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Transhydrogenase and TPNH oxidase activities of rat-liver mitochondria

Because of recent controversies[1–5] as to the transhydrogenase and TPNH oxidase activities of rat-liver mitochondria, and as to the role played by these pathways in the aerobic oxidation of isocitrate, it was of interest to re-examine previous data published from this laboratory[2–3] on this subject. As a test system, that employed by PURVIS[4] was chosen, consisting in a measurement of the rate of aerobic oxidation of TPNH and its increment ensuing the addition of DPN. Two types of assay were performed in parallel: (a) the decrease in the concentration of TPNH was followed at 340 mμ in a recording DK2 Beckman spectrophotometer, and (b) the oxygen consumption was recorded polarographically, be means of the rotating platinum electrode method[6*]. The mitochondria were depleted of endogenous pyridine nucleotides by aging for 1 h[4] in a sucrose–phosphate medium at 30°. The values in Table I show a good agreement between the two types of assay. The mean values, expressed in terms of μmoles TPNH/min/g liver, are 0.05 for the TPNH oxidase and 0.12 for the transhydrogenase. Previous values[2–3], obtained with a slightly different test system, were 0.05 and 0.08, respectively.

The mean values in Table I, converted to μmoles TPNH/min/mg mitochondrial protein (assuming that mitochondria from 1 g liver contain 20 mg protein[3,8]), are 0.0025 for the TPNH oxidase and 0.006 for the transhydrogenase, which markedly differ from those recently reported by PURVIS[4] 0.000 for TPNH oxidase, 0.026 for the transhydrogenase. Although the explanation of these discrepancies must await the publication of the experimental details underlying these values in PURVIS's paper[4], the following comments may be helpful in clarifying the situation.

The TPNH oxidase activity reported above was fairly consistent from one experiment to another. It could be increased considerably, however, by increasing the concentration of TPNH. Similar observations were made when cytochrome *c* rather than oxygen was used as a terminal oxidant. A 20-fold increase of the TPNH

Abbreviations: DPN, DPNH, oxidized and reduced diphosphopyridine nucleotide; TPN, TPNH, oxidized and reduced triphosphopyridine nucleotide; PNH, reduced pyridine nucleotide.

* I am indebted to Dr. H. BALTSCHEFFSKY for his kind help with the use of this technique.

TABLE I

SPECTROPHOTOMETRIC AND POLAROGRAPHIC MEASUREMENTS OF THE AEROBIC OXIDATION OF REDUCED PYRIDINE NUCLEOTIDES (PNH) IN AGED PREPARATIONS OF RAT-LIVER MITOCHONDRIA

Mitochondria, prepared as described elsewhere[24], were aged for 1 h at 30° in a medium of 0.225 *M* sucrose–0.05 *M* phosphate (pH 7.5). The aging suspension contained mitochondria from 0.5 g wet wt. liver (about 10 mg biuret protein)/ml. PNH disappearance was followed at 340 mμ in a recording DK2 Beckman Spectrophotometer, and O_2 consumption was measured polarographically with the rotating platinum electrode method[6]. The test system contained 0.05 *M* K phosphate (pH 7.5) and, where indicated, 0.15 m*M* DPNH or TPNH and 0.5 m*M* DPN or TPN. The samples for spectrophotometry contained mitochondria from 0.1 g liver and had a final volume of 3 ml, and those for polarography contained mitochondria from 0.5 g liver and had a final volume of 1.1 ml. The temperature was about 25°. The reaction rates were constant in both types of assay for at least 5 min in test systems (*d*) and (*f*),and for 1 to 3 min in test system (*b*). The values are expressed in terms of 1 g wet weight liver and minute.

Added pyridine nucleotide	*μmoles PNH oxidized*	*μatoms O_2 consumed*
(*a*) None	—	0.000
(*b*) DPNH	0.465	0.548
(*c*) DPN	—	0.036
(*d*) TPNH	0.044	0.052
(*e*) TPN	—	0.000
(*f*) TPNH + DPN	0.175	0.198
Calculated reaction rates		
DPNH oxidase (*b*)-(*c*)	0.465	0.512
TPNH oxidase (*d*)-(*e*)	0.044	0.052
Transhydrogenase (*f*)-(*e*)-(*d*)-(*c*)	0.131	0.110

concentration, for example, caused a 6- to 8-fold increase of the initial rate of oxidation. As reported previously[9], treatment of mitochondria with 0.05 %–0.1 % deoxycholate results in a similar effect, causing a 6- to 8-fold increase of the TPNH cytochrome *c* reductase and TPNH diaphorase activities as measured with low concentrations of TPNH. The same treatment inactivates the transhydrogenase, in accordance with the earlier observation of KAPLAN *et al.*[10] that this enzyme is sensitive to bile salts. These findings point to the involvement of structural factors in the interaction of external TPNH with the mitochondrial terminal electron transport system. It would appear from these data that the failure of PURVIS[4] to demonstrate a TPNH oxidase activity might be due to such factors rather than, as he implies, to an absence of TPNH-cytochrome *c* reductase from rat-liver mitochondria, with transhydrogenase being the only pathway for mitochondrial TPNH oxidation. This latter conclusion is also in conflict with the earlier finding of REYNAFARJE AND POTTER[11] that aging of rat-liver mitochondria for 2 h at 30° in a hypotonic medium resulted in a release of all TPNH-cytochrome *c* reductase activity into the medium at the same time as the transhydrogenase activity was recovered in the recentrifuged mitochondrial pellet.

