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MOUSE LIVER MICROSOMAL HEXOSE-6-PHOSPHATE DEHYDROGENASE

NADPH GENERATION AND UTILIZATION IN MONOOXYGENATION REACTIONS*

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Abstract—Hexose-6-phosphate dehydrogenase (H6PD) activity in washed hepatic microsomes from male ICR mice, when assayed with $NADP^+$ and deoxyglucose-6-phosphate, was partially latent. Brief sonication or detergents activated H6PD causing an approximately 4- and 8.5-fold increase in NADPH generation respectively. The sonicated microsomes exhibited H6PD-linked *N*-demethylase activity toward aminopyrine. This activity was best sustained in the presence of deoxyglucose-6-phosphate, while galactose-6-phosphate, glucose-6-phosphate, and glucose were less effective. Reaction media containing sonicated microsomes, $NADP^+$ and deoxyglucose-6-phosphate also catalyzed *N*-demethylation of *p*-chloro-*N*-methylaniline, *N,N*-dimethylaniline and nicotine, *O*-demethylation of *p*-nitroanisole, *p*-hydroxylation of aniline, ring hydroxylation of biphenyl at the 2- and 4-positions, dearylation of parathion, and the *N*-oxidation of *N,N*-dimethylaniline. In general, the hexose-6-phosphate dehydrogenase-linked monooxygenation rates were 60% or more of those observed in the presence of exogenous NADPH.

Hexose-6-phosphate dehydrogenase (H6PD) (β-D-glucose:$NAD(P)^+$ 1-oxidoreductase, EC 1.1.1.47), first described as glucose dehydrogenase by Harrison in 1931 [1], is a constituent enzyme of the endoplasmic reticulum [2] present in several organs [3] of many animal species [4], including man [5]. H6PD differs from the cytosolic glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) in many respects such as substrate specificity, subcellular localization, response to activators and inhibitors, immunological, chromatographic and electrophoretic behaviors, and response to diet, starvation, hormones and inducers [6].

Although H6PD is a well-characterized enzyme, its metabolic function is not fully explored. Only two reports [7, 8] indicate its importance in the generation of NADPH utilized in cytochrome P-450-dependent microsomal monooxygenase reactions. Stegeman and Klotz [7] reported that H6PD in intact hepatic microsomes from female CD mice is capable of sustaining benzpyrene hydroxylase activity. This activity was quite low, being only 14 and 20% of that obtained with an exogenous NADPH-generating system when either galactose-6-phosphate (Gal-6-P) or glucose-6-phosphate (G-6-P) was used as a substrate for H6PD. However, up to 60% of the maximum activity was observed with fish liver microsomes [7]. Kimura *et al.* [8], working with intact liver microsomes from Sprague–Dawley rats, found that aminopyrine *N*-demethylase activity was best sustained when deoxyglucose-6-phosphate (dG-6-P) was used as an H6PD substrate as compared to Gal-6-P and G-6-P, while glucose was ineffective. However, brief sonication of microsomes increased both NADPH generation and monooxygenation 2- to 3-fold while the order of effectiveness of H6PD substrates remained unchanged. Whether the reported low monooxygenase activity in mouse hepatic microsomes is due to the latency of H6PD or to species specificity remains unknown. Similarly, the extent to which H6PD-dependent NADPH generation can sustain other monooxygenase reactions is not yet resolved.

Our interest in this subject stems from the unusual interactions of *n*-octylamine with the midgut microsomes of the tobacco hornworm [9, 10]. In these and subsequent investigations, it was demonstrated that insect and mammalian microsomes possess the necessary cofactors and enzyme(s) to reduce $NAD(P)^+$ to NAD(P)H, which in turn reduced both cytochrome b_5 and cytochrome P-450 [11, 12]. In this communication, we demonstrate that H6PD is a partially latent enzyme in hepatic microsomes of male ICR mice and is capable of effectively supplying the NADPH necessary for monooxygenation of a number of xenobiotics.

MATERIALS AND METHODS

Male ICR/Duplin mice (6- to 8-weeks-old) were

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purchased from Flow Laboratories, Duplin, VA. $NADP^+$, NADPH, glucose, G-6-P, Gal-6-P, dG-6-P, and G6PD from Torula yeast were obtained from the Sigma Chemical Co., St. Louis, MO. All other chemicals were of the highest analytical grade commercially available and were used without further purification.

