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N-DEMETHYLATION AND *N*-OXIDATION OF IMIPRAMINE BY RAT AND PIG LIVER MICROSOMES*

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Abstract—With rat hepatic microsomes, *N*-demethylation of imipramine was optimal when the medium was supplemented with Mg^{2+} (18 mM) and EDTA (1 mM). Substitution of chemically reduced Nicotinamide adenine dinucleotide phosphate (NADPH) for the NADPH-generating system (NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase) did not alter this finding. The enhanced metabolic activity by Mg^{2+} was therefore not due to a stimulation of the NADPH-generating system. *N*-oxidation of imipramine, however, was optimal when ethylenediaminetetraacetate (EDTA) (1 mM) was added in the absence of Mg^{2+}. Nicotinamide had no effect on either pathway. SKF 525-A (1 mM) inhibited only the *N*-demethylation, KCN (1 mM) did not affect either pathway and phenobarbital-treatment of the rats increased the *N*-demethylation and decreased the *N*-oxidation.

With pig liver microsomes, *N*-demethylation of imipramine was optimal with EDTA (1 mM) in the absence of Mg^{2+}. *N*-oxidation was optimal in the absence of both substances.

In the presence of Mg^{2+} and EDTA, rat hepatic microsomes demethylated imipramine 3·3 times faster than pig liver microsomes. Under the same conditions, no species difference was observed for the *N*-oxidation. When imipramine-*N*-oxide was incubated with rat hepatic microsomes, it could be shown that this substrate did not undergo any conversion to imipramine or desmethylimipramine. In most assays, a fluorometric determination method was used to quantify the metabolites. Their formation was not altered when rat or pig liver microsomes were used which had been stored as frozen pellets for 1–4 days at −18°.

THE METABOLIC fate of imipramine (IP)† in different species has been reported by several authors.[1–5] Both *in vivo* and *in vitro* studies present a great deal of information as to the number of metabolites resulting from biotransformation in liver and other organs (lung, kidney) and to their pharmacological activity.[6,7] It has been established that IP undergoes metabolic changes mostly by the action of the enzymes localized in the endoplasmic reticulum of the liver and that DMI and IPNO together with 2-OH-IP and 2-OH-DMI are the quantitatively most representative metabolites.[4,8] There remains no doubt that the demethylation reaction is catalyzed by the mixed function oxygenase system requiring NADPH and O_2 and involving cytochrome P-450 as terminal oxidase. For the *N*-oxidation reaction it has been found that it is

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† Abbreviations used: IP = imipramine; DMI = desmethylimipramine; IPNO = imipramine-*N*-oxide; 2-OH-IP = 2-hydroxy-imipramine; 2-OH-DMI = 2-hydroxy-desmethylimipramine; DDMI = desdimethylimipramine; SKF 525-A = 2-diethylaminoethyl-2,2-diphenylvalerate HCl; TEA = triethanolamine; DMA = *N*,*N*-dimethylaniline; DMAO = *N*,*N*-dimethylaniline-*N*-oxide.

also catalyzed by microsomal enzymes, but most likely not involving cytochrome P-450. Once IPNO has been formed, it is further reduced to IP and demethylated to DMI by extramicrosomal systems not requiring additional cofactors.[9,10]

The present study was aimed at further elucidating some factors influencing the *in vitro* metabolism of IP to DMI and to IPNO by rat and pig liver microsomes. Varying the components of the incubation mixture, incubating IP in the presence of inhibitors and using microsomes from phenobarbital-treated rats was taken as means to differentiate the *N*-demethylation and *N*-oxidation pathway. Incubations carried out over time periods up to 1 hr were set up to show species differences respective to the amount of DMI and IPNO formed.

MATERIALS AND METHODS

Male Wistar rats weighing 190–250 g were used. They were allowed free access to laboratory chow and tap water. The animals were killed by decapitation; the livers were immediately excised, chilled and homogenized in a Potter–Elvehjem homogenizer with 4 vol. of ice-cold 0·02 M tris–HCl buffer (pH 7·4) containing 1·15% KCl. The homogenate was centrifuged at 9000 *g* for 20 min in a MSE High Speed 18 refrigerated centrifuge. The supernatant fraction was then centrifuged at 100,000 *g* for 1 hr in a MSE Superspeed 50 refrigerated centrifuge. The microsomal pellet was resuspended in tris–KCl buffer. All operations were carried out between 0° and 4°.

