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Effect of Temperature Extremes and Cortisone on Toxicity of Diphosphopyridine Nucleotide in Mice. (25234)

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It was reported recently(1) that analogs of nicotinamide cause toxic effects in mammals. Degenerative changes in the central nervous system appeared as prominent features of intoxication in mice and rats. Selective neuropathological damage was seen in nuclei of the brain stem and in gray matter of the anterior horn. Kaplan and coworkers(2) showed that analogs of nicotinamide participate in enzymatically catalysed exchange reactions with diphosphopyridine nucleotide (DPN), and they suggested that the toxicity of analogs is due to such reactions. These findings were confirmed and extended by Burton(3). Burton and others(4) could also demonstrate that reserpine and promazine prolong elevation of DPN concentration of tissues caused by injection of nicotinamide in mice. To examine DPN effects in a different manner, we determined first the toxicity of injected DPN. Then we investigated the effect of injected DPN on mice, as animals were subjected to stress of extreme environmental temperatures. Glock and McLean(5) found that starvation or administration of a variety of hormones alter DPN content of tissues considerably. It is well established that under stress of temperature extremes, especially cold, activity of the adrenal cortex increases significantly. Scherr reported(6) that toxicity of cortisone in mice varies according to environmental temperatures. After repeating his experiments we administered DPN and cortisone simultaneously, for evidence was offered by Munch *et al.*(7), that there is a specific interaction between steroid hormones and coenzymes like DPN.

Method. DPN was injected intraperitoneally to 20 Swiss mice, each weighing 24 g. The animals were observed behaviorally and histological studies of brain stem, muscles, kidneys and liver were performed.

Result. 105 mg of DPN/animal killed all mice in 4 hours. Before death, animals exhibit dysmetria, paralysis of hind legs, and marked general irritability. Neuropathological findings were similar to those described by Sternberg and Philips(1). Striped muscles, kidneys or liver showed no histopathological changes. Fifty mg of DPN is well tolerated intraperitoneally. Daily injection of this dose for 15 days, or more, produced no deleterious effects.

DPN injection and environmental temperatures. Method. Swiss mice, each weighing 24 g, in groups of 10, were exposed to hot environmental temperatures (37°C). Others, also in groups of 10, were subjected to cold environmental temperatures (7°C). They received 50 mg of DPN intraperitoneally. Control groups did not receive DPN.

Result. Mice exposed to environmental

temperature of 37°C, others to 7°C, in groups of 10, endured them without mortality. 50 mg of DPN intraperitoneally caused no mortality at room temperature. 50 mg of DPN became acutely toxic for mice at extreme temperatures. At 37°C it caused 50% mortality in 24 hours and 100% mortality in 72 hours. At 7°C it caused 50% mortality in 4 hours and 100% mortality in 24 hours.

DPN injection and cortical steroid. Method. Mice, in groups of 10, were given 0.5 mg cortisone intraperitoneally at room temperature and at temperature extremes. Other groups were administered 50 mg of DPN and 0.5 mg of cortisone simultaneously at room temperature and at temperature extremes.

Result. 0.5 mg of cortisone is well tolerated at room temperature. The same amount became lethal at high temperature. This confirms the results of Scherr(6). At 37°C it caused 50% mortality in 48 hours and 100% mortality in 144 hours. Toxicity of DPN is significantly altered by simultaneous cortisone administration. At room temperature 50 mg of DPN with simultaneous cortisone, each of which alone causes no mortality, produces marked lethal effects. The combined effect of DPN and cortisone is most pronounced under stress of cold environment. At 37°C they produced 50% mortality in 4 hours and 100% mortality in 38 hours. At 7°C they caused 50% mortality in 2 hours and 100% mortality in 6 hours. At room temperature they caused 50% mortality in 6 hours and 100% in 48 hours.

Discussion. We showed that DPN given intraperitoneally in mice has marked toxic effects. DPN proved especially toxic when animals were subjected to stress of hot or cold environment. These toxic effects are much enhanced by simultaneous administration of cortisone. Our data are in good accord with the view(7) that steroid hormones and coenzymes form a steroidcoenzyme complex.

