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Soluble Diaphorase in Animal Tissues

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The soluble fraction obtained upon centrifugation of rat liver homogenates made in 0.25 M sucrose-3 % polyvinylpyrrolidone at 105 000 *g* for 60 min exhibits a high diaphorase activity as revealed by measuring the rate of reduction of 2,6-dichlorophenolindophenol by reduced di- or triphosphopyridine nucleotide (DPNH of TPNH). As emerges from the typical data shown in Table 1, this activity is, in contrast to those found in mitochondria and microsomes, virtually equally high with DPNH and TPNH, and it exceeds, in terms of total protein content, the diaphorase activities of the particulate fractions more than twice in the case of DPNH and about twenty times in the case of TPNH. The soluble diaphorase resembles the particulate one in that it reacts with methylene blue at about half the rate obtained with 2,6-dichlorophenolindophenol, but differs from the latter in being inactive towards cytochrome c or ferricyanide; treatment of the enzyme with ferric chloride according to Mahler and Elowe [1] did not alter this state of affairs. The following findings indicate that the soluble DPNH- and TPNH-diaphorase reactions are catalyzed by one common enzyme: 1) the two activities are not additive; 2) they are equally sensitive to various inhibitors such as *p*-chloromercuribenzoate, atebrine, or flavin adenine dinucleotide (flavin mononucleotide is not inhibitory); 3) they show no tendency to separate upon ammonium sulphate fractionation of the soluble fraction. Preliminary data indicate that this type of soluble diaphorase is ubiquitous as to its occurrence among animal tissues, although the highest activity hitherto found is that present in rat liver.

Table 1. Cytoplasmic distribution of diaphorase activities in rat liver.

Homogenates of rat liver were made in 0.25 M sucrose solution containing 3 % polyvinylpyrrolidone (pH adjusted to 7). 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, mitochondrial, microsomal and soluble fractions, refer to the pellets obtained in *b* and *c*, and the supernatant obtained in *c*, respectively. The mitochondrial pellet was washed, and the microsomal pellet rinsed, twice with 0.25 M sucrose, and both pellets were suspended in 0.25 M sucrose. The test system contained 0.5 ml 0.3 M tris buffer (pH 7.5), 2.3 ml 0.25 M sucrose (containing, in the case of the soluble fraction, 3 % polyvinylpyrrolidone, pH adjusted to 7), 0.1 ml 3 mM DPNH or TPNH, 0.1 ml 1.7 mM 2,6-dichlorophenolindophenol, and 0.1 ml of the fraction to be tested, containing 5 mg wet weight liver equivalent in the case of mitochondria or microsomes, and 0.5 mg in the case of the soluble fraction. 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.

Fraction	Total protein content, mg	Diaphorase activity (μmoles dye reduced per min) DPNH	TPNH
Mitochondrial	23.3	4.3	1.0
Microsomal	31.1	13.1	1.9
Soluble	29.2	39.0	35.0

1. Mahler, H. R. and Elowe, D. G. *J. Biol. Chem.* **210** (1954) 165.

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