The livers from three to five mice were pooled, and microsomes were isolated according to the procedure described previously [13]. Only freshly isolated washed microsomes were used. Each experiment was repeated three or more times with different enzyme preparations, and the data are reported as either the mean ± standard error or are the results of a typical experiment.

In our hands, the ethanol precipitation procedure to quantitate NADPH, as described by Kimura *et al.* [8], was not satisfactorily reproducible. Therefore, H6PD activity was assayed spectrophotometrically as follows. The reaction medium in each cuvette contained the following components in a total volume of 1.0 ml: 0.1 M Tris buffer, (pH 7.9), microsomes (1.0–1.5 mg protein), 2.0 mM $MgCl_2$, 0.1 mM EDTA, 1.0 mM nicotinamide, 1.0 mM *p*-chloromercuribenzoate (*p*-CMBA), 0.25 mM $NADP^+$, and the indicated level of an activator. After 5 min of preincubation at 37°, the reaction was initiated by the addition of 2.5 mM dG-6-P to the sample cuvette and an equal volume of buffer was added to the reference cuvette. The increase in absorbance at 340 nm was monitored at 37°, aerobically, with continuous stirring, using an Aminco DW-2 UV/vis spectrophotometer. Under these conditions, the concentrations of dG-6-P and $NADP^+$ were not limiting and the $NADP^+$ reduction rates were linear with protein content. The linear portion of the reaction curve (between 30 and 90 sec) was used to calculate the quantity of NADPH formed, using 6.22 as the millimolar extinction coefficient for NADPH.

Metabolism of various xenobiotics was compared using three methods of NADPH supply. The assay medium contained either (A) 1.0 mM NADPH, or (B) an artificial NADPH-generating system consisting of 0.5 mM $NADP^+$, 5.0 mM G-6-P and 1.5 units of G6PD from Torula yeast, or (C) NADPH generated *in situ* by mouse liver microsomal H6PD in the presence of 0.5 mM $NADP^+$ and 5.0 mM dG-6-P. These cofactor concentrations were not rate limiting. Other components of the reaction mixture included 2.0 mM $MgCl_2$, 0.1 mM EDTA, 1.0 mM nicotinamide, substrate, microsomes (~3 mg protein), and 0.1 M Tris buffer, (pH 7.9). *N*-Demethylation was assayed using 2.0 mM aminopyrine, 1.5 mM *p*-chloro-*N*-methylaniline or 3.0 mM nicotine, the quantity of formaldehyde formed being estimated by the method of Nash [14] as modified by Cochin and Axelrod [15]. The metabolism of *N,N*-dimethylaniline by either demethylation or *N*-oxidation was measured at a substrate concentration of 1.0 mM. *N*-Demethylase activity was quantified by measuring formaldehyde whereas the formation of *N,N*-dimethylaniline-*N*-oxide was estimated according to the method of Ziegler and Pettit [16]. Biphenyl-2-hydroxylase and 4-hydroxylase activities were determined fluorometrically according to the procedure described by Burke and Prough [17], while *O*-demethylation of *p*-nitroanisole (0.5 mM; [18]) and of aniline-4-hydroxylase (15.0 mM; [19]) was assayed according to published methods. Parathion (0.1 mM) dearylation was assayed spectrophotometrically by monitoring *p*-nitrophenol formation at 405 nm using an Aminco DW-2 spectrophotometer. In all assays, the rate of metabolism was linear with respect to time and, depending upon the assay in question, an incubation time of 10–30 min was employed. Protein was estimated by the method of Lowry *et al.* [20] using crystalline bovine serum albumin, fraction V, as standard.

RESULTS AND DISCUSSION

Under aerobic conditions, incubation of mouse hepatic microsomes with $NADP^+$, either alone [11] or together with dG-6-P resulted in the generation of NADPH although, due to reoxidation by endogenous NADPH oxidase activity of the microsomes, accumulation of NADPH was difficult to demonstrate. This problem was also encountered by other investigators [8, 21]. Generation of NADPH under these conditions can, however, be inferred from the concomitant reduction of the microsomal

Table 1. Effects of chemical agents and sonication on the H6PD activity of mouse hepatic microsomes*

