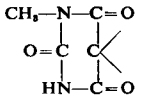
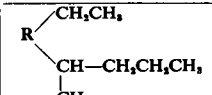
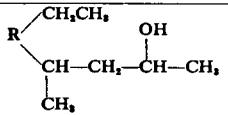
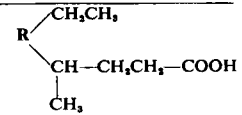
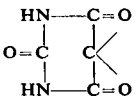
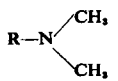
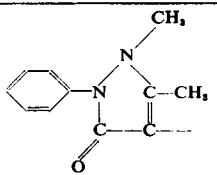
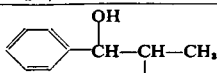
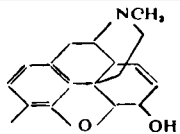
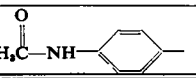
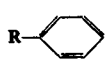
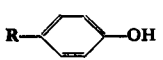
METABOLIC ROUTES

It seemed to us that if a number of drug metabolic pathways were susceptible to the same inhibitor they should have certain factors in common. Using liver homogenates, studies were undertaken to learn what these factors might be. A number of compounds, all oxidatively metabolised along a variety of metabolic pathways, were studied; and here, are described in detail our findings about the oxidation of hexobarbitone to ketohexobarbitone. In general, almost identical findings apply to a number of metabolic pathways including sidechain oxidation, dealkylation, deamination, ether cleavage and hydroxylation[16], examples of which are listed in Table IV.

Unfortified rabbit liver homogenates showed only slight activity in metabolising the drugs and this activity was completely lost after dialysis. Considerable activity was restored by the addition of nicotinamide and triphosphopyridine nucleotide (TPN), but diphosphopyridine nucleotide (DPN) could not replace TPN. Nicotinamide served presumably to protect TPN against enzymatic destruction[24]. Oxygen was also required, little drug metabolism being observed under anaerobic conditions. In addition, activity was enhanced by the addition of Mg^{++} and glucose-6-phosphate. The requirement for both TPN and oxygen first suggested that the various oxidative pathways in Table IV were carried out by the transfer of hydrogen from the various drugs, activated by dehydrogenase systems, with TPN acting as a hydrogen acceptor. This explanation, as we will see later, is far from being the correct one.

Having learned that the metabolic pathways depicted in Table IV all needed TPN, other requirements were sought that they might have in common. Rabbit liver homogenate was centrifuged at 9000 × g to separate the nuclei and mitochondria from the rest of the cell. The various drugs were metabolised in the supernatant fraction, but almost no activity was associated with nuclei or mitochondria. On centrifugation of the supernatant fraction at 80,000 × g the submicroscopic particles (microsomes) were separated from the soluble part of the cell. Neither

TABLE IV

Type of reaction	Substrate and structure	Products and structures			Structure of R group	Reference
Barbiturate side chain oxidation	 Hexobarbitone		or	 Ketohexobarbitone		15
	 Pentobarbitone	 Pentobarbitone alcohol	and	 Pentobarbitone carboxylic acid		17
Dealkylation	 Amidopyrine	$R-NH_2$ 4-aminoantipyrine	and and	2HCHO Formaldehyde		18
	$R-NH-CH_2CH_3$ Monoethylaniline	$R-NH_2$ Aniline	and and	CH_3CHO Acetaldehyde		19
	$R-NH-CH_3$ Ephedrine	$R-NH_2$ Norephedrine	and and	HCHO Formaldehyde		20
Deamination	$R-CH_2-CH-CH_3$ NH_2 Amphetamine	$R-CH_2-C-CH_3$ O Phenylacetone	and and	NH_3 Ammonia		21
Ether Cleavage	$R-O-CH_3$ Codeine	R—OH Morphine	and and	HCHO Formaldehyde		22
	$R-O-CH_2CH_3$ Phenacetin	R—OH *p*-Hydroxyacetanilide	and and	CH_3CHO Acetaldehyde		22
Hydroxylation	 Aniline or acetanilide	 *p*-Hydroxyaniline	or	*p*-Hydroxyacetanilide	NH_2- Aniline or $CH_3-C(=O)-NH-$ Acetanilide	23

of these fractions by itself possessed any activity but when added together the recombination was about as effective as the original whole homogenate (Table V). It was evident that factors present in both microsomes and soluble supernatant were necessary to carry out the metabolism of the various drugs. Activity was increased if more microsomes were added to the mixture but maximal effect was still retained even in the presence of only a small volume of soluble fraction. It seemed reasonable to presume, therefore, that the various enzymatic mechanisms were localised in microsomes and that the soluble fraction supplied something, normally present in excess, that they all needed. The finding, previously mentioned, that the activity of dialyzed homogenates was enhanced by the addition of Mg^{++} and glucose-6-phosphate, together with the known presence of considerable glucose-6-phosphate dehydrogenase in the soluble fraction, suggested that the form of TPN required in the complete system was actually TPNH, generated through the following reaction:

