norepinephrine levels in the brainstems of cat and dog obtained with the single extraction method were also comparable to those found when the separate extraction procedures were used. Tryptamine, metanephrine, normetanephrine and 3,4-dihydroxybenzylamine, which are also extracted by *n*-butanol from a salt-saturated acid solution, do not interfere with the determination of norepinephrine or serotonin.

5-Hydroxytryptophan (5HTP), which has fluorescence characteristics similar to those of serotonin, is partially extracted at the acid pH level. Usually this does not create a problem because 5HTP is not present in detectable amounts in tissues. However, on administration of 5HTP to raise the tissue level of serotonin, it is advisable to analyze the brain for serotonin by the method of Bogdanski *et al.*, which incorporates a wash to remove the 5HTP.

Laboratory of Chemical Pharmacology,
National Heart Institute,
Bethesda, Maryland.

J. A. R. MEAD
K. F. FINGER

REFERENCES

1. D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, *J. Pharmacol.* **117**, 82 (1956).
2. P. A. SHORE and J. S. OLIN, *J. Pharmacol.* **122**, 295 (1958).
3. P. A. SHORE, *Pharmacol. Rev.* **11**, 276 (1959).

Inhibition of the enzymic oxidation of some dihydropyridines by polycyclic aromatic hydrocarbons

(*Received* 13 *January* 1961)

A SOLUBLE flavoprotein that catalyzes the oxidation of reduced ribosyl nicotinamide (NRH) by vitamin K_3 was recently isolated from rat kidney and seminal vesicle.[1] This enzyme also catalyzes the oxidation of certain N^1-alkyl derivatives of dihydronicotinamide, but it is completely inert toward the reduced forms of triphosphopyridine nucleotide, diphosphopyridine nucleotide, and nicotinamide mononucleotide (TPNH, DPNH and NMNH, respectively). The NRH-oxidizing enzyme can be separated from other soluble proteins which oxidize either TPNH or DPNH, or both, in the presence of vitamin K_3 and other quinones or dyes,[2, 3, 4] and does not seem to be identical with any flavoprotein previously described. We have recently observed that the enzymatic oxidation of N^1-(*n*-propyl) dihydronicotinamide is inhibited by very low concentrations ($< 10^{-8}$ M) of benz(*a*)anthracene and some derivatives of this hydrocarbon, as shown in Table 1. The oxidation of NRH was also inhibited by these hydrocarbons to about the same extent. On the contrary, the oxidation of DPNH by vitamin K_3, catalyzed by a kidney enzyme with properties very similar to the flavoprotein described by Märki and Martius[2], was quite unaffected by benzo(*a*)pyrene and 7,12-dimethylbenz(*a*)anthracene at a concentration of 10^{-7} M. Experiments carried out under different degrees of illumination suggested that light was not necessary for the inhibition of the NRH-oxidizing enzyme by benzo(*a*)pyrene. Analysis of the inhibition by the latter aromatic hydrocarbon, according to the method of Lineweaver and Burk[5], showed that it was not competitive with respect to vitamin K_3.

It was also observed that those polycyclic aromatic hydrocarbons that inhibited the NRH-oxidizing enzyme formed red-colored 1:1 molecular complexes with naphthoquinones such as vitamins K_1, $K_{2(5)}$, $K_{2(10)}$ and K_3. Spectrophotometric studies of equimolar solutions (10^{-2} M in methylene chloride) of these benz(*a*)anthracene derivatives and vitamin K_3 revealed that the complex formation was accompanied by the appearance of broad, unstructured absorption bands with maxima in the

range of 480–490 mμ. The intensity of these bands was greatly enhanced by cooling to −78 °C, a process which was readily reversible by warming the solutions to room temperature. Intermolecular charge transfer[6, 7] almost certainly occurs in these complexes. The relationship of the electron-donating properties of polycyclic aromatic hydrocarbons[8] to their ability to inhibit the NRH-oxidizing enzyme remains to be determined.

TABLE 1. INHIBITION OF THE ENZYMATIC OXIDATION OF N^1-(*n*-PROPYL) DIHYDRONICOTINAMIDE BY POLYCYCLIC AROMATIC HYDROCARBONS

Aromatic hydrocarbon	Percentage inhibition		
Final concentration	6×10^{-7} M	6×10^{-8} M	6×10^{-9} M
Benz(*a*)anthracene	72	38	7
7-Methylbenz(*a*)anthracene	79	61	26
7,12-Dimethylbenz(*a*)anthracene	73	46	22
Benzo(*a*)pyrene	79	51	33
3-Methylcholanthrene	32	28	9
1,2-Cyclopentenophenanthrene	0	0	0
Anthracene	0	0	0
Phenanthrene	0	0	0
Naphthalene	0	0	0
Fluorene	0	0	0

The reactions were carried out at 25° in a final volume of 3·0 ml containing 250 μmoles of tris(hydroxymethyl)amino methane buffer of pH 8·5, 0·15 μmole of vitamin K_3 (in 0·01 ml of ethanol), 0·35 μmole of N^1-(*n*-propyl)dihydronicotinamide and suitable amounts of enzyme. The polycyclic aromatic hydrocarbons were freshly dissolved in ethanol (with precautions to combat photo-oxidation), and were added to the reaction mixture in a volume of 0·01 ml (the same volume of ethanol was added to the control cuvettes). The oxidation of the dihydropyridine was followed by decrease in absorbancy at 360 mμ, and was corrected for any non-enzymatic reactions. The rates of reaction were obtained from zero-order plots of the initial rate of change in absorbancy versus time, the oxidations being initiated by the addition of enzyme. The enzyme was isolated from ultracentrifuged homogenates of rat kidney by a procedure involving (a) fractionation with ammonium sulfate (50–60 per cent saturation), (b) removal of inert protein after heating to 40° for 20 minutes at pH 4·2, (c) treatment with protamine sulfate, and (d) adsorption to and elution from calcium phosphate gel. One mg of the purified enzyme catalyzed the oxidation of 15 μmoles of N^1-(*n*-propyl)-dihydronicotinamide per min under these conditions.

The Ben May Laboratory for Cancer Research, and
The Department of Biochemistry,
University of Chicago,
Chicago 37
Ill., U.S.A.

SHUTSUNG LIAO
H. G. WILLIAMS-ASHMAN

REFERENCES

1. H. G. WILLIAMS-ASHMAN and S. LIAO, *Fed. Proc.* **19**, 32 (1960).
2. F. MÄRKI and C. MARTIUS, *Biochem. Z.* **331**, 111 (1960).
3. L. ERNSTER, M. LJUNGGREN and L. DANIELSON, *Biochem. Biophys. Res. Comm.* **2**, 88 (1960).
4. W. D. WOSILAIT and A. NASON, *J. Biol. Chem.* **208**, 783 (1954).
5. H. LINEWEAVER and D. BURK, *J. Amer. Chem. Soc.* **56**, 658 (1934).
6. R. S. MULLIKEN, *J. Amer. Chem. Soc.* **74**, 811 (1952).
7. A. SZENT-GYÖRGYI, *An Introduction to a Submolecular Biology*. Academic Press, New York (1960).
8. A. SZENT-GYÖRGYI, I. ISENBERG and S. L. BAIRD, JR., *Proc. Nat. Acad. Sci. U.S.* **46**, 1444 (1960).