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Impaired mitochondrial oxidative energy metabolism following paracetamol-induced hepatotoxicity in the rat

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1 Effects of paracetamol treatment *in vivo* at subtoxic ($375\,mg\,kg^{-1}$ body weight) and toxic ($750\,mg\,kg^{-1}$ body weight) doses on energy metabolism in rat liver mitochondria were examined.

2 Paracetamol treatment resulted in a significant loss in body weights without affecting the liver protein contents. Toxic doses, however, resulted in 21% decrease in the yield of mitochondrial proteins.

3 Subtoxic doses of paracetamol did not, in general, affect the respiratory parameters in the liver mitochondria except in the case of succinate where both the state 3 respiration and the ADP-phosphorylation rates increased by 28%.

4 Toxic doses of paracetamol caused 25 to 47% decrease in the state 3 respiration rates depending on the substrate used. ADP/O ratios also decreased significantly with pyruvate + malate and succinate as the substrates. Consequently, ADP-phosphorylation was impaired significantly from 20 to 63%.

5 Subtoxic doses of paracetamol resulted in increased contents of cytochrome $c + c_1$ while the toxic doses caused lowering of the cytochromes aa_3 and b contents.

6 Glutamate and succinate dehydrogenase activities decreased in both the experimental groups while Mg^{2+}-ATPase activity was impaired only after toxic dose-treatment.

7 The results show that toxic doses of paracetamol result in impaired energy coupling in the liver mitochondria. Effects of subtoxic doses were also demonstrable in terms of impaired dehydrogenases activities.

Introduction

Actaminophen (N-acetyl-*p*-aminophenol, 4-hydroxyacetanilide), commonly known as paracetamol, is a widely used analgesic and antipyretic drug (Mitchell *et al.*, 1973; Hinson *et al.*, 1981; Breen *et al.*, 1982; McClain, 1982). In the U.S.A., the sale of this drug constitutes about 25% of the total analgesic sale over-the-counter (McClain, 1982). When used at normal therapeutic dose levels, it is considered to be a safe and an effective analgesic drug; however, at high doses this drug produces acute hepatotoxic and nephrotoxic effects, both in experimental animals and in man (Mitchell *et al.*, 1973; Dixon *et al.*, 1975; Hinson *et al.*, 1981; Dixon, 1984). Massive hepatic centrilobular necrosis and acute renal failure are reported following paracetamol overdoses (Hinson *et al.*, 1981; Cobden *et al.*, 1982; Newton *et al.*, 1983; Dixon, 1984). Self-poisoning with paracetamol has been a major problem in the past few years in that paracetamol overdose has become a common means of attempted suicide; cause of death was often acute hepatic necrosis (McClain, 1982).

Paracetamol toxicity is believed to result from interaction of paracetamol and/or its metabolites formed by cytochrome P-450 system, with cellular processes (Hinson *et al.*, 1981; Dixon, 1984). Histochemical studies have shown that liver necrosis is associated with structural damage to subcellular components including mitochondria in man and in experimental animals (Dixon, 1984). Consequently, the serum transaminase levels were also found to be elevated (Dixon, 1984). Swelling of mitochondria,

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disruption of cristae and transient inconsistent increase in succinate dehydrogenase activity at 6h followed by a uniform loss in this enzyme activity have also been reported, based on histological and histochemical studies (Dixon *et al.*, 1975; Dixon, 1984). However, there are few reports demonstrating the *in vivo* effects of paracetamol toxicity on cellular processes at biochemical levels.

In view of the observations that paracetamol toxicity causes structural alterations in mitochondria (Dixon, 1984), it is possible that the toxic effects could be traced to the functional attributes of these organelles. We have, therefore, carried out studies to examine the effects of paracetamol-induced hepatoxicity on mitochondrial functions.

Methods

Animals

Male albino rats of Wistar strain weighing between 250–260 g were used. The average weights of the animals in the control and the experimental groups were matched within 5 g at the start of the experiment. The animals were fasted overnight and were given paracetamol injections next morning (Walker *et al.*, 1982). Saturated solutions of paracetamol ($35\,mg\,ml^{-1}$) were prepared in warm (45–50°C) saline (0.9% w/v) and animals were injected intraperitoneally (i.p.) with a dose of $750\,mg\,kg^{-1}$ body weight (Hinson *et al.*, 1981). This dose which is known to result in massive hepatic necrosis is hereafter referred to as 'toxic' dose. A second group of animals was injected with half-saturated solutions of paracetamol ($17.5\,mg\,ml^{-1}$ of saline at 45–50°C) at a dose of $375\,mg\,kg^{-1}$ body weight. This dose is hereafter referred to as a 'subtoxic' dose. The control animals received an equivalent volume of warm saline. The reason for employing two doses was that increase in the liver necrosis with graded subtoxic doses of paracetamol has been described by Hinson *et al.* (1981). After the injections, the animals were returned to their respective cages and had free access to food and water. The animals were killed after 24 h of paracetamol administration for further studies since maximum liver necrosis is reported to be seen at this time interval (Dixon, 1984).