In contrast to the TPNH oxidase activity, the transhydrogenase activity varied widely from one preparation to another. Out of ten experiments, eight gave lower transhydrogenase values than the one shown in Table I, and only in one experiment was a higher value, approaching PURVIS's figure, obtained. It should be pointed out in this connection that also in PURVIS's paper[4], a relatively low liver transhydrogenase

activity, about 3 times lower than the one stated in his final scheme, can be deduced* from his experiment (Fig. 1, right) where the reduction of 2,6-dichlorophenolindophenol by isocitrate was measured in the presence of TPN and TPN + DPN. In the present experiments, the transhydrogenase activity could not be increased by increasing the concentration of TPNH or DPN, indicating that structural factors were not involved to the same extent as in the case of the TPNH oxidase. One way of explaining the observed fluctuations might be that, besides true transhydrogenase, mitochondria also contain so-called dual-specific dehydrogenases, which, depending on the presence of trace-amounts of their substrates, may transfer hydrogen from TPNH to DPN, thus adding to the total transhydrogenase activity of the system. Similar mechanisms have recently been proposed to operate in the extra-mitochondrial space of the cytoplasm, mediated by lactic dehydrogenase[13] and by certain steroid dehydrogenases[14]. It may be recalled in this connection that liver glutamic dehydrogenase, a mitochondrial enzyme[15] of potent activity[16], is known[17–19] to react with DPN and TPN at comparable rates. Investigations along these lines are now in progress.

In a recent paper, STEIN *et al.*[5] reported liver mitochondrial transhydrogenase activities considerably exceeding those obtained both in this laboratory[2–3] and by PURVIS[4]. These data are based on the use of acetylpyridine-DPN as a hydrogen acceptor. However, since this compound has a more positive potential than has DPN, the specificity and quantitative adequacy of this method may appear doubtful when applied to such a complex system as mitochondria. Acetylpyridine-DPN has recently been shown by the same authors[20] to react with the purified mitochondrial DPNH diaphorase. The reported failure[5] of the same compound to react with the purified TPNH diaphorase as well, or with TPNH in heat-treated mitochondria, does not seem conclusively to eliminate the possibility that it may react with TPNH diaphorase or TPNH-cytochrome *c* reductase in the enzymically more complex, non-heat-treated mitochondrial system. STEIN *et al.*[5] also criticize, on purely theoretical grounds, the concept previously advanced by this laboratory[12] that lactic dehydrogenase may act as a regulator of cytoplasmic TPN-linked oxidations. Since the submission of STEIN *et al.*'s paper[5], three reports[21–23] have appeared implicating an interaction between lactic dehydrogenase and TPNH (or TPN) in intact cells.

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* This deduction was made as follows: The difference in slopes between the TPN- and (TPN + DPN)-lines in PURVIS's[4] Fig. 1 (right) was estimated at 6 min to be 0.13 ΔE_{600}-units. Converted on the basis of the indicated protein content of 0.37 mg and final volume of 3 ml, and with an extinction coefficient of $21 \cdot 10^3$ l. mole^{-1} for 2,6-dichlorophenolindophenol[12], this value yields a transhydrogenase activity of $0.13 \cdot 3/(0.37 \cdot 6 \cdot 21) = 0.0084$ μequiv. 2H/min/mg protein. The corresponding value in PURVIS's[4] final scheme is 0.026.

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The assimilation and metabolism of guanidine by a strain of *Pseudomonas aeruginosa*

Although it is known that certain bacteria can desimidate substituted guanidines[1–6] and a creatinine desimidase has been partially purified[7], there has been no report on the bacterial metabolism of guanidine.

A strain of *Pseudomonas aeruginosa* was grown in 100 ml of Bacto Nutrient Broth for 24 h at 35°. After washing in water by repeated centrifugation the cells were suspended in 10 ml 0.05 *M* Na-K-phosphate, pH 7.6, and 0.5 ml of this used in a final volume of 2.0 ml. After incubation at 37°, 0.2 ml 20 % trichloroacetic was added and after centrifugation aliquots of the supernatant were taken for the estimation of guanidine by the method of WEBER[8] using a 490 mμ filter. Methylguanidine was also estimated by this method and equivalent amounts gave 85 % of the color produced by guanidine.

When 100 μg guanidine·HCl were added to the washed cells 10–15 μg disappeared in 15 min. After that no further disappearance was evident unless an oxidizable substrate such as pyruvate was added. 30–60 min after the addition of pyruvate the rest of the guanidine began to disappear and continued to completion. This is shown in Fig. 1. This disappearance, *i.e.* assimilation, of guanidine depended on the presence of an oxidizable substrate because assimilation ceased when oxidation ceased. Thus, within limits, the amount of guanidine assimilated should be proportional to the amount of substrate added. Fig. 2 shows that this is so for pyruvate in the range of 1.0–2.5 mg. The assimilation of NH_4^+ is also dependent on oxidation and NH_4^+ completely inhibited guanidine assimilation until no extracellular NH_4^+ remained.

Following assimilation, guanidine was metabolized since none was found in the cells after they were broken by sonic vibration. The most likely mechanism is desimidation to form NH_4^+ and urea followed by the breakdown of urea by urease. Urease