Note

No Influence of an Injection of Paraquat and Exposure to Hyperoxia on Liver Quinolinic Phosphoribosyltransferase Activity in Rats

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Quinolinic phosphoribosyltransferase (EC 2.4.2.19, QPRTase), a key enzyme in *de novo* NAD⁺ biosynthesis, was reported to be rapidly inactivated in *Escherichia coli* (*E. coli*) exposed to hyperbaric oxygen¹⁾ and paraquat.²⁾ However, Gardner and Fridovich³⁾ have recently reported that QPRTase in *E. coli* is not inactivated by exposure to hyperbaric oxygen. In rats, Brown *et al.*⁴⁾ reported that an injection of a large amount of niacin alleviates the toxicity of paraquat, and they suggested, but did not prove, that damage by paraquat is a consequence of the inactivating of QPRTase. We reported on QPRTase from *Alcaligenes eutrophus* subsp. *quinolicus*,^{5,6)} hog liver,^{6,7)} hog kidney,^{6,8)} rat liver,⁹⁾ and rat kidney⁹⁾ and that the properties of QPRTases from bacteria are very different from that of mammals.⁶⁾ Accordingly, we are interested in the effects of paraquat and exposure to hyperoxia on rat liver QPRTase activity. In this connection, it is considered that superoxide is a common intermediate in the mechanisms of toxicity for paraquat and exposure to hyperoxia.¹⁰⁾

Paraquat (1,1'-dimethyl-4,4'-bipyridinium (cation) dichloride) was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). [2,3,7,8-¹⁴C]Quinolinic acid (0.23 Ci/mol) was obtained from Daiichi Pure Chemical Co. (Tokyo, Japan). 5-Phosphoribosyl-1-pyrophosphate (tetrasodium salt) and NAD⁺ were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). All the other chemicals used were of the highest commercial grade available.

Rats were purchased from Clea Japan Inc. (Tokyo, Japan). The rats were kept individually in wire-bottomed cages. Food and water were supplied *ad libitum*. The

experimental schedule in experiments I and II are described in the tables. The room temperature was $22 \pm 2^\circ\text{C}$ and humidity was about 60%. The lighting schedule was 06:00–18:00 (light) and 18:00–06:00 (dark). The rats were killed by decapitation and the livers were removed. QPRTase activity⁹⁾ and liver NAD (NAD⁺ + NADH) content¹¹⁾ were measured by the methods reported previously.

As is shown in Tables I and II, the activity of rat liver

Table I. EFFECTS OF PARAQUAT ON THE LEVELS OF NAD AND THE ACTIVITY OF QPRTASE IN THE LIVER RATS (EXPERIMENT I)

Male rats of Sprague-Dewley strain (5 weeks old; body weight 107.7 ± 3.8 g (mean $\pm$ S.D.)) fed on a commercial diet CE-2 (obtained from Clea Japan; average niacin content, 12.7 mg/100 g of diet) were housed for 10 days. The rats (body weight, 200.5 ± 9.8 g) which obtained normal body weight gain, were injected intraperitoneally with 30 mg of paraquat per kg of body weight dissolved in 0.5 ml of sterilized saline at 09:00 hr on the 11 th day of the experiment and after 24 hr, the same amount of paraquat was again injected. The rats were killed at the times indicated in this Table. One rat died during 46–47.5 hr, one rat at about 50 hr, and two rats during 53–65 Hr. The changes in body weight were -25.7 ± 2.1 , -39.8 ± 3.0 , and -53.5 ± 4.6 g at 24, 48, and 72 hr, respectively.

Time after the injection of paraquat (hr)	Liver NAD (NAD ⁺ + NADH) (nmol/g liver)	Liver QPRTase (nmol/hr/g liver)
0	755 ± 63 (5)	463 ± 71 (5)
24 ^a	750 ± 54 (4)	439 ± 25 (4)
48	764 ± 16 (4)	408 ± 52 (4)
72	777 ± 59 (4)	444 ± 39 (4)

^a Just after the second injection of paraquat, the rats were killed.

Values are means $\pm$ S.D.; the numbers in parentheses are the number of rats used.

Table II. EFFECTS OF EXPOSURE OF RATS TO 100% OXYGEN ON THE ACTIVITY OF THE LIVER QPRTase (EXPERIMENT II)

Male rats of the Wistar strain (11 weeks old) fed on a CE-2 were kept in air or 100% oxygen for 2 and 3 days.

	Liver QPRTase ^a (nmol/hr/g liver)
Air	170.1 ± 48.1 (24)
O ₂ (2 days)	120.1 ± 27.6 (6)
O ₂ (3 days)	150.8 ± 41.4 (32)

Values are means $\pm$ SD; the number in parentheses are the number of rats used.

^a Frozen liver (stored at -80°C) was used.

QPRTase was not reduced by the paraquat injection or exposure to hyperoxia, although some of the rats died after these treatments. These results are consistent with those of Gardner and Fridovich on *E. coli*³⁾ but not with those of Brown *et al.* on *E. coli*.^{1,2)} Liver NAD content was not decreased by the injection of paraquat as shown in Table I, although, Brown *et al.*⁴⁾ reported that its content is decreased.

In conclusion, our findings clarified that QPRTase is not the oxygen-sensitive site of NAD⁺ biosynthesis. Gardner and Fridovich³⁾ have suggested that one candidate for the oxygen sensitivity is the quinolinic acid synthetase. This enzyme exists only in microorganisms, not in mammals.^{12,13)} Therefore, it is suggested that NAD⁺ biosynthesis may not be inhibited by paraquat and exposure to hyperoxia in mammals.

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