By means of the same procedure, pig liver microsomes were prepared either from fresh livers which had been kept on ice for about 30 min or from frozen liver pieces which had been stored at −18° for 1–4 days. In the former case, the microsomes were used immediately after their preparation or stored as frozen pellets at −18° for 1–4 days; in the latter case, they were used immediately.

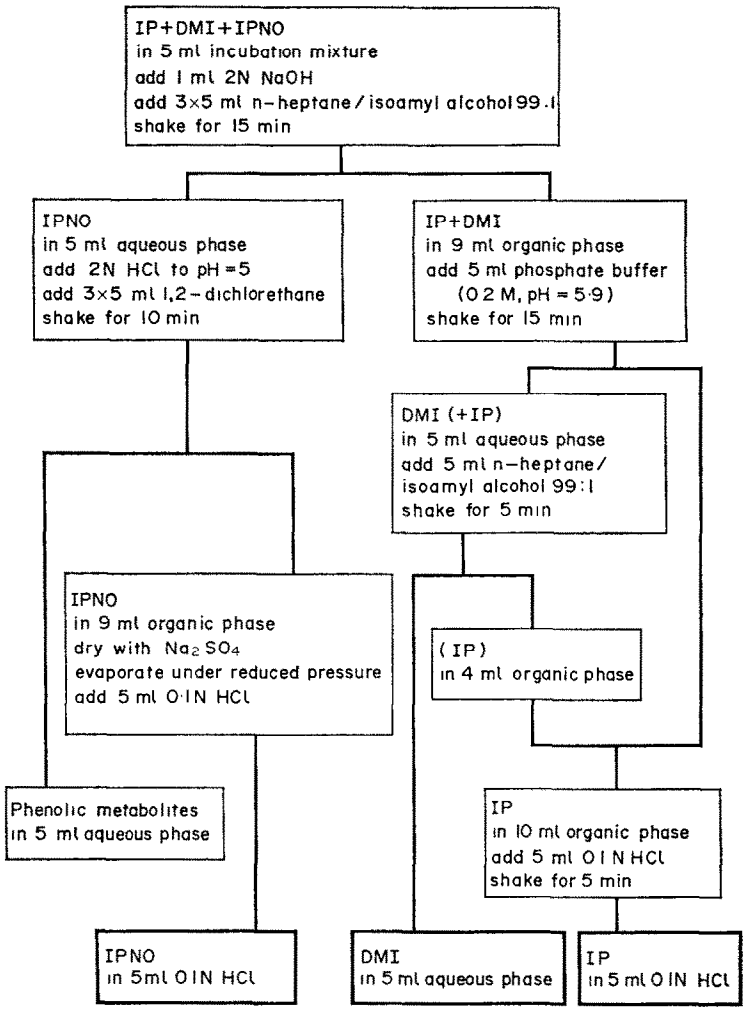
Microsomal protein concentrations were determined by the method of Lowry *et al.*[11] Crystalline bovine serum albumin was used as a standard.

Unless otherwise stated, the following incubation mixture was used: 1·2 μmoles NADP, 50 μmoles glucose-6-phosphate, 1·4 I.U. G-6-P-dehydrogenase, 90 μmoles $MgCl_2$, 100 μmoles nicotinamide, 0·5 μmoles EDTA, 2 μmoles IP and 0·15 M tris–HCl buffer (pH 7·4) to a total volume of 5 ml. The incubations were started by adding 5 mg microsomal protein and then carried out in scintillation glass vials under air at 37°. The reactions were stopped by the addition of 1 ml 2N NaOH to the incubation mixture and the metabolites DMI and IPNO were separated by the extraction procedure shown in Scheme 1. DMI and IPNO standards were carried through the separation procedure. The fluorescence of the two metabolites contained in 2 ml aqueous solution was measured after addition of 0·5 ml 2 N NaOH on an Aminco–Bowman Spectrophotofluorometer at 415 nm (excitation at 295 nm). The values reported represent nmoles metabolites/5 mg microsomal protein.