Agent (concn)	H6PD activity [nmoles · min^{-1} · (mg protein)$^{-1}$]	Relative
Control	1.56 ± 0.06	100
Ammonium sulfate (0.5 M)	0.91 ± 0.15	58
Ammonium chloride (0.5 M)	1.96 ± 0.44	126
Ammonium acetate (0.5 M)	2.45 ± 0.26	157
Acetone (1.0 M)	2.65 ± 0.26	170
α,α-Bipyridyl (1.0 mM)	1.52 ± 0.28	97
Sodium cholate (1 mg/ml)	8.68 ± 0.91	556
Triton X-100 (0.5 mg/ml)	12.76 ± 0.95	818
Octyl glucoside (12.5 mM)	13.35 ± 0.89	856
Sonication†	6.17 ± 0.29	391

* H6PD activity was assayed by monitoring the increase in absorbance at 340 nm in the presence of 1.0 mM *p*-CMBA as described in Materials and Methods.

† Washed microsomes were sonicated for 45 sec.

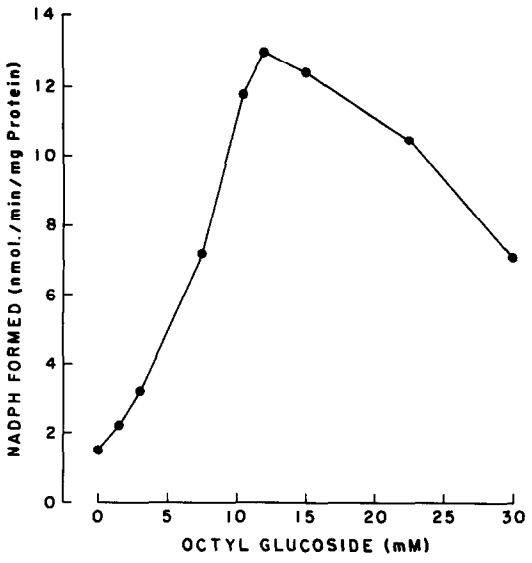

Fig. 1. Activation of mouse liver microsomal H6PD by octyl glucoside. Assays were performed as described in Materials and Methods in the presence of different concentrations of octyl glucoside, as shown.

hemoproteins [11]. Although *p*-CMBA is a potent inhibitor of microsomal NADPH–cytochrome *c* reductase [22], it is without effect on H6PD even at high concentrations [8, 11]. Thus, when *p*-CMBA was incorporated into the reaction medium containing mouse liver microsomes, $NADP^+$ and dG-6-P, NADPH accumulated with an initial velocity of about 1.5 nmoles·min^{-1}·(mg protein)$^{-1}$ (Table 1). The activity reported for rat liver microsomes under similar conditions is approximately six times as high [8].

The latency of mouse liver microsomal H6PD was evident from the activation observed when membrane perturbation was brought about by different chemical or physical agents (Table 1). Thus, ammonium chloride, ammonium acetate and acetone caused a 25–70% increase in activity over control values. Ammonium sulfate was inhibitory at the concentration used while α,α-bipyridyl was without any effect. All of the detergents tested activated the enzyme and a maximum activation of approximately 8.5-fold was observed in the presence of octyl glucoside, while approximately 4-fold activation was observed when briefly sonicated microsomes were used. The dependence of the activation process on the concentration of octyl glucoside is shown in Fig. 1. The results of several such experiments indicated that, under the experimental conditions employed, a maximum activation of H6PD could be achieved with 10–15 mM octyl glucoside or 0.4–0.6 mg of Triton X-100/ml.

Although our observations on detergent activation of the mouse hepatic microsomal H6PD are in accord with those of others [8, 21] on rat liver microsomes, some interesting facts emerge when the data are compared. While the maximal specific activity of about 13 nmoles of NADPH formed·min^{-1}·(mg protein)$^{-1}$ with either Triton X-100 or octyl glucoside-treated mouse liver microsomes (Table 1) is close to the values of 15.7 [8] and 10.7 [23] reported for the rat liver microsomes, the unactivated levels are much lower. In contrast to over 8.5-fold activation of the mouse enzyme, rat liver enzyme showed only 43% activation [8]. Therefore, presuming the maximum activity achieved represents full expression of enzyme activity, it appears that only 11–12% of the mouse liver H6PD was freely accessible for catalytic activity while about 90% was latent. Similar calculations of the data reported by Kimura *et al.* [8] indicate that, in the rat, only about 30% of the enzyme is latent. Based on H6PD activity under an anaerobic or CO atmosphere as well as that observed in the presence of detergents or *p*-CMBA, Kimura *et al.* [8] also reached similar conclusions. However, this is in contrast to the conclusions of Hori and Takahashi [21, 23] who consider H6PD in rat liver microsomes to be a latent enzyme. The conclusions are based solely on the ease of activation and solubilization of H6PD by detergents. These authors did not use *p*-CMBA in the reaction media during the H6PD assay and, therefore, distinction between luminal and cytosolic surface localization of the enzyme cannot be made. However, their data on inhibition of H6PD by antibody in phospholipase *c* or deoxycholate-treated, but not in intact, microsomes support the view that at least part of H6PD activity resides in the luminal surface. These authors also suggest that failure of $NADP^+$, but not of G-6-P, to penetrate microsomal membranes also contributes to the latency of H6PD.