Isolation of mitochondria

The animals were killed by decapitation and their livers were quickly removed, weighed and 12% (w/v) homogenates were made in chilled (0–4°C) 0.25 M sucrose with a Potter-Elvehjeim type glass-teflon homogenizer. Measured aliquots of homogenates (70 ml) were taken for isolation of mitochondria essentially according to the procedure described previously (Satav & Katyare, 1982). The mitochondria were washed once and the pellets from 1 g tissue were finally suspended in 1.0 ml of 0.25 M sucrose. All operations were carried out at 0–4°C.

Aliquots of homogenates and mitochondria were saved for protein measurements and paracetamol estimation.

Oxidative phosphorylation

Measurements of oxidative phosphorylation were carried out at 25°C with a Clark-type oxygen electrode as described earlier (Chance & Williams, 1955; Satav & Katyare, 1982; Katyare & Rajan, 1988) in the respiration medium (total volume: 1.3 ml) consisting of 225 mM sucrose, 10 mM potassium phosphate buffer, pH 7.4, 10 mM Tris-HCl buffer, pH 7.4, 5 mM $MgCl_2$ and 2–3 mg of mitochondrial proteins. Final concentrations of substrates were 10 mM; pyruvate was supplemented with 1 mM L-malate and 0.1 mM TMPD was employed in conjunction with ascorbate. Rotenone (1.0 μM) was used in studies with succinate and ascorbate + TMPD. The state 3 respiration rates (initiated by addition of 150 nmol of ADP in 15–20 μl volume) and the state 4 respiration rates (after the depletion of added ADP) were recorded. Calculations of ADP/O ratios (mol ADP phosphorylated per atom of O_2 consumed) and ADP-phosphorylation rates were as described earlier (Chance & Williams, 1955; Katyare *et al.*, 1971; Ferreira & Gil, 1984).

Mitochondrial cytochromes

For determination of cytochrome contents, mitochondrial suspensions in phosphate-buffered isolation medium were solubilized with Triton X-100 at a final concentration of 3–4 mg protein ml^{-1} and the difference spectra of dithionite-reduced minus ferricyanide-oxidized cytochromes were recorded in a Hitachi model 150-20 double-beam spectrophotometer. Cytochrome contents were calculated by using the wavelength pairs and the extinction coefficients as described earlier (Satav & Katyare, 1982).

Enzyme assays

ATPase: ATPase activities were measured in a medium (final volume: 1.0 ml) consisting of 50 mM Tris-HCl buffer, pH 7.4, 75 mM KCl and 0.4 mM EDTA; 6.0 mM $MgCl_2$ and/or 0.1 mM DNP were included wherever indicated (Satav & Katyare, 1982). After preincubating 200–250 μg of mitochon-

Table 1 Effects of paracetamol treatment on body and liver weights

Parameter	*Animals*		
		Paracetamol treated	
	Control (9)	*Subtoxic dose* (11)	*Toxic dose* (11)
Body weight (g)	265.6 ± 12.4	226.8 ± 6.0*	226.9 ± 3.9**
Liver weight:			
(g)	13.0 ± 0.53	12.0 ± 0.58 NS	11.3 ± 0.32*
% body weight	4.93 ± 0.20	5.20 ± 0.18 NS	4.93 ± 0.11 NS

Male Wistar rats weighing between 250–260 g were injected (i.p.) with paracetamol at a dose of 375 mg kg^{-1} (subtoxic) or 750 mg kg^{-1} (toxic) body weights as described in 'Methods'. Control animals received only equivalent volumes of saline vehicle. Animals were killed 24 h later for isolation of mitochondria as given in the text. Results are given as mean ± s.e.mean of the number of observations indicated in parentheses.
* $P < 0.02$, ** $P < 0.01$, NS not significant.

drial proteins in the reaction medium at 25°C for 2 min, the reaction was started by adding 6.0 mM ATP and carried out for 15 min. At the end of the incubation period, the reaction was terminated by adding 0.1 ml of 10% (w/v) SDS and the inorganic phosphate liberated was estimated according to the method of Fiske & Subba Row (1925).