For the assays with pretreated rats, the animals received once daily for 3 days i.p. 80 mg phenobarbital/kg (phenobarbital sodium in 2·5 ml isotonic NaCl/kg); the last dose was administered 15 hr prior to sacrifice.

For the determination of K_m and V_{max} values, the amount of formaldehyde formed as the product of all demethylation pathways was measured by the method of Nash[12] as modified by Davies *et al.*[13]

The kinetic constants were obtained by a least squares method giving best fitting lines of the S/V vs. S plots, using a digital computer.



SCHEME 1.

Separation procedure for imipramine, desmethylimipramine, imipramine-*N*-oxide and phenolic metabolites.

Statistical variations among the experiments were evaluated by the Student *t*-test.[14] P-values $<0{\cdot}05$ were considered to represent significant differences between means.

RESULTS

Preliminary assays were carried out to determine whether rat liver microsomes which had been stored as frozen pellets for 20 hr at $-18°$ showed any differences in their ability to metabolize IP to DMI and IPNO as compared with microsomes used immediately after their preparation. The amounts of DMI and IPNO, measured after 10 and 30 min respectively, were not significantly different whether fresh or frozen stored microsomes had been used in the standard incubation mixture. The following

assays were therefore carried out alternatively with both kinds of microsomes. Furthermore, no change in the metabolism of IP was observed using pig liver microsomes when either liver or microsomal pellets had been stored frozen.

Also, the amounts of DMI and IPNO obtained in incubations run for 10 and 30 min respectively, were not significantly different whether TEA–HCl buffer (0·15 M, pH 7·4) or tris–HCl buffer was used. Tris buffer was ultimately chosen because it did not interfere with the microsomal protein determination.

The comparison of DMI and IPNO formation in incubations using microsomes or the 9000 *g* supernatant fractions as enzyme source showed that both reaction rates were somewhat higher in the latter system, in agreement with an earlier report.[4] The use of microsomes was preferred because the amount of metabolites could be related to the microsomal protein content and because reactions due to soluble enzymes could be excluded.

Effect of EDTA, Mg^{2+} and nicotinamide. In order to determine the effect of EDTA (Fig. 1), incubations were run for 30 min with rat liver microsomes in the presence of EDTA in concentrations up to 10^{-2} M. Since 10^{-4} M EDTA appeared to be optimal for the *N*-demethylation (65·5 per cent increase), this concentration was chosen for the standard incubation mixture. Table 1 presents the effects obtained when Mg^{2+}

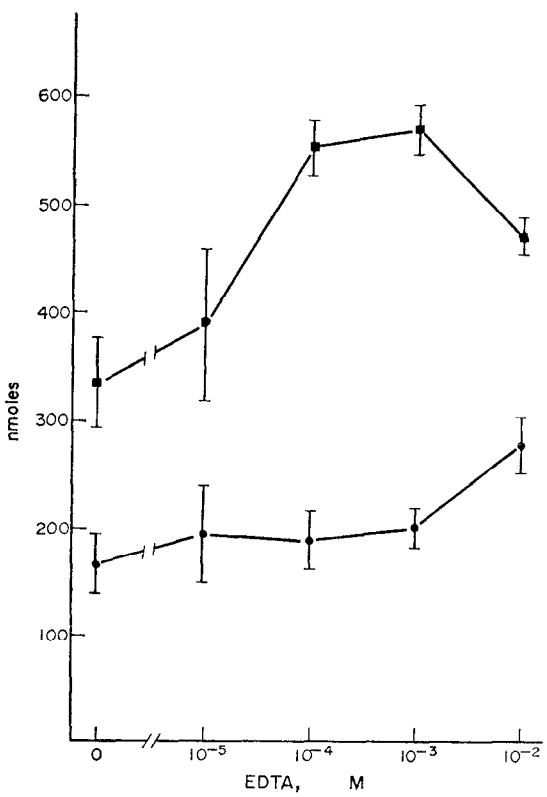


FIG. 1. Effect of EDTA on *N*-demethylation and *N*-oxidation of imipramine by rat liver microsomes. Values represent means ± standard error of five determinations. DMI (■—■) and IPNO (●—● after 30 min in standard incubation mixture (nmoles metabolite/5 mg microsomal protein).