Another factor that should be taken into account while evaluating H6PD is its inhibition by reduced pyridine nucleotides [11, 24]. Therefore, NADPH estimation by the ethanol precipitation method after long incubation times [8] is not suitable for the determination of initial velocities although the extent to which this factor affects kinetic measurements using continuous spectrophotometric assay is unknown. This inhibition was presumed to be negligible in our experiments since observations were made within 90 sec after deoxyG-6-P addition.

Although detergents markedly activated mouse hepatic microsomal H6PD, preliminary experiments indicated a high degree of inhibition of monooxygenase activity when assayed with aminopyrine as a substrate. A species specific inhibition of cytochrome P-450-dependent mixed function oxidation of xenobiotics is well known [25]. Therefore, the experiments reported below were carried out with briefly sonicated microsomes in which H6PD was activated about half as much as with detergents (Table 1). For comparison, data were also obtained with microsomes not previously activated.

Before evaluating the ability of H6PD to support *in vitro* metabolism of several xenobiotic substrates, different factors affecting monooxygenase activity were studied using aminopyrine as a substrate and the amount of formaldehyde formed as the index of *N*-demethylase activity. The results (Fig. 2A) indicate that, although significant *N*-demethylation occurred when microsomes sonicated for 15 sec were used, the optimal sonication time appeared to be 45 sec. Experiments performed with microsomes sonicated for 45 sec indicated that about 2.5 mM dG-6-P (Fig. 2B) and at least 0.25 mM $NADP^+$ (Fig.

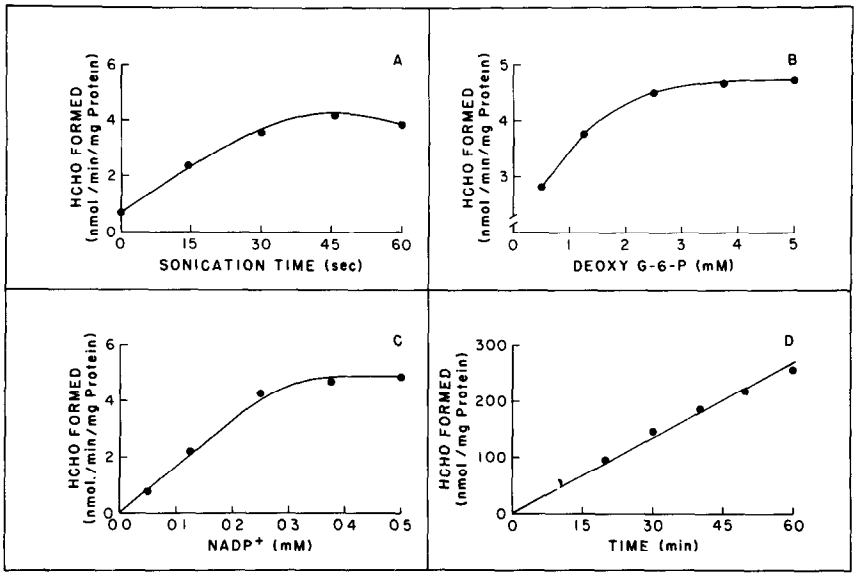

Fig. 2. Effects of assay conditions on H6PD-linked *N*-demethylation of aminopyrine by mouse hepatic microsomes. Each reaction medium contained 2.0 mM aminopyrine, 1.0 mM nicotinamide, 0.1 mM EDTA, 2.0 mM $MgCl_2$ and (A) microsomes sonicated for the indicated time, 0.5 mM $NADP^+$ and 5.0 mM dG-6-P, or (B) microsomes sonicated for 45 sec, 0.5 mM $NADP^+$ and the indicated concentration of dG-6-P, or (C) microsomes sonicated for 45 sec, 5.0 mM dG-6-P and the indicated concentration of $NADP^+$, or (D) microsomes sonicated for 45 sec, 0.5 mM $NADP^+$ and 5.0 mM dG-6-P. The incubation time varied from 0 to 60 min (15 min in A, B and C). See text for further details.