TABLE V

CELLULAR LOCALISATION OF ENZYME ACTIVITY

Various cell fractions were prepared from rabbit liver by differential centrifugation and incubated 1 hour at 37° with 1·0 μM of hexobarbitone at pH 7·4.

	Hexobarbitone metabolized μ moles
Whole homogenate	0·50
Supernatant fraction	0·65
Microsomes	0
Soluble fraction of supernatant ..	0
Microsomes + soluble fraction ..	0·70

$$\text{glucose-6-phosphate} + \text{TPN} \xrightarrow{\text{glucose-6-phosphate-dihydrogenase}} \text{phosphogluconic} + \text{TPNH}$$

Substantiation of this hypothesis was afforded by the finding that all the substrates in Table IV could be metabolised by washed microsomes with the soluble fraction substituted by a TPNH generating system consisting of glucose-6-phosphate dehydrogenase, glucose-6-phosphate and TPN. Direct evidence that TPNH was involved in these reactions was obtained by the demonstration that the addition of chemically prepared TPNH to washed microsomes could replace the soluble fraction (Table VI). Thus we find additional factors possessed in common by a variety of drug metabolic pathways—localisation in liver microsomes and a requirement for TPNH and oxygen.

Now, instead of the familiar picture of oxidation proceeding through dehydrogenation with DPN or TPN acting as hydrogen acceptors, we are faced with a strange kind of oxidation where the pyridine nucleotide is already in a reduced form and therefore unable to accept hydrogen. Obviously oxidation must be proceeding in an unusual way.

Some progress has been made towards the elucidation of the mechanism of these oxidative enzymes. Drs. La Du and Gillette have obtained important information about the role of TPNH[25]. They have demonstrated the presence of an oxidase (TPNH oxidase) in liver microsomes, which catalysed the oxidation of TPNH to TPN even in the absence of drug substrates. This reaction was not blocked by cyanide, showing that

the normal cytochrome system was not involved in this hydrogen transfer. Material with hydrogen peroxide-like properties was demonstrated to be a product of the TPNH oxidation—suggesting that hydrogen peroxide or a peroxide-like intermediate was utilised by a variety of enzymes in microsomes to carry out the various drug reactions. Although other tissue cells were shown to contain microsomes with TPNH oxidase

TABLE VI

REQUIREMENTS FOR TPNH IN OXIDATION OF HEXOBARBITONE BY LIVER MICROSOMES

Microsomes were incubated for 1 hour with 1 μmole of hexobarbitone at pH 7·4.

Components	Hexobarbitone oxidised μ moles
Microsomes + 1·0 μmole TPN	0
Microsomes + 1·0 μmole + 4 μmoles glucose-6-phosphate	0
Microsomes + 1·0 μmoles TPN + 4 μmoles glucose-6-phosphate + glucose-6-phosphate dehydrogenase	0·30
Microsomes + TPNH	0·61
Microsomes + 0·5 ml. soluble fraction + 1 μmole TPN	0·57

activity, only those in liver catalysed the various metabolic pathways outlined in Table IV, thus confirming the generally considered view that drug metabolism usually occurs mainly in liver. Apparently the liver microsomes contain additional catalysts which the microsomes of other tissues lack in order to effect the metabolism of drugs.

Before leaving the subject of the role of the liver in drug metabolism, mention should briefly be made of some preliminary work in our laboratory. We read in the textbooks that the liver is responsible for the metabolism of many drugs; therefore, subjects with liver disease probably present a therapeutic problem. This may not be true with most drugs. Drs. Burns and Weiner showed that subjects with badly diseased livers could still metabolise antipyrine, salicylate, phenylbutazone, amidopyrine, pethidine, pentobarbitone and a number of other drugs as readily as did normal subjects. Thus, the fact that the liver does not function in a normal manner does not necessarily mean that the enzyme systems involved in drug metabolism are impaired.