Dehydrogenases: For estimation of dehydrogenase activities, mitochondria at a concentration of 10–12 mg ml^{-1} in 250 mM sucrose containing 10 mM Tris-HCl, pH 7.4 were sonicated for 2 min (10 s sonication followed by 10 s rest interval) at 20 kHz in a 'Vibro-cell' ultrasonic disintegrator (Sonics and Materials Inc. CT, U.S.A.), temperature being maintained at 0–4°C during sonication. Glutamate dehydrogenase (Leighton *et al.*, 1968). β-hydroxybutyrate dehydrogenase (Miyahara *et al.*, 1981), malate dehydrogenase (Ochoa, 1955) and succinate dehydrogenase (Caplan & Greenawalt, 1968) were determined in sonicated mitochondria.

Paracetamol estimation

Paracetamol estimations in serum, homogenate and isolated mitochondria were carried out according to the procedure of Swanson & Walters (1982). Briefly, samples were deproteinized with freshly prepared *p*-diazobenzene sulphonic acid and released paracetamol was extracted in ethyl acetate which was estimated under alkaline conditions using Folin-Ciocalteau's reagent (Meola, 1978). Separate recovery experiments indicated that the extent of recovery of paracetamol in these experiments was from 90–102% (data not given).

Protein estimations were carried out according to Lowry *et al.* (1951) with crystalline bovine serum albumin used as the standard.

Chemicals

Paracetamol (A.R.) was purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A. Sodium salts of L-glutamic acid, β-hydroxybutyric acid, L-malic acid, pyruvic acid, ascorbic acid, succinic acid, and ADP, ATP, rotenone, Triton X-100 and sodium dodecyl sulphate (SDS) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. 2,4-Dinitrophenol (DNP) and N,N,N',N'-tetramethyl-*p*-phenylenediamine (TMPD) were from British Drug Houses, Poole, Dorset, U.K. All other chemicals were of analytical-reagent grade.

Results

The results in Table 1 summarize the effects of paracetamol treatment on body and liver weights. It can be noted that treatment with both 'toxic' and 'subtoxic' doses of paracetamol resulted in decrease in the body weights. However, the liver weights decreased by 13% in only those animals given toxic doses of the drug (Table 1). The total protein content in the liver tissue was not significantly affected by paracetamol treatment (Table 2). However, interestingly, the yield of mitochondrial proteins expressed either as mg g^{-1} tissue or as % of liver proteins decreased in the animals receiving toxic doses of the drug (Table 2).

The data on oxidative energy metabolism in rat liver mitochondria as affected by paracetamol treatment are given in Table 3. It can be seen that the treatment with subtoxic doses of paracetamol did not significantly affect the state 3 and state 4 respiration rates nor did it have any effects on the ADP/O ratios and the ADP-phosphorylation rates with any of the substrates tested, except in the case of succinate where both the state 3 respiration rate and

Table 2 Effect of paracetamol treatment on liver protein content and mitochondrial yield

	Animals		
		Paracetamol-treated	
Parameter	*Control* (12)	*Subtoxic dose* (11)	*Toxic dose* (11)
Liver protein content, (mg g^{-1} tissue)	184.61 ± 4.87	181.11 ± 6.19 NS	176.77 ± 6.86 NS
Yield of mito-chondrial protein:			
(mg g^{-1} tissue)	18.95 ± 1.05	18.37 ± 1.09 NS	14.60 ± 1.39*
% liver protein	10.46 ± 0.78	10.36 ± 0.83 NS	8.27 ± 0.67*

Experimental details are as given in Table 1 and in 'Methods'. Results are given as mean ± s.e.mean of the number of observations given in parentheses.
* $P < 0.05$, NS not signficant.

the ADP-phosphorylation rate increased by 28% (Table 3).

By contrast, when the animals were treated with toxic doses of paracetamol (Table 3), the state 3 respiration rates decreased with all the substrates used (from 25 to 47%); the highest decrease being seen with glutamate and pyruvate + malate. The state 4 respiration rates were generally unchanged for glutamate, β-hydroxybutyrate and succinate but increased with pyruvate + malate and decreased for ascorbate + TMPD. The ADP/O ratios decreased by 19% and 27% for succinate and pyruvate + malate respectively. The ADP-phosphorylation rates registered a decrease corresponding to the lowered state 3 respiration rates in the case of glutamate, β-hydroxybutyrate and ascorbate + TMPD; these values for pyruvate + malate and succinate decreased to an even greater extent because of the impairments in the ADP/O ratios referred to above (Table 3).

