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Effect of Ethanol Oxidation on Levels of Pyridine Nucleotides in Liver and Yeast.* (27374)

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In the animal organism the liver is the prime site for ethanol oxidation. In the liver ethanol is oxidized partially to acetaldehyde and acetate, whereas the complete oxidation occurs mainly outside the liver. Oxidation of ethanol and acetaldehyde both require DPN as hydrogen acceptor and Forsander *et al.*(1) showed that the DPN/DPNH ratio decreased in the rat liver during ethanol oxidation. This observation was confirmed by Smith and Newman(2).

In the present investigation the levels of di- and triphosphopyridine nucleotides in the rat liver were studied during fasting and during ethanol oxidation. The results obtained from the studies on liver were compared with changes occurring in baker's yeast during oxidation of ethanol.

Methods. White laboratory rats weighing between 200 and 250 g were used. Ethanol in a dosage of 3 mg/g was administered either intraperitoneally in 10% solution or by stomach tube in a 20% solution. The animals were killed by a blow on the neck ½ to 4 hours after administration of ethanol. The liver tissue was rapidly excised and prepared for determination of nucleotides. In the experiments on fasting animals the time of fasting varied between 2 to 5 days.

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The experiments on yeast were performed with commercial baker's yeast produced by the Rajamäki Factories of the State Alcohol Monopoly, Rajamäki. The 20% yeast suspension was aerated vigorously at 30°C and after 45 min. 38.5 mg of ethanol per g of fresh yeast was added. In this suspension the pyridine nucleotides were determined before and 5 min. after addition of ethanol without separation of the yeast.

Determination of pyridine nucleotides. DPN, DPNH, TPN and TPNH were determined in the liver extracts and in yeast preparations according to the enzymatic method of Holzer *et al.*(3). The recoveries were determined by adding known amounts of the pyridine nucleotides to tissue preparations during extraction. Recoveries for DPN, DPNH, TPN and TPNH were 93%, 91%, 90% and 86% respectively. All enzymes and substrates for the determinations were obtained from the Boehringer Biochemical Co., Mannheim.

Results. Results of the animal experiments are seen in Table I. Fasting decreases the DPN/DPNH ratio slightly from 3.95 to 3.25, mainly due to a decrease of DPN. Total DPN + DPNH is also slightly decreased by fasting. During ethanol oxidation the DPN/DPNH ratio decreases to 1.44 in the fed ani-

TABLE I. Levels of Di- and Triphosphopyridine Nucleotides in Fed and Fasting Livers of Control Rats and Rats Metabolizing Ethanol. Results are expressed as mμmoles per g liver wet weight.

	No. of rats	DPN	DPNH	DPN + DPNH	DPN/DPNH	TPNH
Fed	6	542 ± 29	138 ± 12	680 ± 27	3.95 ± 1.40	208 ± 36
Fed metabolizing alcohol	6	403 ± 25	285 ± 50	688 ± 55	1.44 ± .81	203 ± 36
Fasted	10	476 ± 51	149 ± 21	625 ± 157	3.25 ± 1.40	220 ± 79
Fasted metabolizing alcohol	16	453 ± 65	288 ± 39	741 ± 227	1.57 ± .61	204 ± 45

mals and to 1.57 in the fasted animals, mainly because of an increase of DPNH. This decrease is statistically highly significant in both cases ($p<0.001$). Total DPN + DPNH is increased in the fasted animals metabolizing ethanol. The levels of TPN are below 40 mμmoles/g liver in all cases and TPNH concentration is slightly over 200 mμmoles/g and is not significantly affected by fasting or oxidation of ethanol. The triphosphopyridine nucleotide is thus mainly present in its reduced form. The decrease in DPN/DPNH ratio during oxidation of ethanol is not progressive. It was measured from 30 minutes to 4 hours after administration of ethanol and was maintained at a fairly steady level as long as ethanol was oxidized.

Table II shows concentrations of DPN and DPNH in baker's yeast before and after incubation with ethanol. A marked increase in DPNH accompanied by a slight decrease in DPN is found during ethanol oxidation. The DPN/DPNH ratio is lowered from 3.36 to 1.67 and total DPN + DPNH increases from 838 to 970 mμmole/g during the oxidation of ethanol.