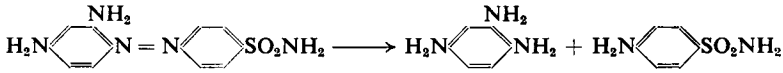
A number of oxidative pathways of drug metabolism including side-chain oxidation, dealkylation, deamination, ether cleavage and hydroxylation all require TPNH and oxygen and are harboured in the microsomes of the liver cell. These pathways may have a step in common—the production of a peroxide-like intermediate—which under the influence of other catalysts in liver microsomes is utilised in the biotransformation of the various types of drugs.

But in considering the mechanism of drug oxidation we are left with a number of unsolved problems. Does each type of metabolic pathway in Table IV involve the same enzyme system? This is of course extremely unlikely and, in fact, a number of observations indicate that this is not so. For example, microsomes prepared at pH 7·0 rapidly lose their ability to hydroxylate aniline and acetanilide but not to dealkylate amidopyrine[23], indicating that the hydroxylation mechanism is more labile than the dealkylation one. Although it may be assumed that the different

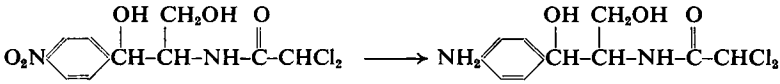
metabolic pathways require different enzyme systems, does the demethylation of methylamines require only a single system, and can this system also dealkylate ethyl and propylamines? Again, although the evidence indicates that the various types of reactions require a common peroxide-like intermediate, there is no definitive information concerning the nature of the reaction of this substance with drugs. A main difficulty in learning more about the mechanisms lies in the lability of the various enzyme systems which so far have suffered inactivation when microsomes are subjected to various solubilisation procedures.

Previous work from this laboratory has shown that at least two of the metabolic pathways in Table IV, hydroxylation and ether cleavage, can occur in the presence of a model system consisting of ascorbic acid, oxygen and inorganic iron chelated with versene[26]. There is evidence that the action of this system is mediated through the generation of hydroxyl groups produced by a reaction product of hydrogen peroxide with ascorbic acid[27]. Will sidechain oxidation, dealkylation and deamination also occur in the presence of this system? If so, this would suggest that the various oxidative pathways can occur *in vitro*, non-enzymatically, with hydroxylation as a common step. Although the reactions *in vivo* require enzymes, we are looking into the possibility that a common step in the microsomal oxidation of drugs may be the production of hydroxyl groups.

Another unsolved puzzle is how SKF 525–A acts. This problem has taken an unusual turn. The original premise that led to our studies on the oxidative drug enzymes was that since SKF 525–A inhibited so many pathways of drug metabolism, these must have certain factors in common. An obvious thought is that the compound blocks the oxidation of TPNH. But this step is not affected by the inhibitor[25]. Furthermore, there are a number of instances where the metabolism of a drug which follows one of the pathways outlined in Table IV is not antagonised by SKF 525–A. The demethylation of methylaniline to aniline which is catalysed by an enzyme system in microsomes and requires TPNH and O_2 is not affected by the inhibitor even at high concentrations[19], inhibition not even being restricted to oxidative pathways. Thus, Dr. Fouts has shown that the enzymatic reductions of prontosil and chloramphenicol in rabbit liver homogenates are also inhibited by SKF 525–A[28].



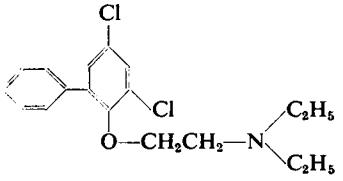
Prontosil — Sulphanilamide



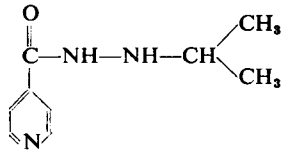
Chloramphenicol — Reduced chloramphenicol

These reactions also require TPNH but proceed anaerobically. Still more surprising is the finding that SKF 525–A inhibits drug enzymes in microsomes that do not even require TPNH, for example the formation of a glucuronide with morphine[14] and the de-esterification of procaine[29].

To further complicate the story, Dr. Fouts has shown that Lilly 18947[30] and Marsilid[31], compounds structurally unrelated to SKF 525-A, block



Lilly 18947
2,4-dichloro-6-phenylphenoxy-ethyl diethylamine



Marsilid
2-*iso*propyl-1-*iso*nicotinyl hydrazide

the same drug enzyme systems. The possibility is entertained that the inhibitors act in some physico-chemical manner perhaps by preventing, in some way, the entrance of certain drugs into microsomes.