2C) were needed for maximum *N*-demethylase activity with aminopyrine as a substrate. Furthermore, when optimum levels of these cofactors were used, the rate of formaldehyde production was linear up to 60 min (Fig. 2D).

In contrast to the cytosolic G6PD, which is specific for G-6-P and $NADP^+$, microsomal H6PD can oxidize glucose, G-6-P, Gal-6-P, and dG-6-P using either NAD^+ or $NADP^+$ as an electron acceptor [1–4, 6–8, 21–24]. In view of the reported [8] effectiveness of dG-6-P, as compared to G-6-P, Gal-6-P and glucose, in supporting aminopyrine *N*-demethylation by rat liver microsomes, this reaction was investigated using mouse liver microsomes. The data obtained (Table 2) clearly suggest that the very low *N*-demethylase activity observed with intact mouse liver microsomes may be related to a low level of NADPH supply. The results suggest that in intact mouse liver microsomes the latency of H6PD described earlier with dG-6-P also exists with other substrates. However, a significant increase in *N*-demethylase activity was observed when briefly sonicated microsomes were used. Although glucose was found to be least effective, about 20 and 25% *N*-demethylase activity was observed with either G-6-P or Gal-6-P, while dG-6-P was about 60% as effective as NADPH. These results, in general, agree with those reported for the rat liver microsomes [8].

Table 2. Aminopyrine *N*-demethylase activity supported by NADPH generated by H6PD using different substrates of H6PD*

H6PD substrate	Aminopyrine *N*-demethylation [nmoles HCHO · min^{-1} · (mg protein)$^{-1}$]	Relative
Intact microsomes		
Control	9.65 ± 0.67	100.00
Glucose	0.13 ± 0.05	1.37
G-6-P	0.30 ± 0.05	3.15
Gal-6-P	0.28 ± 0.08	2.90
dG-6-P	0.74 ± 0.11	7.63
Sonicated microsomes		
Control	6.91 ± 0.36	100.00
Glucose	0.62 ± 0.12	8.98
G-6-P	1.48 ± 0.11	21.46
Gal-6-P	1.71 ± 0.02	24.80
dG-6-P	4.09 ± 0.24	59.17

* Aminopyrine *N*-demethylase activity was assayed using intact or sonicated (for 45 sec) microsomes as described in Materials and Methods. Incubation medium contained either 1.0 mM NADPH (control) or 0.5 mM $NADP^+$ and 5.0 mM H6PD substrate besides aminopyrine and microsomes.

Table 3. *N*-Demethylation of different substrates by mouse hepatic microsomes by NADPH generated *in situ* by microsomal H6PD*

Substrate	*N*-Demethylase activity [nmoles HCHO formed·min^{-1}·(mg protein)$^{-1}$] A†	B‡	C§
Intact microsomes			
Aminopyrine	9.65 ± 0.67 (100)	10.07 ± 1.05 (104)	0.74 ± 0.11 (8)
p-Chloro-*N*-methylaniline	16.91 ± 1.39 (100)	15.72 ± 1.76 (93)	1.17 ± 0.08 (7)
N,N-Dimethylaniline	10.50 ± 1.20 (100)	10.61 ± 1.31 (101)	2.23 ± 0.24 (21)
Nicotine	0.72 ± 0.06 (100)	0.67 ± 0.04 (93)	0.31 ± 0.00 (43)
Sonicated microsomes			
Aminopyrine	6.90 ± 0.36 (100)	7.32 ± 0.51 (106)	4.09 ± 0.24 (59)
p-Chloro-*N*-methylaniline	15.20 ± 1.21 (100)	15.53 ± 0.96 (102)	9.41 ± 0.48 (62)
N,N-Dimethylaniline	12.38 ± 1.27 (100)	11.95 ± 1.03 (97)	8.45 ± 0.76 (68)
Nicotine	0.60 ± 0.04 (100)	0.60 ± 0.04 (100)	0.57 ± 0.01 (95)

* Assays were performed as described in Materials and Methods. The data are means ± S.E.M.; the values for relative activity are given in parentheses. Sonication time was 45 sec.

