when the data are plotted individually for 8-MOP and UVA (table). In order to compare the effects of various 8-MOP concentrations with different exposure lengths of UVA, the individual data were plotted semilogarithmically as the product of psoralen concentration and UVA dose (figure). This product may be called the phototoxic index (PTI). As can be seen on the figure, the incorporation rates show a linear decrease with an increasing PTI. The minimal effective PTI value was approximately 0.01, whereas complete inhibition was found at a PTI of 10 (figure). The inhibitory range is therefore three orders of magnitude independent of the individual dose of light or psoralen used. The linear curve in the figure may be calculated by the following formula:

$$\text{cpm} = c \times \ln \frac{\text{J/cm}^2 \times \mu\text{g 8-MOP/ml}}{\text{PTI}_{max}}$$

Abbreviations:
c = constant relative to cell type under study
ln = natural logarithm
$\text{PTI}_{max}$ = complete inhibition of $^3$H-TdR incorporation

The results show that the inhibitory effect of psoralen and 8-MOP upon scheduled DNA synthesis in cultured cells is a product of the effect of psoralen and light. By this method, information on the photosensitizing effects of a given 8-MOP concentration and the applied UVA dosage can be obtained rapidly, e.g. the absolute sensitivity of these cells is obtained by determining the minimum PTI, whereas the relative sensitivity is shown by the slope of the curve.

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## Hepatic UDP-glucuronyltransferase activity in acrylamide neuropathy[1]

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*Summary.* Acrylamide was found to be tissue selective in its toxicity. Doses which were clearly neurotoxic to rats were without effect on hepatic UDP-glucuronyltransferase, total hepatic protein or microsomal protein.

Endoplasmic reticulum (ER) in nerves becomes disordered in certain toxic neuropathies. Dorsal root ganglia and anterior horn cells exhibit freeing and dispersion of granular ER and polyribosomes in cats with acrylamide neuropathy[2]. Tubulovesicular profiles of ER become evident in the axoplasm of animals given vincristine[3] or bis-(N-oxopyridine-2-thionato) zinc II[4]. Amino acid incorporation into the nerves is depressed prior to pathological evidence of the neuropathy[5] and then becomes enhanced when the neuropathy is established[6].

Acrylamide is distributed in the total body water[7] and hence might be expected to exert a similar disruptive action on non-neural ER. Liver UDP-glucuronyltransferase (UDP-GT) from acrylamide intoxicated rats provides a readily accessible substrate upon which to assess this action since it is located in the ER, is affected by changes in its microenvironment[8-10] and is important in the metabolism of many xenobiotics.

*Methods.* Male Wistar rats (120–200 g) were given monomeric acrylamide dissolved in sterile saline (30 mg/kg s.c. daily for 14 days); control animals received an equivalent volume of saline. Animals were judged to be neuropathic when they exhibited splaying of the hindlimbs, motor paralysis and loss of body weight. On the 15th day, when neurotoxicity was evident, the rats were decapitated, their livers rapidly removed and chilled in ice-cold 1.15% KCl and microsomal fractions prepared as previously described[11].

UDP-GT activity was measured in microsomal fractions using either o-aminophenol (OAP) or p-nitrophenol (PNP) as substrates. Typical incubation mixtures contained in a final volume of 0.5 ml: 1-3 mg microsomal protein; 50 mM Tris-HCl buffer, pH 7.4; 5.0 mM $MgCl_2$; 0.5 mM of phenolic substrate and 5.0 mM UDP-glucuronic acid. Reactions were started by adding UDP-glucuronic acid and run for 15 min at 37 °C. OAP glucuronidation was estimated by the method of Dutton and Storey[12]. PNP glucuronidation was estimated by determining the disappearance of PNP as previously reported[11]. All determinations were run in duplicate including blanks, which contained all reactants

Table 1. Effect of chronic acrylamide treatment on hepatic UDP-GT activity. Rats were treated for 14 days with either acrylamide (30 mg/kg, s.c.) or an equivalent volume of saline vehicle. Hepatic microsomal fractions were prepared 24 h later and UDP-GT activity was determined

| Treatment | UDP-GT activity* (nmoles/15 min/mg protein) | |
|---|---|---|
| | OAP | PNP |
| Control | 4.0±0.39 | 26.2±4.9 |
| Acrylamide | 4.7±0.14 | 23.0±4.1 |

* The values are the mean ± SEM for 4 animals.