Discussion. During ethanol oxidation the pyridine nucleotide system is maintained at a new steady state at which the level of DPNH is increased as compared to the level during normal respiration. When ethanol is added as a substrate to the liver it rapidly distributes throughout the tissue fluids and is oxidized, due to the availability of liver alcohol dehydrogenase which has a high affinity for ethanol and DPN. The increased DPN reduction which occurs is mainly opposed by the oxidative action of the respiratory chain but since the oxygen consumption of the liver does not increase during ethanol oxidation(4, 5) the DPNH reoxidizing mechanisms do not maintain the previous normal DPN/DPNH ratio and a new steady state is reached.

According to Leloir and Munoz(4) and Lundqvist(6) 75% of the oxygen used by the liver is used in the partial oxidation of ethanol. If the over-all oxygen consumption is to be kept fairly constant a corresponding decrease should occur in the oxidation of other substrates. Further studies are needed to explain the effect of the changed DPN/DPNH ratio on other metabolic processes in the liver.

The increase in total DPN + DPNH which occurs in both liver and yeast cells during ethanol oxidation is a surprising finding. Whether the increase is due to a more rapid synthesis or a conversion from other nucleotides can not be answered on the basis of the present experiments. The observation in liver tissue that the TPN and TPNH levels do not change significantly during ethanol oxidation, however, indicates that a transformation from TPN to DPN does not take place.

Sumrnary. The levels of di- and triphos-

TABLE II. Levels of Diphosphopyridine Nucleotides in Commercial Baker's Yeast before and after Addition of 38.5 mg of Ethanol per g of Fresh Yeast. Results are expressed as mμmoles per g yeast fresh matter.

	DPN	DPNH	DPN + DPNH	DPN/DPNH
Yeast metabolizing carbohydrate reserves	646	192	838	3.36
Yeast metabolizing added ethanol	607	363	970	1.67

phopyridine nucleotides have been studied in rat liver after fasting and during oxidation of ethanol. The changes in diphosphopyridine nucleotides have also been studied in baker's yeast during ethanol oxidation. By fasting alone a slight decrease of DPN/DPNH ratio mainly due to a fall in the concentration of DPN is noted. Total DPN + DPNH is also decreased slightly. When the animals are metabolizing ethanol the DPN/DPNH ratio decreases further, due to an increase of DPNH, and especially in the fasted animals a fairly marked increase of the total DPN + DPNH can be noted. Similar changes are observed in baker's yeast during ethanol oxidation. Triphosphopyridine nucleotide is mainly present in the liver in its reduced form, and the TPN/TPNH does not change significantly during ethanol oxidation.

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Inhibition of Reticulocyte Lysis by Alkyl Polyamines. (27375)

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The participation of the alkyl polyamines in many diverse biological and biochemical phenomena has made this class of organic compounds a source of increasing interest. Spermine, spermidine and related substances are widely distributed in animal tissues and microorganisms; yet specific information about their function(s) is lacking. A variety of biological effects has been demonstrated in selected systems(1) including: 1) promotion of growth of microorganisms, 2) stabilization of bacteria, bacterial protoplasts, mitochondria, ribosomes, bacteriophages, and transforming principle, 3) binding to nucleic acids, heparin, and certain lipids, and 4) stabilization of enzyme activity.

This report deals with the stabilizing effect of spermine and related polyamines on the reticulocyte undergoing spontaneous lysis.

Methods. Blood samples, anticoagulated with dry dipotassium versenate, were obtained from human subjects and Sprague-Dawley rats. Reticulocyte-rich blood was obtained from female Sprague-Dawley rats weighing 150-200 g, previously given subcutaneous injections of phenylhydrazine hydrochloride(2). Reticulocyte counts of up to 80% were obtained. Reticulocyte counts were made on all blood samples using the methods of Brecher(3,4).

Dilute suspensions of red blood cells and reticulocytes were incubated in phosphate buffered saline following the osmotic fragility method of Dacie(5). For each blood sample tested, a duplicate series of tubes was used containing 2 ml aliquots of serial dilutions of 0.150 M NaCl in 0.01 M sodium phosphate buffer at pH 7.40. To one series of tubes 0.01 ml of an alkyl amine solution was added so that the final concentration of amine was 0.5 mM. A second series of tubes without alkyl amine served as a control. Whole blood (0.02 ml) was then delivered into each tube and mixed immediately by gentle inversion. Tubes were incubated at room temperature (23°-25°C) for 30 minutes and then centrifuged at 2000 rpm for 5 minutes. The amount of hemolysis (referred to as immediate hemolysis) was determined by reading the optical density of the supernatant solution at 540 mμ in a Coleman Junior Spectrophotometer. The tubes were then reinverted for mixing, covered to prevent evaporation, and incubated at specific temperatures