TABLE 1. EFFECT OF Mg^{2+} AND/OR NICOTINAMIDE ON *N*-DEMETHYLATION AND *N*-OXIDATION OF IMIPRAMINE BY RAT LIVER MICROSOMES

Incubation mixture	nmoles DMI/5 mg microsomal protein	
	10 min	30 min
+ Mg^{2+} + Nicotinamide	298 ± 16 (17)	512 ± 21 (13)
− Mg^{2+} + Nicotinamide	246 ± 15 (13)*	367 ± 17 (9)*
+ Mg^{2+} − Nicotinamide	338 ± 20 (13)	541 ± 31 (9)
	nmoles IPNO/5 mg microsomal protein	
	10 min	30 min
+ Mg^{2+} + Nicotinamide	157 ± 10 (17)	175 ± 15 (13)
− Mg^{2+} + Nicotinamide	254 ± 27 (13)*	296 ± 20 (9)*
+ Mg^{2+} − Nicotinamide	178 ± 13 (13)	185 ± 18 (9)

Values represent means ± standard error. Number of determinations in brackets. Standard incubation mixture as described in methods.
* $P < 0.05$ as compared with Mg^{2+}.

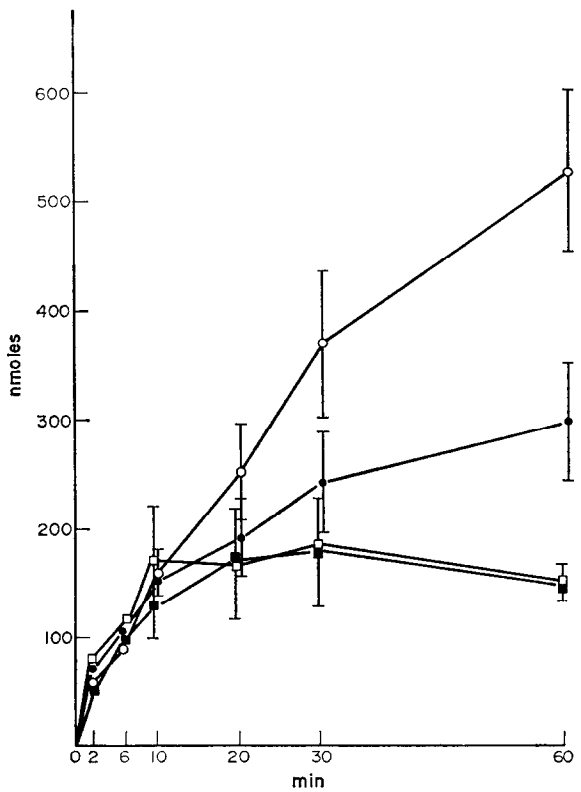


FIG. 2. Effect of Mg^{2+} on *N*-demethylation and *N*-oxidation of imipramine by pig liver microsomes. Values represent means ± standard error of four determinations, except for the 2 and 6 min values which represent means of two determinations DMI in the presence (■—■) and absence (□—□) of Mg^{2+} and IPNO in the presence (●—●) and absence (○—○) of Mg^{2+}. Standard incubation mixture as described in methods (nmoles metabolite/5 mg microsomal protein).

or nicotinamide were omitted from the standard incubation mixture with rat liver microsomes. In the absence of Mg^{2+} there was 17·5 and 28·3 per cent less DMI after 10 and 30 min respectively. The amount of IPNO, however, was increased 61·8 and 69·1 per cent respectively. The absence of nicotinamide did not alter either reaction rate. In incubations run for 10 min, the substitution of NADPH (1·2 μmoles) for the NADPH generating system did not produce significant differences in the amounts of DMI and IPNO respectively. The effects of EDTA, Mg^{2+} and nicotinamide were similar on both metabolic pathways when TEA–HCl buffer was substituted for tris–HCl buffer.

