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SHORT COMMUNICATIONS

Effects of some chemical radioprotectors on mouse liver NAD+ and NADH levels

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SOME preliminary experiments previously reported[1] indicated that cysteamine (MEA), 2 aminoethylisothiouronium bromide hydrobromide (AET) and serotonin (5HT) given in radioprotective doses caused a rapid fall in NAD+ (nicotinamide adenine dinucleotide, diphosphopyridine nucleotide or coenzyme 1) levels in mouse or rat liver. The possible involvement of this induced biochemical change in the mechanism of radioprotection was also considered. As a prelude to further work these experiments have now been extended. Measurements of NADH have also been made to determine whether the NAD+ loss is merely a reduction of the oxidised to the reduced form.

Experiments have been carried out with Balb/c mice, aged 10–12 weeks. Animals of either sex have been used according to availability.

The chemical radioprotectors used were: Cysteamine (MEA) 150 mg/kg in phosphate buffer at pH 7·5. Cysteine prepared by neutralization of the hydrochloride with 1 N NaOH 1 g/kg. 2 aminoethylisothiouronium bromide hydrobromide (AET) 300 mg/kg in distilled water. Serotonin (5HT) 95 mg/kg in distilled water. Sodium monofluoroacetate 4 mg/kg in distilled water. All compounds were given by intraperitoneal injection.

NAD+ and NADH were estimated by the enzymatic methods described by Klingenberg.[2] In the case of the NADH measurements it was found essential to freeze the tissue in liquid nitrogen immediately the animals were killed. Otherwise considerable losses occurred very rapidly and experiments were not repeatable.

The results are shown in Figs. 1–5 inclusive. In all experiments the data are shown as percentages of control values taken at the same time. This is to overcome the problem of diurnal variation in NAD+ levels.[3] Each experimental value represents the mean of three to five individual readings.

In all cases the radioprotective agents have caused a fall in NAD+ levels. With the exception of serotonin the NADH levels have shown little deviation from normal values. In the case of serotonin there is a rise after 1 hr. These readings may be erroneous since, although serotonin solutions have

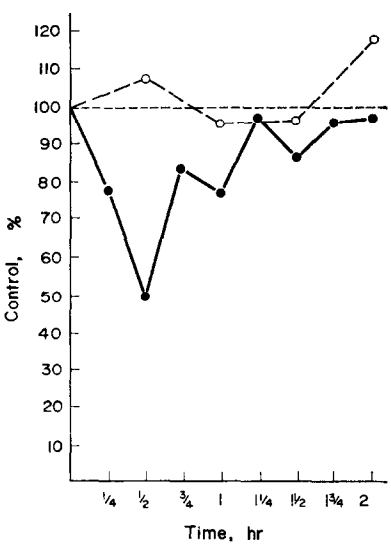

FIG. 1. Effects of cysteamine on NAD+ and NADH levels. In this and all subsequent figures NAD+ levels are shown as the full line and NADH levels as a dash line.

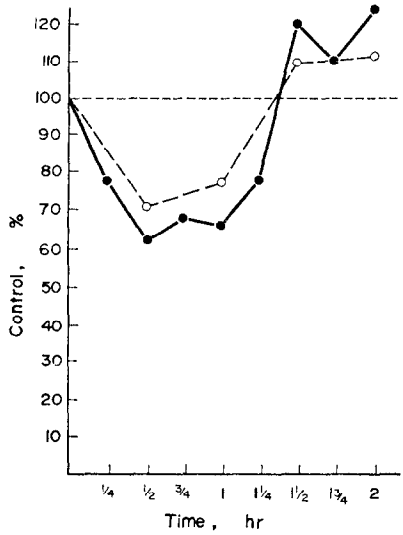

FIG. 2. Effects of cysteine on NAD^+ and NADH levels.

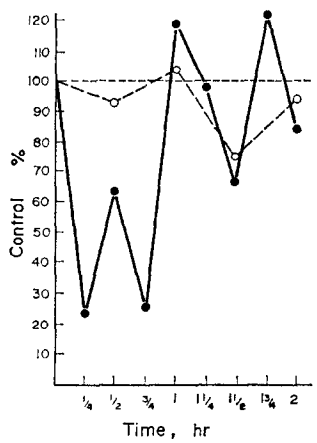

FIG. 3. Effects of AET on NAD^+ and NADH levels.

little optical absorbance at the wavelength (340 nm) used for the estimations their additions to liver extracts immediately increases absorbance. So it may not have been NADH which was measured, but some addition product of serotonin or a metabolite with a tissue component.

Since in these experiments the basal tissue levels of NAD^+ and NADH were found to be roughly equal the findings do not support any view that a reduction of NAD^+ has occurred. It would appear that NAD^+ has leaked out of the tissue. Supporting evidence comes from two other experiments. Firstly, the *in vitro* incubation of NAD^+ with aminothiols failed to give any reduction. Secondly, isolated ascites tumour (NK lymphoma) cells were divided into a number of equal portions. The addition of MEA or AET prior to spinning off the cells and estimating NAD^+ showed a loss of NAD^+ in comparison with untreated cells. If, however, the estimates were made on the cells plus the suspending medium no NAD^+ loss was found. This indicates the direct transfer of NAD^+ from the cells to the surrounding medium.