With a view to understanding the lesion underlying the impairment in the coupled (state 3) respir-

Table 3 Effects of paracetamol treatment on oxidative energy metabolism in rat liver mitochondria

			Respiration rate (nmol O_2 mg protein min^{-1})		*ADP-phosphorylation rate* (nmol ATP formed mg^{-1} protein min^{-1})
Substrate	*Treatment*	*ADP/O ratio*	+ADP	−ADP	
Glutamate	Control (16)	2.28 ± 0.10	38.7 ± 1.6	2.5 ± 0.5	177.2 ± 10.7
	Subtoxic dose (12)	2.30 ± 0.11 NS	41.7 ± 2.0 NS	3.7 ± 0.8 NS	192.6 ± 15.3 NS
	Toxic dose (12)	2.03 ± 0.20 NS	21.6 ± 2.5††	3.8 ± 1.2 NS	89.3 ± 15.2††
β-Hydroxy-butyrate	Control (14)	2.39 ± 0.10	37.8 ± 2.8	2.4 ± 0.6	178.3 ± 12.1
	Subtoxic dose (12)	2.32 ± 0.11 NS	43.1 ± 2.6 NS	3.2 ± 0.8 NS	196.9 ± 9.5 NS
	Toxic dose (12)	2.42 ± 0.13 NS	28.9 ± 2.9*	3.4 ± 0.6 NS	143.7 ± 17.8 NS
Pyruvate + malate	Control (12)	2.46 ± 0.17	24.7 ± 1.3	2.3 ± 0.4	120.9 ± 9.5
	Subtoxic dose (14)	2.52 ± 0.14 NS	25.7 ± 1.1 NS	5.0 ± 1.5 NS	127.4 ± 7.2 NS
	Toxic dose (12)	1.80 ± 0.17**	13.2 ± 1.0††	5.4 ± 1.4*	45.1 ± 6.3††
Succinate	Control (12)	1.48 ± 0.08	70.3 ± 3.1	8.7 ± 2.3	202.8 ± 8.7
	Subtoxic dose (12)	1.46 ± 0.08 NS	90.0 ± 3.6††	10.6 ± 2.3 NS	261.1 ± 15.2***
	Toxic dose (12)	1.20 ± 0.10*	48.9 ± 4.8†	10.7 ± 2.1 NS	123.3 ± 19.1††
Ascorbate + TMPD	Control (12)	0.43 ± 0.01	41.3 ± 3.0	19.4 ± 1.6	36.1 ± 2.9
	Subtoxic dose (12)	0.44 ± 0.01 NS	46.4 ± 2.3 NS	23.9 ± 2.2 NS	40.8 ± 2.5 NS
	Toxic dose (11)	0.42 ± 0.02 NS	30.8 ± 2.0**	15.1 ± 1.2*	26.0 ± 2.4***

Animals were given subtoxic or toxic doses of paracetamol as described in the text. Other experimental details are as described in Table 1 and 'Methods'. Results are given as mean ± s.e.mean of the number of observations indicated in parentheses.
* $P < 0.05$; ** $P < 0.02$, *** $P < 0.01$, † $P < 0.002$, †† $P < 0.001$, NS not significant.

Table 4 Effects of paracetamol treatment on the dehydrogenase activities in rat liver mitochondria

Dehydrogenases	*Control*	*Paracetamol-treated* *Subtoxic dose*	*Toxic dose*
Glutamate dehydrogenase	182.2 ± 11.3 (11)	142.6 ± 7.4* (9)	142.7 ± 9.1* (10)
β-Hydroxybutyrate dehydrogenase	510.7 ± 19.7 (13)	525.2 ± 29.7 (12) NS	516.0 ± 28.2 (18) NS
Malate dehydrogenase	3512.3 ± 209.2 (9)	3654.5 ± 209.5 (10) NS	3405.4 ± 216.8 (14) NS
Succinate dehydrogenase	113.0 ± 8.2 (13)	81.3 ± 6.2* (13)	82.5 ± 6.2* (17)

Dehydrogenases activities were determined in sonicated mitochondria as described in 'Methods'. Other experimental details are as given in Table 1 and in the text. Dehydrogenases activities are expressed as nmol substrate transformed mg^{-1} protein min^{-1}, except for succinate dehydrogenase which is expressed as μmol substrate transformed mg^{-1} protein min^{-1}. Results are given as mean ± s.e.mean of the number of observations indicated in parentheses.
* $P < 0.01$, NS not significant.