† A: NADPH.

‡ B: an artificial NADPH-generating system consisting of $NADP^+$, G-6-P, and G6PD from Torula yeast.

§ C: *in situ* generation of NADPH by mouse liver microsomal H6PD in the presence of dG-6-P [1.48 ± 0.12 and 6.23 ± 0.41 nmoles·min^{-1}·(mg protein)$^{-1}$ with intact and sonicated microsomes respectively].

Subsequently, the ability of NADPH, generated *in situ* by microsomal H6PD, to support *in vitro* metabolism of several xenobiotics using $NADP^+$ and dG-6-P as substrates was investigated, and the data obtained are given in Table 3. A comparison of the rates of various substrate oxidations observed in reaction media containing externally added NADPH and those supplemented with an exogenous NADPH-generating system indicates only a small difference, if any, when either intact or sonicated microsomes were used. Although the sonication of microsomes activated H6PD, it caused either no change or up to 30% loss of optimal monooxygenase activity shown toward different substrates by the other two systems.

Preliminary experiments on each monooxygenase reaction suggested that the concentrations of $NADP^+$ and deoxyG-6-P employed were not limiting. Thus, it appears from the data (Table 3) that H6PD-dependent generation of NADPH not only supported *N*-demethylation of aminopyrine but also of *p*-chloro-*N*-methylaniline, *N,N*-dimethylaniline and nicotine. The H6PD-linked activity was quite low with intact microsomes, being 8–21% for all substrates except nicotine. However, H6PD-linked reactions showed an efficiency of 60–70% when rates observed with sonicated microsomes were compared with those in the presence of NADPH. The 43 and 95% efficiencies in the formation of nornicotine from nicotine in the H6PD-linked reaction by intact and sonicated microsomes, respectively, may have been due to several other factors. However, the low turnover number for this reaction observed in the presence of externally added NADPH may actually have been the cause.

NADPH, generated endogenously by the microsomal H6PD, also supported a number of other monooxygenation reactions including *O*-demethylation of *p*-nitroanisole, *p*-hydroxylation of aniline, ring hydroxylation of biphenyl at the 2- and 4-positions, and dearylation of parathion (Table 4). Although intact microsomes exhibited low activity

Table 4. H6PD-linked monooxygenation of model substrates in mouse hepatic microsomes*

	Monooxygenase activity [nmoles product formed·min^{-1}·(mg protein)$^{-1}$] Intact microsomes			Sonicated microsomes		
	A	B	C	A	B	C
p-Nitroanisole *O*-demethylase	0.68 ± 0.04 (100)	0.71 ± 0.04 (113)	0.14 ± 0.02 (22)	0.58 ± 0.02 (100)	0.55 ± 0.03 (95)	0.46 ± 0.02 (79)
Aniline *p*-hydroxylase	0.92 ± 0.07 (100)	1.03 ± 0.01 (112)	0.22 ± 0.01 (24)	0.62 ± 0.10 (100)	0.65 ± 0.14 (105)	0.40 ± 0.06 (65)
Biphenyl 4-hydroxylase	5.60 ± 0.18 (100)	6.23 ± 0.20 (111)	3.23 ± 0.14 (58)	5.02 ± 0.42 (100)	5.01 ± 0.30 (100)	3.32 ± 0.11 (66)
Biphenyl 2-hydroxylase	0.18 ± 0.02 (100)	0.17 ± 0.02 (94)	0.08 ± 0.01 (44)	0.16 ± 0.02 (100)	0.13 ± 0.01 (81)	0.10 ± 0.01 (63)
Parathion dearylase	1.35 ± 0.12 (100)	1.22 ± 0.13 (92)	0.16 ± 0.01 (12)	0.88 ± 0.06 (100)	0.80 ± 0.07 (91)	0.51 ± 0.01 (58)
N,N-Dimethylaniline *N*-oxidase	4.05 ± 0.22 (100)	4.15 ± 0.20 (103)	0.87 ± 0.03 (22)	4.20 ± 0.20 (100)	4.28 ± 0.19 (102)	2.64 ± 0.09 (63)

* Details are the same as given in the footnotes to Table 3.

of about 12 and 24% when parathion and aniline, respectively, were used as substrates, relatively higher rates of metabolism were observed with biphenyl and this may have been due to the presence of Tween-80 in the reaction medium. The preparation of this substrate requires the use of detergent [17] which apparently activates H6PD. This view is further supported by the observations that, as compared to the rates of H6PH-linked monooxygenations in intact microsomes, the rates observed with sonicated microsomes were three to eight times higher for most substrates (Tables 3 and 4), while in the case of biphenyl an increase of only 8 and 19% in 4- and 2-hydroxylase, respectively, was observed (Table 4).