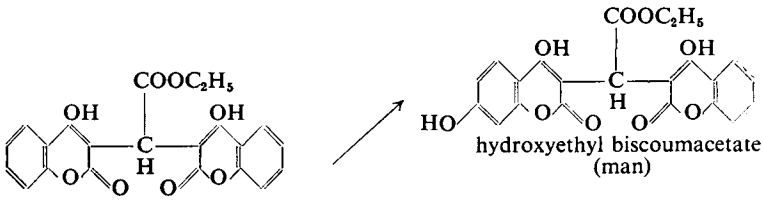
Species Differences and their Relation to Drug Metabolism

Species differences are of some concern to the pharmacologist who screens drugs in animals and on the basis of the results so obtained has to decide which ones to try in man. It is well known that various animal species react differently to the same drug. Some of the differences are mainly qualitative in nature; for instance, morphine depresses man but stimulates racehorses and cats. It is probable, however, that most species differences are in the duration of action of drugs and here, a few examples would be pertinent.

Phenylbutazone, an antirheumatic drug, is metabolised unusually slowly in man, about 10 to 15 per cent. per day[32] but in mice, rabbits, dogs, guinea-pigs and horses it disappears from the body in a few hours. It is not surprising that the antirheumatic action of this compound was first observed in man by chance, since relatively enormous doses are needed, because of its rapid metabolism, to show an anti-inflammatory effect in rats.

Pethidine, a narcotic analgesic, is metabolised in man at the rate of about 17 per cent. per hour, a single dose exerting an analgesic effect lasting three to four hours. In a study of its fate and distribution, Dr. Burns in our laboratory decided to give a dog 20 mg. per kg., which in man would be considered a huge dose. He was prepared for artificial respiration since it was fully expected that the animal would experience dire effects including loss of breathing. The drug was infused over a period of 20 minutes following which, to his surprise, the dog leaped from the table and walked away. The explanation for the relative immunity to the effects of the narcotic lies in the extraordinarily rapid rate of biotransformation in the dog, 70 to 90 per cent. per hour. The rapid disappearance of pethidine may also explain the difficulty of producing either tolerance or addiction to the drug in dogs. One wonders how many useful analgesics are still sitting neglected on the dusty shelves of pharmaceutical houses because they showed only minimal effects on test animals, merely because they were metabolised too rapidly in the particular species in which they were tested.

Another example of species differences may be seen with ethyl biscoumacetate, an anticoagulant similar in structure to dicumarol. It was introduced as a safer anticoagulant on the basis of its rapid metabolism compared to dicumarol, so that recovery from bleeding difficulties due to overdosage would also be rapid. It so happens that ethyl biscoumacetate was first screened in the rabbit, an animal that metabolises the drug at the same rate as in man. But this was sheer coincidence since the two species metabolise the drug entirely differently, man, by hydroxylation of an aromatic ring and the rabbit by de-esterification.



If the drug had been screened in dogs, it would probably have been discarded since in this species it is metabolised almost as slowly as dicumarol, about 3 per cent. per hour.

One of the most dramatic examples of species differences was uncovered by us in a programme in which, in collaboration with Mr. William Lott of E. R. Squibb, we were trying to develop a barbiturate that would be rapidly metabolised in man. One of the compounds investigated, a bromo*cyclo*hexenyl derivative of barbituric acid (thialbarbitone without the bromine atom), when given to dogs, produced a deep anæsthesia. But no matter how long the dogs were kept under anæsthesia, by intermittent infusion of the drug, the animals showed almost complete recovery shortly after termination of the infusion. Plasma level measurements showed that the drug was metabolised in dog at an enormous rate compared to thiopentone. Very hopefully, thinking that,

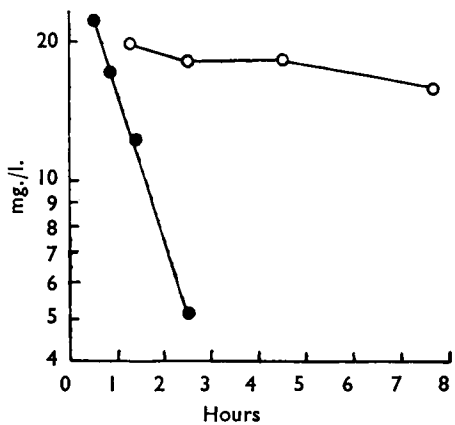


FIG. 6. Comparison of plasma levels of a bromo*cyclo*hexenyl derivative of barbituric acid in man and dog.