The kinetics of the IP *N*-demethylation and *N*-oxidation over a 1-hr incubation period with pig liver microsomes are presented in Fig. 2. After the formation of 127 nmoles DMI per 5 mg microsomal protein within the first 10 min in the standard incubation mixture, there was only a small increase of this metabolite during the following 50 min. The absence of Mg^{2+} only slightly altered the amounts of DMI. The formation of IPNO, however, was increased 51·9 and 79·6 per cent after 30 and 60 min respectively in the absence of Mg^{2+}.

Table 2 shows the combined effects of EDTA and Mg^{2+} on the *N*-demethylation and *N*-oxidation of IP by rat and pig liver microsomes for 10 and 30 min. If we consider the amounts of metabolites obtained in the incubation mixture without EDTA and Mg^{2+} as 100 per cent, the addition of EDTA (10^{-4} M) caused a stimulation of the *N*-demethylation in both species. When Mg^{2+} (18 mM) was already present, EDTA still increased the amounts of DMI in both species. The addition of Mg^{2+} in the absence of EDTA led to a slight stimulation of the *N*-demethylation with

TABLE 2. EFFECT OF EDTA AND/OR Mg^{2+} ON *N*-DEMETHYLATION AND *N*-OXIDATION OF IMIPRAMINE BY RAT AND PIG LIVER MICROSOMES

Incubation mixture	Rat (5)		Pig	
	(nmoles DMI/5 mg microsomal protein)			
	10 min	30 min	10 min	30 min
+ Mg^{2+} + EDTA	365 ± 25‡	556 ± 23‡	109 ± 13 (10)	163 ± 29 (7)
+ Mg^{2+} − EDTA	236 ± 28†	336 ± 41†	77 ± 13 (3)	125 ± 5 (3)*
− Mg^{2+} + EDTA	278 ± 21†	392 ± 21*†‡	126 ± 24 (10)	161 ± 28 (7)
− Mg^{2+} − EDTA	226 ± 9†	295 ± 21*†	90 ± 20 (3)	136 ± 5 (3)
	nmoles IPNO/5 mg microsomal protein			
	10 min	30 min	10 min	30 min
+ Mg^{2+} + EDTA	179 ± 20	189 ± 27	176 ± 19 (10)	276 ± 44 (7)*
+ Mg^{2+} − EDTA	167 ± 30	167 ± 27‡	168 ± 8 (3)	173 ± 3 (3)‡
− Mg^{2+} + EDTA	256 ± 47	329 ± 19†	177 ± 21 (10)	374 ± 46 (7)*
− Mg^{2+} − EDTA	223 ± 46	268 ± 34	233 ± 30 (3)	410 ± 46 (3)*

Values represent means ± standard error. Number of determinations in brackets. Standard incubation mixture as described in methods.

* $P < 0.05$ as compared with the 10 min values.

† $P < 0.05$ as compared with + Mg^{2+} +EDTA.

‡ $P < 0.05$ as compared with −Mg^{2+} −EDTA.

rat microsomes and to a slight decrease with pig microsomes. When EDTA was already present, Mg^{2+} further increased the amounts of DMI with rat microsomes. With pig microsomes, however, Mg^{2+} further decreased *N*-demethylation (10 min) or did not change it (30 min). Thus the *N*-demethylation of IP was increased in both species by the addition of EDTA whether Mg^{2+} was present or not. Mg^{2+} itself stimulated the formation of DMI with microsomes to a higher degree when EDTA was present. With pig microsomes it caused a slight decrease whether EDTA was present or not. The addition of both EDTA and Mg^{2+} as in the standard incubation mixture led to an increased *N*-demethylation in both species. With rat microsomes, the two components potentiated their effects, resulting in 61·5 and 88·5 per cent more DMI after 10 and 30 min respectively. With pig liver microsomes, the more potent effect of EDTA overrode the effect of Mg^{2+} and it resulted in 21·1 and 19·9 per cent more DMI after 10 and 30 min respectively.