ation rates, we extended our observations to examine the possible functional alterations/defects in the mitochondrial electron transport chain components. From the data in Table 4 it can be noted that paracetamol treatment did not significantly affect either β-hydroxybutyrate dehydrogenase or malate dehydrogenase activities. The glutamate and succinate dehydrogenase activities were lowered under these conditions (Table 4). Interestingly, both toxic and subtoxic doses of the paracetamol brought about reduction in the activities of these enzymes to the same extent (24 to 28% decrease). Thus even with subtoxic doses, the paracetamol-induced lesion was already apparent at the primary dehydrogenase level although this was not manifested in terms of coupled state 3 respiration rates (Table 3).

The examination of intramitochondrial cytochromes content (Table 5) revealed that treatment with subtoxic doses of paracetamol resulted in a 13% increase in the cytochrome $c + c_1$ content. By contrast, treatment with toxic doses of paracetamol brought about 11% and 20% decrease respectively in the contents of cytochrome aa_3 and b without affecting the cytochrome $c + c_1$ content (Table 5).

The data in Table 6 show that the mitochondria from the control animals were characterized by low basal ATPase activity obtained in the absence of both Mg^{2+} and DNP. This activity was not stimulated by Mg^{2+} alone but could be fully evoked by DNP or DNP + Mg^{2+}, thus emphasizing the intactness of the mitochondrial membranes (Katyare *et al.*, 1970). This was also reflected in terms of high ratio of DNP/Mg^{2+} ATPase activities (Table 6). A similar trend was noted also for mitochondria from animals receiving subtoxic doses of paracetamol. However, when the animals were given toxic doses of paracetamol, this apparently caused damage to the mitochondrial membranes as evident from a greater stimulation of basal ATPase activity by Mg^{2+}. At the same time DNP and DNP + Mg^{2+} failed to evoke fully the ATPase activity. Consequently, the ratio of DNP to Mg^{2+}-ATPase activity was low (Table 6).

Experiments to estimate paracetamol levels in serum, tissue homogenates and isolated mitochondria revealed the presence of detectable levels of the drug only in the tissue homogenates (4.6 ± 1.3 and 4.8 ± 1.2 μg g^{-1} liver respectively in the two groups).

Table 5 Effect of paracetamol treatment on cytochrome content in rat liver mitochondria

Cytochrome	*Cytochrome content* (nmol mg^{-1} protein) *Control* (12)	*Paracetamol-treated* *Subtoxic dose* (10)	*Toxic dose* (16)
aa_3	0.131 ± 0.006	0.138 ± 0.010 NS	0.116 ± 0.004*
b	0.236 ± 0.006	0.257 ± 0.013 NS	0.188 ± 0.012**
$c + c_1$	0.434 ± 0.014	0.490 ± 0.021*	0.410 ± 0.013 NS

Intramitochondrial cytochrome contents were calculated from the difference spectra of dithionite-reduced and ferricyanide-oxidized mitochondria solubilized with freshly prepared Triton X-100 as described in 'Methods'. Results are given as mean ± s.e.mean of the number of observations indicated in parentheses.
* $P < 0.05$, ** $P < 0.02$, NS not significant.

Table 6 Effect of paracetamol treatment on ATPase activities in rat liver mitochondria

	ATPase activity (μmol Pi, mg^{-1} protein h^{-1})		
		Paracetamol-treated	
Additions	*Control* (12)	*Subtoxic dose* (12)	*Toxic dose* (14)
None	1.33 ± 0.23	1.09 ± 0.25 NS	1.28 ± 0.15 NS
+ Mg^{2+}	1.66 ± 0.15	1.87 ± 0.30 NS	2.50 ± 0.31*
+ DNP	13.89 ± 0.74	15.93 ± 0.95 NS	13.16 ± 1.23 NS
+ DNP + Mg^{2+}	16.21 ± 0.65	15.90 ± 1.10 NS	13.24 ± 0.73**
$\frac{DNP}{Mg^{+}}$ activity ratio	8.4	8.8	5.3

ATPase activity in mitochondria was measured as described in the 'Methods'. Results are given as mean ± s.e.mean of the number of observations given in parentheses.
* $P < 0.05$, ** $P < 0.01$, NS not significant.

No detectable levels of paracetamol were found either in the serum or mitochondria (data not given).

