Summary. In proper doses administration of reserpine phosphate subsequent to adrenergic blockade with phentolamine will produce a more rapid decline in blood pressure than is commonly obtained by administration of reserpine alone. After pretreatment with amine oxidase inhibitor, choline p-tolyl ether, reserpine will produce pressor responses. These data are interpreted as supporting the hypothesis that reserpine releases sympathomimetic humoral agents which mask the early onset of the cardiovascular actions commonly attributed to reserpine.

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Received May 14, 1957. P.S.E.B.M., 1957, v95.

Effect of Increased Diphosphopyridine Nucleotide Levels on Rate of Ethanol Metabolism in the Mouse. (23282)

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The rate-limiting reaction in total oxidation of ethanol to CO_2 is the first step in the sequence of oxidation of ethanol to acetaldehyde(1). Three separate entities, ethyl alcohol, diphosphopyridine nucleotide,* and the enzyme alcohol dehydrogenase, participate in this reaction. It is generally agreed that the rate curve for disappearance of ethanol from blood follows a straight line rather than an exponential curve, except at low concentrations of blood alcohol(2). The enzyme, therefore is soon saturated with its ethanol substrate, and the availability of the alcohol does not appear to be a physiologically significant rate-limiting factor in its oxidation. This study was initiated in an attempt to determine whether the DPN concentration or amount of alcohol dehydrogenase present in liver may be the controlling factor in the disappearance of ethanol from the body.

Kaplan and coworkers(3) have induced DPN levels 10 times that of normal in the mouse liver by injection of nicotinamide 8-12 hours previously. Mice preinjected in this manner have been used in this work to study possible effects of the heightened DPN level on rate of ethanol oxidation. Both *in vivo* and *in vitro* studies have been performed.

Methods. In vivo experiments. Blood alcohol concentration was determined by the method of Newman and Newman(4). Adult mice of the C57 BL strain maintained on Purina Laboratory Chow were used. Six hours before administration of alcohol, the mice were injected intraperitoneally with 500 mg/kg nicotinamide in a solution containing 25 mg/ml. This time interval was chosen so that during the latter part of the 5 hours after alcohol administration, the DPN would be at optimum level in the liver as reported by Kaplan *et al.*(3). The ethyl alcohol was injected in 20% aqueous solution w/v in a single intraperitoneal injection at 4 g/kg. Alcohol injections were made to both mice preinjected with nicotinamide and to their untreated controls. At hourly intervals for 5 hours after alcohol injection, nicotinamide treated animals and controls were anaesthetized with chloroform and blood withdrawn from the heart which resulted in their death.

In vitro experiments. Slices. Webster Swiss mice were injected with 500 mg/kg nico-

* Diphosphopyridine nucleotide will be abbreviated to DPN

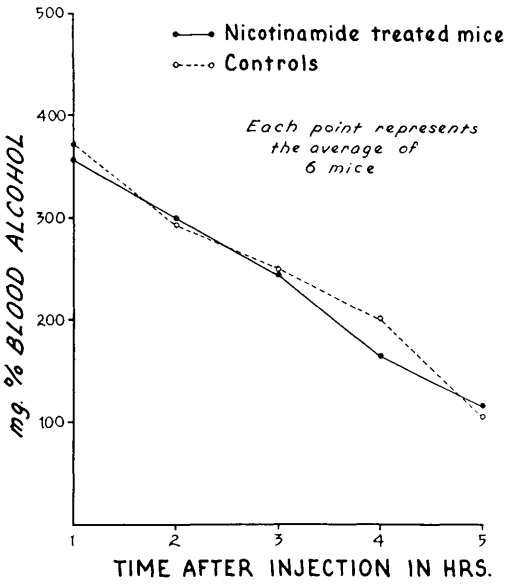

FIG. 1. Fall in blood alcohol levels after ethanol inj. in mice pre-inj. with nicotinamide and their controls.

tinamide 8 hours before killing. These and untreated mice were killed by decapitation, the livers quickly excised and sliced to a thickness of approximately 0.5 mm by a Stadie-Riggs microtome. The slices were immersed in 25 ml Erlenmeyer flask which contained 3.5 ml 0.1 M phosphate buffer at pH 7.5 and 4.5 mg ethyl alcohol. About 400 mg of slices were used flask. Air above the mixture was replaced with oxygen, then the flask was stoppered and allowed to incubate in Dubnoff Metabolic Shaker at 37°C. After 3 hours, 1 ml of 50% trichloroacetic acid was added to stop the reaction. Appropriate controls contained trichloroacetic acid in the original incubation medium. The contents of the flasks were centrifuged and the supernatant fluid was analyzed for alcohol by the enzymatic method of Marshall and Fritz(2).