○——○ Man (dose 15 mg./kg.).
●——● Dog („ „).

at long last, we might have discovered the intravenous anæsthetic of choice, the compound was administered in man only to find that it was one of the most stable barbiturates we had ever studied (Fig. 6).

These examples indicate in a general way the importance of drug metabolism in causing species differences. These differences were studied in a more detailed fashion by comparing the duration of action and the rate of biotransformation of hexobarbitone in a number of animal species. The duration of action of the barbiturate bore an inverse relationship to its rate of disappearance from the body (Table VII). The drug was

TABLE VII

SPECIES DIFFERENCES IN METABOLISM OF HEXOBARBITONE
DOSE OF BARBITURATE 100 MG./KG.

Figures in brackets refer to number of animals in each species.

	Sleeping time minutes	Hexobarbitone half-life minutes	Enzyme activity µg./g./hr.
Mice (12) ..	12 ± 8	19 ± 7	598 ± 187
Rabbits (9) ..	49 ± 12	60 ± 11	294 ± 28
Rats (10) ..	95 ± 15	139 ± 54	134 ± 51
Dogs (5)* ..	315 ± 105	261 ± 20	36 ± 29

* Dogs received 50 mg./kg. of hexobarbitone.

metabolised extremely rapidly in the mouse having a half-life of only 15 minutes. In other animal species it was metabolised more slowly and in dog it was found to be relatively stable with a half-life of over 300 minutes (about the same as in man). Although the mouse, in general, metabolises hexobarbitone and a number of other drugs more rapidly than do other animal species, there is no clear-cut relationship between the size of the species and the rate of biotransformation. For example, the rat, though smaller than the rabbit, inactivates hexobarbitone more slowly.

Since Nature has packaged a number of her drug enzyme systems in microsomes, they can be easily separated from the rest of the cell. It became possible, therefore, to ascertain directly whether species differences with hexobarbitone could be explained in terms of the activity of an enzyme system in microsomes. When the activity of the hexobarbitone enzyme system in microsomes was assayed it, also, was found to bear an inverse relationship to the duration of drug action. For example, the activity of the hexobarbitone oxidising enzyme system in mouse microsomes was about 17 times that in the dog (Table VII). Thus with hexobarbitone we have demonstrated that species differences in duration of action can be expressed in terms of the activity of a single enzyme system, the microsomal system which "detoxicates" it. We now have accumulated evidence that species differences can be similarly explained in terms of a single "detoxication" enzyme system with a number of other drugs.

As though species differences are not enough trouble to the pharmacologist, strain differences must also be considered. We were fortunate in having available a number of inbred strains of mice. Individual mice of a given strain given a 100 mg. per kg. of hexobarbitone slept for a

remarkably uniform interval of time. But different strains slept for different times varying from an average of 12 minutes in strain SWR/HEN to 55 minutes in strain 1/LN.

In addition there are sex differences. It has been known for a number of years that female rats anæsthetised with certain barbiturates slept considerably longer than did males[34,35]. While certain important differences between the two sexes have long been recognised and investigated in some detail, this manifestation seemed rather curious and worth while studying.

TABLE VIII

SEX DIFFERENCES IN THE METABOLISM OF HEXOBARBITONE IN RATS
DOSE OF BARBITURATE 100 MG./KG.

Figures in brackets refer to number of animals in each series.

Sex	Sleeping time minutes	Plasma level at 60 minutes μg./ml.	Enzyme activity μg./g./hr.
Females	90 ± 15 (10)	65 ± 8	134 ± 51
Males	22 ± 5 (11)	23 ± 9	682 ± 102

TABLE IX

INFLUENCE OF ŒSTRADIOL ON THE METABOLISM AND DURATION OF ACTION OF HEXOBARBITONE IN MALE RATS

DOSE OF BARBITURATE 100 MG./KG.

Figures in brackets refer to number of animals in each series.

	Sleeping time minutes	Plasma level at 60 minutes μg./ml.	Enzyme activity μg./g./hr.
Control	22 ± 5 (11)	23 ± 9	682 ± 102
Œstradiol ..	84 ± 22 (9)	71	177 ± 33

When male and female rats were given 100 mg. per kg. of hexobarbitone, the females slept about four times as long as the males. A comparison of the plasma levels indicated that the drug was metabolised much more rapidly in males than in females. In accord with this finding, microsomes isolated from livers of male rats showed considerably higher enzyme activity than those of females in oxidising hexobarbitone (Table VIII). Thus we see that the female rats were not actually more susceptible to hexobarbitone but were merely unable to metabolise it as rapidly.