For the *N*-oxidation it was observed that the addition of EDTA caused an increase with rat and a decrease with pig microsomes. When Mg^{2+} was already present, EDTA slightly increased the amounts of IPNO in both species. Addition of Mg^{2+} caused a decreased *N*-oxidation in both species. When EDTA was already present, Mg^{2+} caused a further decrease in rat and no change (10 min) or further decrease (30 min) with pig microsomes. Thus the *N*-oxidation of IP with rat microsomes was increased by EDTA whether Mg^{2+} was present or not. With pig microsomes it was decreased in the absence of Mg^{2+} and increased in its presence. Mg^{2+} itself decreased the *N*-oxidation in both species, however, to a greater extent in the absence of EDTA than in its presence. The addition of both EDTA and Mg^{2+} as in the standard incubation mixture resulted in both species in a decreased *N*-oxidation. With rat liver microsomes the decreasing effect of Mg^{2+} overrode the increasing effect of EDTA to give 19·7 and 25·5 per cent less IPNO after 10 and 30 min respectively. With pig liver

TABLE 3. *N*-DEMETHYLATION AND *N*-OXIDATION OF IMIPRAMINE BY RAT LIVER MICROSOMES IN THE PRESENCE OF SKF 525-A OR KCN AND IN PHENOBARBITAL-TREATED ANIMALS

Incubation mixture	+ SKF 525-A	+ KCN	Phenobarbital-treated
	(nmoles DMI/5 mg microsomal protein)		
+ Mg^{2+} + Nicotinamide	143 ± 7*	274 ± 17	570 ± 31*
− Mg^{2+} + Nicotinamide	117 ± 2*†	210 ± 13†	406 ± 19*†
+ Mg^{2+} − Nicotinamide	134 ± 6*	310 ± 21	673 ± 34*
	(nmoles IPNO/5 mg microsomal protein)		
+ Mg^{2+} + Nicotinamide	186 ± 26	174 ± 9	128 ± 8
− Mg^{2+} + Nicotinamide	257 ± 19	265 ± 18†	169 ± 8†
+ Mg^{2+} − Nicotinamide	154 ± 24	210 ± 13	111 ± 4*

Values represent means ± standard error of four determinations for 10 min incubations in the presence of SKF 525-A and KCN (both 10^{-3} M) and five determinations for the phenobarbital-treated animals. Standard incubation mixture as described in methods.

* $P<0·05$ as compared with controls (Table 1).

† $P<0·05$ as compared with + Mg^{2+}.

microsomes addition of both components gave 23·6 and 32·7 per cent less IPNO after 10 and 30 min respectively.

Effect of SKF 525-A, KCN or phenobarbital-treatment. Table 3 presents the effects of SKF 525-A and KCN (both 10^{-3} M) and of phenobarbital-treatment on the *N*-demethylation and *N*-oxidation of IP by rat liver microsomes in 10 min incubations. Preliminary assays have shown that the increase of the *N*-demethylation rate as a function of the number of daily phenobarbital injections from two to four days was insignificant, whereas the amount of IPNO remained unchanged or was decreased as compared with the controls. Therefore the rats received one daily injection for three days as described in methods.

In the presence of SKF 525-A the formation of DMI was decreased more than 50 per cent. The *N*-oxidation, however, was not altered. KCN did not affect either pathway. The effects reported earlier (Table 1) due to the absence of Mg^{2+} were observed here too.

After pretreatment with phenobarbital, the *N*-demethylation of IP was 90 per cent higher when Mg^{2+} was present and 65 per cent in its absence. The amounts of IPNO