Discussion

The present studies have clearly demonstrated that subtoxic doses of paracetamol did not affect any of the respiratory parameters except in the case of succinate where a stimulatory effect was seen (Table 3). This may perhaps correlate with an increased content of cytochrome c + c_1 in mitochondria from this group of animals (Table 5). The regulatory role of cytochrome c in succinate oxidation has previously been demonstrated by us (Katyare *et al.*, 1970). Interestingly, paracetamol-induced lesions could be seen even in the subtoxic group in terms of impaired glutamate and succinate dehydrogenase activities (Table 4). However, the subtoxic dose did not affect the liver protein content and the mitochondrial protein content, though this treatment had a definite adverse effect on the body weights (Tables 1 and 2). Toxic doses of paracetamol, on the other hand, caused impairments in active state 3 respiration rates from 25 to 47% depending on the substrate used, besides lowering the ADP/O ratios with succinate and pyruvate + malate. Consequently, the rate of ATP synthesis (ADP-phosphorylation) decreased from 20 to 63%. The observed decrease in the respiration rates correlates well with the decreased contents of cytochrome aa_3 and b. The decrease in glutamate and succinate dehydrogenase levels may also be responsible for the lowering of respiration rates but their contribution may be less important when viewed in the context of the results in the subtoxic group (Table 3) and the decreased cytochrome aa_3 and b contents would seem to play a major role. The structural damage inflicted on mitochondrial membrane integrity can also be inferred from the Mg^{2+}-ATPase activity and decreased mitochondrial yield. These results have thus demonstrated, for the first time the effects of paracetamol toxicity at the level of organelle dysfunction. Disruption of the mitochondrial cristae and the transient increase in the succinate dehydrogenase activity followed by a decrease have been demonstrated by earlier workers with histological and histochemical techniques (Dixon *et al.*, 1975; Dixon, 1984). The results of our present studies corroborate these reported observations. Our findings on damage to mitochondrial membrane structure in terms of ATPase activity also correlate well with the reported elevated levels of serum transaminase activities (Dixon, 1984).

In our other studies we have also observed that the kinetic properties of membrane-bound enzyme systems such as succinoxidase, NADH oxidase and ATPase were significantly altered by both subtoxic and toxic doses of paracetamol (Satav & Katyare, unpublished observations).

In separate experiments, levels of paracetamol in serum, whole liver homogenate and isolated mitochondria were estimated with a view to examining whether the observed effects on oxidative energy metabolism in mitochondria (Tables 3 to 6) are direct effects of paracetamol resulting from the accumulation of the drug in these subcellular organelles. As is to be expected, there were no detectable levels of paracetamol in the serum since most of the drug is known to be detoxified within 4–6 h of ingestion (Green & Fisher, 1981). While the tissue homogenates contained barely detectable concentrations of paracetamol, the mitochondria were totally devoid of it. These results therefore clearly indicate that the observed effects are not due to accumulation of the drug by mitochondria but may be a consequence of initial toxic insult to the hepatocytes. In this connec-

tion it is of interest to note that only very high (6–10 mM) concentrations of paracetamol could inhibit respiration in isolated kidney tubules or kidney mitochondria (Porter & Dawson, 1979). It is also likely that paracetamol may be altering intracellular Ca^{2+} homeostatis in the hepatocytes and thereby impairing the mitochondrial functions (Schanne *et al.*, 1979). However, this possibility has not been examined in the present studies.

Paracetamol toxicity is believed to be mediated by adduct formation of its metabolites with subcellular components, mostly proteins (Hinson *et al.*, 1981; Dixon, 1984). It seems likely that the observed effects on mitochondrial functions may result from the modifications in respiratory chain components because of such an adduct formation. It is also possible that impairment in mitochondrial functions may have occurred much earlier i.e. within hours of paracetamol treatment. We have, however, not examined this possibility and restricted our studies only to 24 h post-paracetamol treatment. Nevertheless, the results of the present studies have clearly shown that significant effects of paracetamol persist even 24 h after its ingestion and the overall effect is the impaired rate of ATP synthesis.

Mitochondria are the major site of ATP synthesis in cellular metabolism and cellular synthetic processes are known to be energy-dependent (Lehninger, 1982). While paracetamol is known to deplete the cellular glutathione levels (Hinson *et al.*, 1983), impairments in ATP synthesis will lead to a further set-back in recovery from the necrosis.

In conclusion, the results of the present studies have shown for the first time that paracetamol toxicity can lead to dysfunction in mitochondrial energy-linked functions. As far as we are aware, such *in vivo* effects at the organelle level have not been described earlier.

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