McIlwain and Rodnight(8) showed that brain tissue DPN-ase activity is exceptionally high. They suggested that in unimpaired brain tissue the rate of DPN decomposition is checked by special inhibitors and by peculiarities of cellular architecture. Burton and others reported(4) that brain DPN content increases with nicotinamide injection, and this increased level is further extended by administration of reserpine or promazine. They also found that compounds related to reserpine or promazine which are not "tranquilizing" agents, fail to extend the elevated DPN levels. Thus tranquilizing effects seem to inhibit DPN-ase activity of tissues. Furthermore, DPN requiring phosphorylations are also inhibited(9) by tranquilizing compounds. Thus it is likely that tranquilizing agents act selectively in such manner that particular brain tissue DPN-ase is inhibited. A recent report of Woolley(10) comes to similar conclusion. He studied tranquilizing and antiserotonin activity of nicotinamide. He found evidence that inhibition of DPN-ase is a likely mechanism of the antiserotonin and tranquilizing action of large doses of nicotinamide. Stress itself demands an increase in DPN requiring phosphorylations(11,12).

Given and Knox reported(13) that tryptophan and/or hydrocortisone treatment produced a marked increase in tryptophan-transaminase levels of the liver. This "stress of substrate injection"(13) was potentiated by injection of hydrocortisone. In an analogous manner, our findings, support the hypothesis that stress is concomitant with increase of DPN-ase activity and reduction of DPN-ase activity parallels reduction of stress.

Summary. 1. DPN injection produces toxic effects in mice similar to those described for nicotinamide analogs. 2. Toxicity of injected DPN is influenced by environmental temperatures; both cold and hot environment enhances toxicity. 3. Simultaneous cortisone potentiates the effect of heat and cold. 4. These effects are discussed as they bear on inhibition of DPN-ase activity of reserpine, promazine and nicotinamide.

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Effect of Desiccated Thyroid and Thiouracil on Dental Caries Experience of Adult Rats.* (25235)

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Many previous studies(1-7) from these laboratories have shown that feeding desiccated thyroid to rats reduces the incidence of dental caries and administration of either I^{131} or propylthiouracil increases the caries experience. In all previous studies, the thyroid drugs were administered to weanling rats and continued for periods varying from 90 to 140 days. Since previous experience has shown that the proteolytic activity of the secretions of salivary glands of control animals increases with age, but that the effect of propylthiouracil on proteolytic activity is independent of age(8), it was considered of importance to study the relationship between thyroid activity and dental caries in adult animals. Since the proteolytic activity of the salivary glands increases with age with continued administration of desiccated thyroid(8), such information may be helpful in explaining the mechanism of action of the thyroid gland on the caries experience of the rat.

Method. A total of 120 weanling male Sprague-Dawley strain rats was divided equally into 3 groups according to body weight. All of the animals were housed in pairs in raised screen cages and placed on a low caries producing diet (this diet would produce about a mean of 1.5 carious lesions in 100 days) consisting of (in g %): granulated sugar, 72; vitamin test casein, 18; cottonseed oil, 5; vitamin mixture, 11; salts, 4; plus 20 drops of Percomorph oil per kilo of diet. After 100 days (123 days of age) they were divided into 3 groups: Group I animals received a stock corn cariogenic diet consisting of (in g %): yellow corn grits, 52.7; ground yellow whole corn, 11.3; powdered whole milk, 30; alfalfa, 4.8; iodized sodium chloride, 1; and, irradiated yeast, 0.2. The animals in Group II received the same diet to which 0.1% proplythiouracil had been added, and those in Group III the same diet as those in Group I except that it contained 250 mg of desiccated thyroid per kilo of diet. All of the animals received fluoride-low (F = 0.05 mg/ml) drinking water *ad libitum* and remained on their respective diets for an additional 100 days. They were then sacrificed, their heads prepared for dental caries evaluation by methods previously described(9), and thyroid and salivary glands removed for histologic examination.

Results. The data pertaining to body weight gain of the animals and the effect of thyroid drugs on the caries experience are seen in Table I. As previously reported, administration of propylthiouracil is associated with a failure to gain weight. In this study the final body weight of the control group had a mean value of 333 g while that of the animals receiving thiouracil was 214 g. In previous studies the animals receiving desiccated thyroid similarly failed to gain as much weight as the controls, due undoubtedly to their high rate of metabolism. In this study, however, amount of desiccated thyroid was adjusted so that their weight gain was comparable to the controls.

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