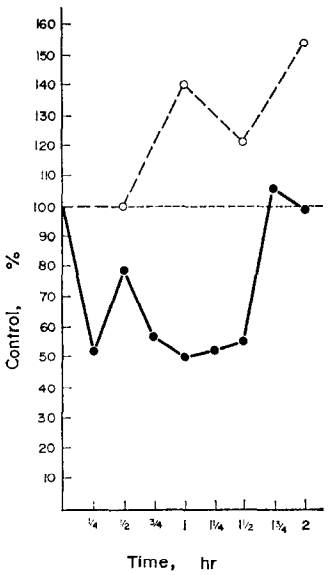

FIG. 4. Effects of serotonin on NAD^+ and NADH levels.

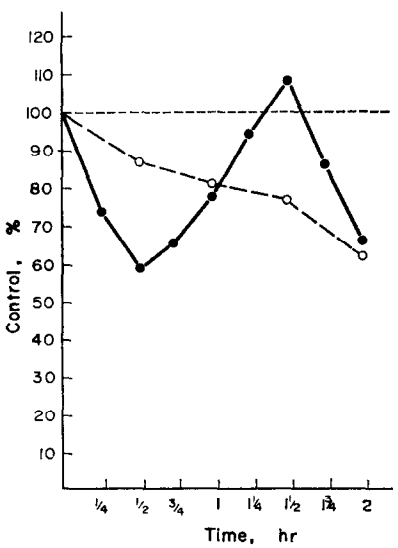

FIG. 5. Effects of sodium monofluoroacetate on NAD^+ and NADH levels.

Studies with other radioprotective agents have shown that glutathione (G.SH—1 g/kg) causes a rapid fall in mouse liver NAD^+ levels similar to that induced by cysteine. The two weak protectors methylamine hydrochloride (4 mg/mouse) and ethyl alcohol (0·5 ml of 10% ethanol per mouse) both cause an immediate small loss of NAD^+. So a group of widely different chemicals having a common biologic property (that of radioprotection) are now shown to exercise a common biochemical property.

Since NAD^+ is a powerful electron transfer agent and as an obligate coenzyme is intimately involved in many oxidative metabolic processes its possible further role in questions of radiation response is worthy of further detailed investigation.

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3. S. M. TING, unpublished data.

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Carbon tetrachloride induced peroxidation of liver lipids in vitamin E pretreated rats

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IT HAS been demonstrated that carbon tetrachloride induces a peroxidative reaction of the structura lipids of the hepatic cell.[1–6] It has also been suggested that lipoperoxidation is an important factor in the pathogenesis of CCl_4 hepatotoxicity.[7–9] Recently McLean[10] observed that α-tocopherol (vit. E), which is an antioxidant, afforded only minor protection against the CCl_4-induced liver damage. It was thus deduced that lipid peroxidation does not play a major role in the hepatotoxic effect of carbon tetrachloride.

It seemed, therefore, of interest to us to investigate whether lipid peroxidation can still be detected in liver cells after pretreatment of rats with vit. E and subsequent poisoning with carbon tetrachloride. Should vit. E prevent lipid peroxidation, then the biochemical changes produced by CCl_4 cannot be related to a peroxidative hypothesis, but if not then the alterations produced by carbon tetrachloride, may well be mediated through lipoperoxidation.

Male Sprague–Dawley rats were used. The animals to be pretreated with vit. E were given *dl*-α-tocopherol acetate (Roche) in a small volume of olive oil, by mouth. Two levels of vit. E were examined: 25 mg/100 g body wt. (an experimental situation identical to that of McLean's experiments) and 75 mg/100 g body wt. Twenty-four hr after vit. E pretreatment, one-half of the rats received CCl_4 (0·25 ml/100 g body wt.) by gastric intubation, the others mineral oil. These rats not pretreated with vit. E received an equal volume of olive oil and then either CCl_4 or mineral oil as above. All rats were starved for 18 hr before CCl_4 administration. Spectrophotometric analysis of microsomal lipids over the u.v. range was performed according to the method of Recknagel and Ghoshal[3,4] with minor modifications. Liver triglycerides were determined by the method of Van Handel and Zilversmit.[11]

One hr after CCl_4 administration, liver microsomal lipids of the rats pretreated with 25 mg/100 g body wt. of vit. E showed the diene conjugation absorption characteristic of peroxidized lipids (Fig. 1 b). The spectral changes are identical to those observed in rats not pretreated with vit. E (Fig. 1 a). However, when the dose of vit. E was increased to 75 mg/100 g body wt., the absorption of "conjugated dienes", although still evident, was reduced, as shown by the reduced magnitude of the difference spectrum (Fig. 1 c). The same results as shown in Fig. 1, were found for the u.v. absorption of microsomal lipids, 5 min after CCl_4 dosing. Even at 4 and 6 hr after poisoning, microsomal lipid peroxidation was not prevented by prior administration of 25 mg/100 g body wt. of vit. E.