All of these mixed function oxidation reactions are known to be catalyzed by the various species of microsomal cytochrome P-450. However, biphenyl 2-hydroxylase [26] and benzpyrene hydroxylase [27] activities are primarily catalyzed by the polycyclic aromatic hydrocarbon inducible form of cytochrome P-450 called cytochrome P-448. Thus, our data on biphenyl 2-hydroxylase and those reported on benzpyrene hydroxylase by fish and mouse liver microsomes [7] suggest that both cytochrome P-450- and P-448-dependent xenobiotic oxidations are catalyzed with equal efficiency when linked with H6PD. Similar results wre obtained with *N,N*-dimethylaniline oxidation (Table 4). The formation of *N*-oxide from this substrate depends upon cytochrome P-450 [28] as well as the FAD containing microsomal monooxygenase (EC 1.14.13.8) [29].

At present it is widely believed that the generation of NADPH in hepatocytes is an extramicrosomal process, primarily catalyzed by G6PD, 6-phosphogluconate dehydrogenase (6PGD; EC 1.1.1.43), isocitrate dehydrogenase (EC 1.6.4.2) and by malic enzyme (EC 1.1.1.40) [30, 31]. However, experiments carried out with liver perfusion techniques [30, 32], isolated hepatocytes [33, 35], whole homogenates [36, 37] or with postmitochondrial supernatant fraction [38] cannot readily distinguish between microsomal and cytosolic NADPH-generating enzyme activities. Although a precise evaluation of the relative importance of liver cytosolic and microsomal NADPH-generating enzymes in supporting *in vitro* microsomal mixed function oxidase activity has not been reported nor can the data gathered in this study shed any light on this problem, the possible *in vivo* significance of H6PD is obvious from the facts that it is located in endoplasmic reticulum and that it can support a variety of mixed function oxidation reactions at significant rates. This contention is further supported by a recent report [39] demonstrating the presence of 6PGD activity in rat microsomes.

The H6PD-linked activity did not equal that observed in the presence of exogenous excess NADPH in any of the monooxygenation reactions studies or in those reported by Kimura *et al.* [8] and Stegman and Klotz [7]. This may, in part, have been due to the fact that all of these experiments were performed under conditions permitting less than half (45%) of the potential activity of H6PD. Further investigations are in progress to develop methods to stabilize the fully activated H6PD in microsomes. The reconstitution of monooxygenase activity using purified H6PD and other components as well as physiological substrates would be of great value from this point of view.

Recent findings of Sawada *et al.* [40] support this view. These authors reported that reconstitution of aromatic aldehyde ketone reductase with H6PD, both purified from the guinea pig liver microsomes, exhibited twice the activity of that observed when yeast G6PD or NADPH alone was used. These results clearly suggest that, although H6PD appears to be a general supplier of NADPH for the different activities of enzymes from the endoplasmic reticulum *in vivo*, it may have a more specific function.