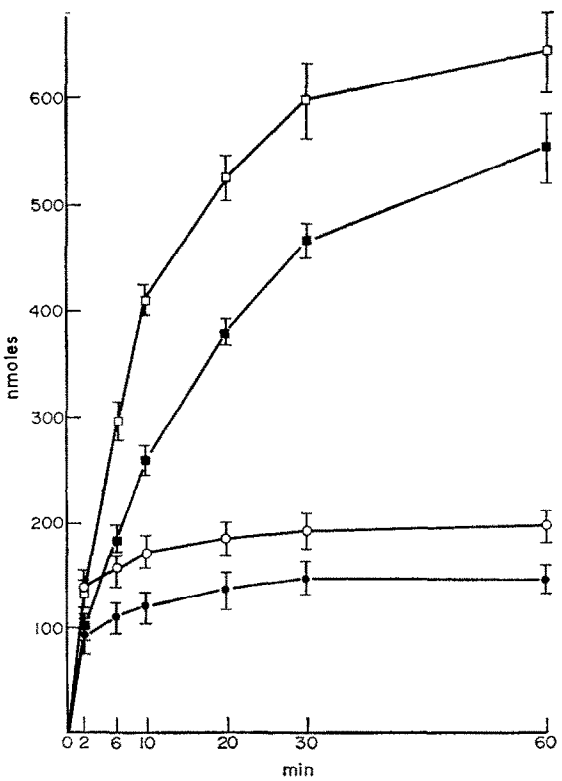


FIG. 3. Influence of microsomal protein concentration on *N*-demethylation and *N*-oxidation of imipramine by rat liver microsomes. Values represent means ± standard error of four determinations. DMI with 5 mg (■—■) and 10 mg (□—□) microsomal protein and IPNO with 5 mg (●—●) and 10 mg (○—○) microsomal protein. Standard incubation mixture as described in methods (nmoles metabolite/incubation).

were decreased significantly only when nicotinamide was omitted. Here again, the effects due to the absence of Mg^{2+} were observed.

Effect of microsomal protein concentration. Figure 3 presents the amounts of DMI and IPNO formed as a function of time in incubations allowed to run for 60 min with rat liver microsomes at two protein concentrations (5 and 10 mg per 5 ml standard incubation mixture). With both protein concentrations the *N*-demethylation proceeded at a linear rate only during the first 10 min. The *N*-oxidation, however, proceeded at the same rate only during the first measured time period (2 min) to level off after 10 min. Doubling the protein concentration increased the initial *N*-demethylation and *N*-oxidation rate only 84·6 and 52·8 per cent respectively, calculated from the relative increases during the first 30 min.

When IPNO was substituted for IP as substrate in the standard incubation mixture, we could not detect any metabolic change.

Kinetic constants. In some assays the *N*-demethylation was measured using both the determination of DMI and of formaldehyde formed. As expected the amount of formaldehyde was somewhat larger than the amount of DMI, since there are additional, if minor, *N*-demethylation reactions: DMI—→DDMI, 2-OH-IP—→2-OH-DMI.[4] The fluorometric determination of DMI yielded 448 ± 46 nmoles/30 min/5 mg microsomal protein as compared with 570 ± 76 nmoles formaldehyde (mean value ± standard error of five determinations).

The overall formaldehyde formation, proceeding at a linear rate up to at least 20 min, was used to determine the apparent K_m value of the *N*-demethylation which was $1{\cdot}3 \times 10^{-4}$ M. The corresponding V_{max} was 8·5 nmoles formaldehyde formed/min/mg microsomal protein.

DISCUSSION

Our finding that storage of rat and pig hepatic microsomes as frozen pellets did not change the rate of IP *N*-demethylation and *N*-oxidation parallels other reports.[16–20] Under similar conditions there was no decrease observed in the hydroxylation of androgens[16,17] and 3,4-benzpyrene,[18] and in the *N*-demethylation of ethylmorphine,[17,19] morphine and codeine.[20] Ziegler and Pettit[21,22] have reported that repeated freezing and thawing of rat and pig liver microsomes, however, increased the DMA *N*-oxidation.

Nicotinamide has long been thought to be a necessary component of the incubation mixture for microsomal drug metabolism *in vitro*.[23,24] It inhibits the pyridine nucleotidase of liver which destroys NADP.[25] Nicotinamide increased the metabolism of *p*-ethoxyacetanilide[24] and pyromycine.[26] More recent investigations, however, have shown that the presence of nicotinamide inhibits the *N*-demethylation of aminopyrine[27] and ethylmorphine[28] and the *p*-hydroxylation of aniline.[27] In our assays with rat hepatic microsomes, nicotinamide had no effect on either pathway studied (Table 1) up to 30 min.