The observed sex differences prompted us to investigate the effects of the sex hormones. Male rats were given œstradiol for several weeks before the administration of a single dose of hexobarbitone. The treated rats now slept much longer than the controls, in fact, as long as the females. Studies with liver microsomes showed that these had lost much of their capacity to metabolise hexobarbitone (Table IX). Female rats were given testosterone for two weeks and then given a single dose of hexobarbitone. Now when given hexobarbitone they slept as short a time as did males. Correspondingly, their liver microsomes had acquired considerably more capacity to metabolise hexobarbitone (Table X).

When recounting these experiments at a gathering of anæsthesiologists, I was told emphatically that they had no clinical impression of any such differences in the reaction of males and females of the human species to barbiturates. We therefore decided to compare the duration of action of hexobarbitone in males and females of a number of other species including

TABLE X

INFLUENCE OF TESTOSTERONE ON THE METABOLISM AND DURATION OF ACTION OF HEXOBARBITONE IN FEMALE RATS

DOSE OF BARBITURATE 100 MG./KG.

Figures in brackets refer to number of animals in each series.

	Sleeping Time minutes	Plasma level at 60 minutes μg./ml.	Enzyme activity μg./g./hr.
Control	90 ± 15 (10)	65 ± 8	134 ± 51
Testosterone ..	38 ± 17 (14)	37 ± 14	543 ± 123

mice, guinea-pigs, rabbits and dogs. To our consternation no differences between the sexes were observed in these animals. Furthermore, œstradiol and testosterone could not induce any appreciable change in the detoxification rate of the barbiturates in mice and rabbits. Just why this sex difference seems to be present only in rats and whether it extends to other drugs we do not yet know. But the considerable amount of basic research with hormones that has been made with the rat gives one considerable food for thought. This animal behaves differently in this respect, might it not in others also?

THE NORMAL ROLE OF THE ENZYME SYSTEMS DISCUSSED

When we first began our studies on drug metabolism we shared the traditional concept that foreign compounds were metabolised by mechanisms which acted on structurally related substances normally present in the body. When our studies made it evident that the body again and again was able to "detoxicate" compounds it has never "seen" before, we began to question this concept. A most striking property of most enzymes is not only their catalytic effect on chemical reactions but also their specificity; that is, a given enzyme can usually catalyse only a small range of reactions and in many cases only a single reaction. After all, if every foreign compound which even faintly resembled a substrate in the body were acted on by the same enzyme system a normal function of the body might soon be overwhelmed in the presence of a foreign compound. Consequently, some of us are gaining the viewpoint that the microsomal enzyme systems in liver may be there just to "detoxicate" foreign compounds. For this function, enzyme systems would need to be extremely nonspecific; one to dealkylate alkylamines, another to hydroxylate aromatic compounds, and so forth.

Indirect evidence is gradually accumulating for the point of view that the microsomal drug enzymes metabolise only foreign compounds. For one thing, Mr. Gaudette in our laboratory found that the normally

occurring alkylamines, such as sarcosine and dimethylaminoethanol, were not demethylated by the microsomal dealkylation system. Yet sarcosine is readily demethylated by an enzyme system present in mitochondria[36] which will not dealkylate foreign alkylamines. Again, Mitoma and Udenfriend[23] showed that a considerable number of normally occurring aromatic compounds, for example, tryptophan and phenylalanine, were not hydroxylated by the hydroxylation system in microsomes but required specific mechanisms present in another part of the cell[37,38]. In what way are these compounds protected from the microsomal system? If we think of the microsomes as particles with a membrane which will, ordinarily, pass non-polar compounds but not polar compounds, we have a plausible picture of the way the body protects its essential substrates from wasteful metabolism due to the non-specific microsomal enzymes. Thus far, I might stress, we have found no normal substrate which is metabolised by the drug enzyme systems in microsomes.

Mr. Gaudette also demonstrated that amidopyrine was not appreciably dealkylated in liver homogenates of turtles, frogs and goldfish and hexobarbitone was not oxidised in liver homogenates of turtles and frogs. In fact, in preliminary experiments, frogs anæsthetised with hexobarbitone excreted it mainly in an unchanged form. The evidence is certainly incomplete, but it is possible that the microsomal drug enzyme systems are absent in lower animals.

Thus, we can consider the possibility that the enzyme systems in microsomes of mammals which have a major role in limiting the duration of action of drugs have developed by a process of evolution to protect the organism from a multitude of foreign compounds ingested in food as well as produced by micro-organisms in the intestines.

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