REFERENCES

1. D. C. Harrison, *Biochem. J.* **25**, 1016 (1931).
2. S. Ohno, H. Payne, M. Morrison and E. Beutler, *Science* **153**, 1015 (1966).
3. B. Mandula, S. K. Srivastava and E. Beutler, *Archs Biochem. Biophys.* **141**, 155 (1970).
4. A. Yoshida, in *Isozymes* (Ed. C. L. Markert) Vol. IV. p. 853. Academic Press, New York (1975).
5. S. K. Srivastava, K. G. Blume, E. Beutler and A. Yoshida, *Nature, Lond.* **238**, 240 (1972).
6. N. Matsuoka and S. H. Hori, *Comp. Biochem. Physiol.* **65B**, 191 (1980).
7. J. J. Stegeman and A. V. Klotz, *Biochem. biophys. Res. Commun.* **87**, 410 (1979).
8. K. Kimura, H. Endou, J. Sudo and F. Sakai, *J. Biochem. Tokyo* **85**, 319 (1979).
9. A. P. Kulkarni and E. Hodgson, *Insect Biochem.* **6**, 385 (1976).
10. A. P. Kulkarni and E. Hodgson, *Chem. Biol. Interact.* **17**, 223 (1977).
11. A. P. Kulkarni and E. Hodgson, *Int. J. Biochem.*, in press.
12. A. P. Kulkarni and E. Hodgson, *Int. J. Biochem.*, in press.
13. A. P. Kulkarni, R. B. Mailman and E. Hodgson, *J. agric. Fd Chem.* **23**, 177 (1975).
14. T. Nash, *Biochem. J.* **55**, 416 (1953).
15. J. Cochin and J. Axelrod, *J. Pharmac. exp. Ther.* **125**, 105 (1959).
16. D. M. Ziegler and F. H. Pettit, *Biochem. biophys. Res. Commun.* **15**, 188 (1964).
17. M. D. Burke and R. A. Prough, in *Methods in Enzymology* (Eds. S. Fleischer and L. Packer), Vol. LII, Part C, p. 399. Academic Press, New York (1978).
18. K. J. Netter and G. Seidel, *J. Pharmac. exp. Ther.* **146**, 61 (1964).
19. T. Fugita and G. J. Mannering, *J. biol. Chem.* **248**, 8150 (1973).
20. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
21. S. H. Hori and T. Takahashi, *Biochim. biophys. Acta* **496**, 1 (1977).
22. N. Oshino, Y. Imai and R. Sato, *J. Biochem., Tokyo* **69**, 155 (1971).
23. T. Takahashi and S. H. Hori, *Biochim. biophys. Acta* **524**, 262 (1978).
24. H. Endou and V. Neuhoff, *Hoppe-Seyler's Z. physiol. Chem.* **356**, 1381 (1975).
25. H. Denk, *Pharmac. Ther.* **6**, 551 (1979).
26. B. D. Burke and J. W. Bridges, *Xenobiotica* **5**, 357 (1975).
27. A. Y. H. Lu and S. B. West, *Pharmac. Ther.* **2**, 337 (1978).
28. P. H. Lavica and S. Hulsmann, *Biochem. J.* **182**, 109 (1979).

29. D. M. Ziegler and C. H. Mitchell, *Archs Biochem. Biophys.* **150**, 116 (1972).
30. J. Chayen, F. P. Altman and R. G. Bucher, in *Fundamentals of Pharmacology* (Ed. S. Dikstein), p. 196. Charles C. Thomas, Springfield, IL (1973).
31. R. G. Thurman, D. P. Marazzo and R. Scholz, in *Cytochrome P-450 and b_5, Structure, Function and Interaction* (Eds. D. Y. Cooper, O. Rosenthal, R. Snyder and C. Whitmer), p. 355. Plenum Press, New York (1975).
32. R. G. Thurman, L. A. Reinke, S. A. Belinksy, and F. C. Kauffman, in *Microsomes, Drug Oxidations and Chemical Carcinogenesis* (Eds. M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette and P. J. O'Brien), p. 913. Academic Press, New York (1980).
33. P. Moldeus, P. Grundin, H. Vadi and S. Orrenius, *Eur. J. Biochem.* **46**, 351 (1974).
34. K. Weigl and H. Sies, *Eur. J. Biochem.* **77**, 401 (1977).
35. J. W. Bridges, P. Wiebkin and J. R. Fry, in *Microsomes, Drug Oxidations and Chemical Carcinogensis* (Eds. M. J. Coon, A. H. Conney, R. W. Estabrook, H. V. Gelboin, J. R. Gillette and P. J. O'Brien), p. 619. Academic Press, New York (1980).
36. F. C. Kauffman, R. K. Evans and R. G. Thurman, *Biochem. J.* **166**, 583 (1977).
37. F. C. Kauffman, R. K. Evans, L. A. Reinke, S. A. Belinksy, C. Ballow and R. G. Thurman, *Biochem. Pharmac.* **29**, 697 (1980).
38. S. Shah and D. J. Pearson, *Biochim. biophys. Acta* **539**, 12 (1978).
39. C. Bublitz, *Biochem. biophys. Res. Commun.* **98**, 588 (1981).
40. H. Sawada, M. Hayashibara, A. Hara and T. Nakayama, *J. Biochem., Tokyo* **87**, 985 (1980).