The presence of Mg^{2+} has been reported to modify the rate of metabolism of several drugs by hepatic microsomes *in vitro*.[19,23,29–31] In some cases, no change could be observed.[24,26] Its effect has been shown either to improve the NADPH-generating system or to stimulate the enzymes directly. We have found that the *N*-demethylation of IP by rat hepatic microsomes was equally decreased by the absence of Mg^{2+} with either NADPH-generating system or NADPH. It can therefore be

assumed that in our assays Mg^{2+} did not stimulate the metabolism by improving the NADPH generation. Similar findings have been reported by Terriere and Chan[19] and Peters and Fouts.[31] The two latter authors have also shown[31] that the addition of EDTA (10^{-2} M) in the absence of Mg^{2+} caused little decrease in metabolism of some drugs. With 100 times less EDTA, we have shown (Table 2) an increase in the *N*-demethylation of IP with rat and pig liver microsomes. Since Mg^{2+} has been assayed only at the concentration (18 mM) reported to be optimal by Bickel and Baggiolini,[4] it could well be that the effect of this ion is dependent on the concentration and that it could either improve, decrease or not change the rates of metabolism. Peters and Fouts[31] have shown these types of dependence on the amount of Mg^{2+} added. Our findings could be explained by the fact that endogenous Mg^{2+} is more active when chelated by EDTA. The addition of Mg^{2+} only slightly increased the IP *N*-demethylation with rat microsomes, but the stimulation became greater when Mg^{2+} was present together with EDTA. With pig microsomes, the addition of Mg^{2+} inhibited the *N*-demethylation, but after addition of EDTA we observed stimulation. Thus stimulation of the formation of DMI by Mg^{2+} in the presence of EDTA was greater than the inhibition in the absence of EDTA.

For the *N*-oxidation of IP, we showed that EDTA increased it with rat microsomes and decreased it with pig microsomes. Mg^{2+} alone inhibited in both species. Thus with rat microsomes the inhibiting effect of Mg^{2+} alone may be greater than its stimulating effect in the presence of EDTA. With pig microsomes, Mg^{2+} decreased the *N*-oxidation, independently whether EDTA was present or absent. In order to give appropriate explanations of these interactions, more work needs to be done in varying the amounts of Mg^{2+} and EDTA added.

In the standard incubation mixture (EDTA and Mg^{2+} present) we could, however, notice some species differences: with rat hepatic microsomes, the rate of *N*-demethylation of IP was 3·3 times that observed with pig microsomes. While there was no difference in the *N*-oxidation after 10 min, the amount of IPNO was about 40 per cent higher with pig microsomes after 30 min. The time-dependent increase of IPNO with pig microsomes was about 60 per cent after 30 min as compared to the amount obtained after 10 min, whereas with rat microsomes there was no increase. In both species, however, the *N*-demethylation was increased 50–70 per cent after 30 min as compared to 10 min. The optimal conditions for the two pathways studied with rat and pig microsomes appeared to be as shown in the following layout:

Rat hepatic microsomes:	*N*-demethylation	in the	presence of Mg^{2+}
			presence of EDTA
	N-oxidation	in the	absence of Mg^{2+}
			presence or absence of EDTA
Pig liver microsomes:	*N*-demethylation	in the	presence or absence of Mg^{2+}
			presence of EDTA
	N-oxidation	in the	absence of Mg^{2+}
			absence of EDTA.

Under these conditions, the *N*-demethylation rate was always higher than the *N*-oxidation with rat liver microsomes and slower with pig liver microsomes.

The effects of SKF 525-A (Table 3), i.e. decrease of the amount of DMI and no change in the *N*-oxidation, may be explained by the assumption that the two path-

ways are catalyzed by different enzyme systems, the *N*-oxidation not requiring cytochrome P-450 as terminal oxidase.[10] With DMA, Ziegler and Pettit,[21] however, have shown that inhibition of *N*-demethylation by SKF 525-A with pig liver microsomes resulted in a higher rate of *N*-oxidation. The fact that CN^- does not alter either metabolic pathway of IP parallels this report[21] for DMA metabolism.

Phenobarbital pretreatment of rats has been shown (Table 3) to stimulate the *N*-demethylation of IP. The rate of IP *N*-oxidation, however, was decreased. This cannot be explained by a higher rate of metabolism of IPNO (reduction or demethylation), since our finding that IPNO did not undergo any metabolic change is in agreement with the report by Bickel, Weder and Aebi,[9] who found that IPNO was reduced to IP or demethylated to DMI by extramicrosomal enzyme systems.

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