When dimethylnitrosamine was added to the normal diet of rats at a level of 50 parts per million a high incidence of malignant tumours of the liver was found between the 26th and 40th weeks [2]. Dimethylnitrosamine is a useful agent for the experimental study of toxic liver injury because its acute effects are regular and reproducible and also it can be readily estimated in tissues and body fluids by the polarographic method of Heath and Jarvis [3]. The compound is rapidly metabolised *in vivo*, mainly, and perhaps exclusively by the liver [4,5]. It is distributed almost uniformly in the different tissues and organs with no preferential accumulation in the liver, and there is evidence that it becomes uniformly distributed in the body water soon after administration [5]. Metabolism occurs *in vitro* by liver slices and broken-cell preparations, but not to a significant extent with other tissues with the possible exception of kidney. There is a strong requirement for oxygen and complete inhibition by anaerobiosis. Metabolic activity of broken-cell preparations can be removed by dialysis and partially restored by the addition of TPN and to a less extent DPN. All the activity occurs in the microsome+cell sap fraction of the liver with negligible metabolism by the nuclear+mitochondrial fraction [6].

Incorporation of labelled amino acids into rat liver protein *in vivo* is markedly reduced 3 h after the animal has received a necrotising dose of dimethylnitrosamine but incorporation into kidney and spleen protein is not altered significantly nor is the labelling of the free amino acids in the liver. Incorporation of ^{32}P into acid-soluble, lipid and residual phosphorus fractions of the liver is not significantly changed 6 h after the same dose of dimethylnitrosamine, but incorporation into a partially purified RNA fraction is reduced at this time [7].

Some histochemical and biochemical changes found in the liver during the development of the necrosis will be described, also some recent work on the metabolism of ^{14}C-dimethylnitrosamine and on the effect of the compound on rat liver slices *in vitro*.

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Structural Factors Involved in the Diaphorase and Cytochrome c-Reductase Activities of Mitochondria and Microsomes

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As indicated by previous studies [1,2], the low activity at which TPNH * is oxidized by way of the cytochrome system may constitute a limiting factor for the function of TPN-dependent oxidations in liver tissue. The great discrepancy in activity, known to prevail between the liver TPNH- and DPNH-cytochrome c reductase systems [3], and recent evidence reported in the literature [4,5] that the former may be selectively regulated by metabolic control mechanisms, prompted a comparative study of the two systems with respect to their catalytic components. Interest was in the first place focused on the diaphorase components, since these are believed to constitute the first catalytic links of the cytochrome c-reductase systems with the reduced pyridine nucleotides.

While the TPNH- and DPNH-cytochrome c reductases are located exclusively in the particulate elements of the cytoplasm [3,6,7], recent studies [8] revealed the occurrence of a considerable diaphorase activity in the soluble cytoplasm. This activity was found to be equally high with DPNH and TPNH, in contrast to those present in mitochondria and microsomes, which are 3—7 times higher with DPNH than with TPNH. Since there are indications that the TPNH- and DPNH-diaphorase activities of the soluble cytoplasm are catalyzed by one common enzyme [8] it appeared to be of interest to investigate whether the same could be valid for the particulate DPNH- and TPNH diaphorases, their discrepancy in activity being due to secondary factors, connected with the organized nature of the structures in which they are located. The data collected in Tables 1 and 2 seem to support this concept.

As emerges from Table 1, precipitation of a suspension of mitochondria or microsomes with acetone resulted in a selective stimulation of the TPNH-diaphorase activity, with a shift of the TPNH/DPNH activity ratio towards unity. A similar effect ensued if mitochondria or microsomes were exposed to a hypotonic

* The symbols, DPNH and TPNH, stand for the reduced forms of di- and triphosphopyridine nucleotide, respectively.

Table 1. Effects of acetone treatment and hypotonicity on the activities of particulate liver diaphorases.

Homogenates of rat liver were made in 0.25 M sucrose solution. 100 ml homogenate, containing 10 g wet weight liver, was centrifuged *a*) at 1 600 *g* for 10 min, *b*) at 4 100 *g* for 15 min, and *c*) at 105 000 *g* for 60 min. The denotions, mitochondria and microsomes, refer to the pellets obtained in *b* and *c*, respectively. The mitochondrial pellet was washed, and the microsomal pellet rinsed, twice with 0.25 M sucrose, and both pellets were suspended in 10 ml 0.25 M sucrose. 4 ml aliquots of both suspensions were added to 36 ml cold (—12°C) acetone, the precipitates separated by centrifugation at 0°C, and dried in a desiccator. The dry precipitates were suspended in 4 ml 0.25 M sucrose. Another aliquot of the mitochondrial and microsomal suspensions was diluted with 9 volumes of distilled water and allowed to stand at 0°C for 30 min before test. The test system contained 0.5 ml 0.3 M tris buffer (pH 7.5), 2.3 ml 0.25 M sucrose, 0.1 ml 3 mM DPNH or TPNH, 0.1 ml 1.7 mM 2,6-dichlorophenolindophenol, and 10 mg wet weight liver equivalent mitochondria or 5 mg equivalent microsomes in a volume of 0.1 ml 0.25 M sucrose. When indicated, ATP was added in a final concentration of 3 mM and Mn++ in a final concentration of 0.3 mM. The decolorization of the dye was recorded in a Beckman DK2 spectrophotometer at 600 mμ, using glass cuvettes of 1 cm light path. The reaction was started by the addition of the reduced pyridine nucleotides, and followed for 4—6 min at room temperature. The values are expressed in terms of 1 g wet weight liver equivalent.