Homogenates. The mice were preinjected as for the slice experiments. Homogenates were made from normal and pretreated livers by homogenizing the liver with 2 parts of 0.1 M phosphate buffer pH 7.5 which contained 50 mg nicotinamide/ml to minimize nucleotide destruction. One ml of this homogenate was added to the incubation medium which contained 3 ml 0.1 M phosphate buffer at pH 7.5 and 4.5 mg ethyl alcohol. The flasks were gassed, incubated, and analyzed as described for slice experiments.

TABLE I. Total Ethanol Metabolized by Liver Slices from Normal Mice and from Mice Pre-injected with Nicotinamide. Complete system contains 3.5 ml 0.1 M phosphate buffer at pH 7.5, 4.5 mg ethanol, and 400 mg liver slices incubated for 3 hr at 37°C under oxygen.

	Ethanol metabolized (mg)	
	Control	Pre-inj.
Exp. 1	1.35	1.15
2	1.47	1.38

Results. As shown in Fig. 1, the nicotinamide-injected animals did not metabolize more alcohol than did the control animals in the 5 hour period tested. There was wide variation among individuals, but except for the 4 hour point, the average blood alcohol levels of the 2 groups followed each other closely. The total fall in blood alcohol concentration in the nicotinamide-treated animals was 240 mg %, while in controls it was 264 mg %.

In the slice experiments the results are similar, as shown in Table I. High levels of DPN in the liver did not increase the rate of alcohol metabolism.

The homogenate experiments show a definite increase in total alcohol metabolized (Table II) by the preinjected animals. Undoubtedly, in spite of the large amounts of nicotinamide added during the homogenization process, the rapid destruction of DPN which takes place in homogenates by nucleotide phosphatases has made this coenzyme the limiting factor. These data serve to confirm the finding of Kaplan and coworkers(3) that a definitely higher level of functioning DPN is induced in the animals pretreated with nicotinamide.

Discussion. Our results show that, in the

TABLE II. Total Ethanol Metabolized by Homogenates of Livers of Normal Mice and Mice Preinjected with Nicotinamide. Complete system contains 3.0 ml 0.1 M phosphate buffer at pH 7.5, 4.5 mg ethanol, and 1 ml liver homogenate. The mixture was incubated under oxygen for 3 hr at 37°C.

	Ethanol metabolized (mg)	
	Control	Pre-inj.
Exp. 1	1.53	2.09
2	1.29	2.07
3	1.01	2.06

well-fed mouse, the physiological level of DPN is sufficient to maintain a maximum rate of conversion of ethanol to acetaldehyde. The alcohol dehydrogenase appears to be fully saturated with DPN as well as with ethanol. The enzyme content in the mouse liver therefore appears to control the rate of the first step of alcohol oxidation, and since this reaction limits the rate of subsequent oxidations, the amount of enzyme present must be the limiting factor in the whole sequence. If this is the case, it is difficult to understand how various metabolites are able to increase the rate of alcohol disappearance in the well-fed animal, as is frequently reported. Vitale *et al.*(5) have emphasized the importance of adequate diet for animals used in research on alcohol metabolism, and studies are in progress in this laboratory to determine the effect of fasting on DPN and alcohol dehydrogenase levels.

Summary. Mice, in which a high level of DPN was induced by injection of nicotinamide, metabolized ethanol no more rapidly than untreated controls. This is interpreted to mean that in an animal receiving adequate niacin in its diet, availability of DPN does not limit the disappearance of ethyl alcohol from the body.

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Received May 17, 1957. P.S.E.B.M., 1957, v95.

Ice as a Mechanical Factor in Death of Spermatozoa on Freeze-Thawing.* (23283)

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A consideration of ice formation has figured prominently in the elucidation of theories concerning the lethal consequences of cooling protoplasm below its freezing temperature. Physical destruction by ice in the medium surrounding the cell, within the cell, or at both sites, has been proposed as a salient causative factor in death on freezing and thawing(1,2). This proposition has provoked the suggestion(3) and application(4) of a theory of survival based upon vitrification by rapid cooling. At the time glycerol was introduced as a protective agent which permitted revival of spermatozoa after cooling to −79°C, mechanical injury by ice and the advantage of vitrification were pertinent considerations in attempts at preservation of cells by freezing. In fact, the term vitrification was used in lieu of freezing in the very paper which reported the protective action of glycerol(5). Further, the results of direct microscopical observations on freezing of fowl semen were said to indicate that glycerol modifies ice formation and dissolution in the medium so that damage due to pressure and other mechanical effects is reduced(6). Recent work(7), however, strongly suggests that damage on freezing and thawing spermatozoa of the bull, rabbit, fowl, and herring, can be attributed to increased salt concentration. Although glycerol has been shown to modify ice formation while favoring survival, on freezing semen of bull and man, no evidence has been presented for physical destruction by ice in the absence of this substance(8,9).

The present report concerns a further study of the possible role of ice as a mechanical factor in death. The object was to ascertain whether or not there is a relationship between

* This investigation was supported, in part, by Research Grant from N.I.H., Public Health Service.