Treatment	*Mitochondria*			*Microsomes*		
	Diaphorase activities (μmoles dye reduced/min)					
	TPNH	DPNH	$\frac{\text{TPNH}}{\text{DPNH}}$	TPNH	DPNH	$\frac{\text{TPNH}}{\text{DPNH}}$
None	1.0	4.3	0.23	1.9	10.2	0.15
Acetone	1.9	3.0	0.65	3.5	3.4	1.03
Hypotonicity	2.8	4.5	0.61	2.9	10.8	0.27
» + ATP	1.5	4.3	0.34			
» + ATP + Mn++	0.9	4.3	0.21			

medium (0.05 M sucrose) at 0°C for 30 min; in this case the effect on the mitochondria could be counteracted by ATP and Mn++, which are known protectors of the mitochondrial structure [9].

Since deoxycholate has previously proved to be a useful tool in studying the biochemical organization of mitochondria [10-12] and microsomes [13] the effect of this agent was investigated in greater detail. Table 2 illustrates the effects of increasing concentrations of deoxycholate on the mitochondrial and microsomal diaphorase and cytochrome c reductase activities. Again, the structural destruction was accompanied by an increase of the TPNH/DPNH activity ratio in both instances and with both types of activities measured. However, whereas in the case of mitochondria this increase was mainly due to an absolute increase of the TPNH-diaphorase and cytochrome c-reductase activities, in the case of microsomes these activities remained virtually constant and the increase of the activity ratios could entirely be accounted for by a decrease of the DPNH-linked activities; yet, the mitochondrial DPNH-cytochrome c-reductase was also strongly inhibited by deoxycholate. Whether the different behaviors of the mitochondrial and microsomal TPNH-diaphorase and cytochrome c-reductase activities towards deoxycholate are related to the known difference existing between the actions of deoxycholate on the mitochondrial [10,11] and microsomal [13] membranes is the subject of continued studies. The data as yet available point to the possibility that the great difference prevailing between the cellular activities of the TPNH- and DPNH-cytochrome c-reductase systems is primarily due to different locations within, or relations to, the cytoplasmic structures rather than to different catalytic compositions.

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Table 2. Effects of varying concentrations of deoxycholate on the diaphorase and cytochrome c-reductase activities of rat liver mitochondria and microsomes.

Diaphorase activities were tested as described in Table 1. The test system used for the determination of the cytochrome c-reductase activity contained the following: 0.5 ml 0.3 M tris buffer (pH 7.5), 2.2 ml 0.25 M sucrose, 0.1 ml 10 mM KCN, 0.1 ml 3 mM DPNH or TPNH, and 0.1 ml 1.5 mM cytochrome c. The reaction was started by the addition of the reduced pyridine nucleotides, and followed by recording the increase in optical density at 550 mμ, due to the reduction of cytochrome c. The amounts of enzyme used in the different tests, expressed in mg wet weight liver equivalents, were as follows: *Mitochondria:* TPNH- or DPNH-diaphorase, 10; TPNH-cytochrome c-reductase, 33; DPNH-cytochrome c-reductase, 3.3. *Microsomes:* TPNH- or DPNH-diaphorase, 3.3; TPNH-cytochrome c-reductase, 17; DPNH-cytochrome c-reductase, 1.7. Deoxycholate was included in the test systems in concentrations as indicated. The values below are expressed in relative terms, giving the value 100 to the activity obtained with DPNH as substrate in the absence of deoxycholate within each individual type of test.

Deoxycholate, %	*Mitochondria*			*Microsomes*		
	TPNH	DPNH	TPNH/DPNH	TPNH	DPNH	TPNH/DPNH
	Relative Diaphorase Activity					
0	15	100	0.15	20	100	0.20
0.005	21	92	0.23	20	106	0.19
0.01	21	97	0.22	17	109	0.16
0.02	39	108	0.36	17	91	0.19
0.035	82	126	0.65	13	93	0.14
0.05	97	131	0.74	20	57	0.35
0.075	87	116	0.75	20	48	0.42
0.1	90	116	0.78	22	48	0.46
	Relative Cytochrome c-Reductase Activity					
0	1.3	100	0.01	5.0	100	0.05
0.005	2.1	87	0.02	4.6	104	0.04
0.01	2.9	74	0.04	4.6	100	0.05
0.02	1.8	74	0.02	5.0	104	0.05
0.035	4.5	65	0.07	5.4	113	0.05
0.05	12.5	45	0.28	5.8	83	0.07
0.075	8.8	16	0.55	5.8	8	0.73
0.1	10.1	6	1.69	5.8	4	1.45

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