Table 2. Effect of chronic acrylamide treatment on liver weight and total and microsomal hepatic protein content. Rats were treated as described in table 1

| Treatment | g liver/100 g b.wt* | Total protein (mg/g liver)* | Microsomal protein (mg/g liver)* |
|---|---|---|---|
| Control | 4.57±0.07 | 231±3.6 | 11.4±0.5 |
| Acrylamide | 4.29±0.14 | 241±3.3 | 12.9±0.7 |

* The values are the mean ± SEM for 4 animals.

except UDP-glucuronic acid. Protein was determined by the Lowry[13] method using bovine serum albumin as the standard. Statistical comparison between means was made by Student's t-test.

*Results.* The majority of rats became neurologically impaired by the time they had received 9–11 injections (270–330 mg/kg) of acrylamide. Neurological deficits included ataxia and paralysis of the limbs, with outward splaying of limbs when placed on a flat surface. All rats were markedly neuropathic after receiving 14 injections (420 mg/kg).

Preliminary results established that the in vitro addition of acrylamide in concentrations up to 10 mM was without effect on UDP-GT activity towards either OAP or PNP. Chronic treatment of rats with acrylamide was also without effect on the activity of UDP-GT. No statistically significant differences in the glucuronidation of substrates was observed between control and treated groups (table 1). Further, acrylamide treatment did not significantly alter the liver/body weight ratio, total hepatic protein and microsomal protein content (table 2).

*Discussion.* These data point out the apparent tissue selectivity of the toxic effects of acrylamide. In neural tissue, acrylamide has been shown to disrupt the integrity of ER[2], alter protein synthesis[5,6] and interfere with normal neurophysiological function[14–17]. The data presented here suggest that acrylamide does not similarly affect hepatic tissue since UDP-GT activity was unchanged and no significant change in the protein content of the whole liver or microsomes was observed. This in itself is not surprising since the funcitons of ER in hepatic and neuronal tissue are quite different. The lack of effect of acrylamide on UDP-GT activity in vitro and in vivo, however, is striking when one considers that UDP-GT activity is known to be regulated by sulfhydryl groups[18–20] and that acrylamide has been shown to bind to these groups[21].

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## Effect of thiamine hydrochloride on the blood level of 2-formyl 1-methyl pyridinium oxime chloride (2-PAM.Cl) in rats

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*Summary.* The biological half-life of 2-PAM.Cl was found to increase in female rats pretreated with thiamine hydrochloride (10 mg/kg i.m.). No such effect was observed in the male rats.

One of the several reactivators of phosphorylated acetylcholinesterase (EC 3.1.1.7), such as 2-formyl 1-methyl pyridinium oxime chloride (2-PAM.Cl), was reported to be excreted rapidly from the body[3–5]. Nicotinamide was tried earlier to prolong its retention, but the results were inconclusive[6]. However, Swartz and Sidell[7] reported prolonged biological half-life of 2-PAM.Cl by thiamine hydrochloride in male human volunteers. We therefore reinvestigated the problem using both male and female rats to establish the influence of thiamine hydrochloride on the biological half-life of 2-PAM.Cl.

*Materials and methods.* Albino rats, weighing 150±5 g, maintained at this establishment were used in the present study. The rats were fed on Hind Lever Diet (approximately 10 g per day) and water ad libitum. Thiamine content in the diet was in the order of 0.6 mg per 100 g of food. The rats were divided into 2 groups, each group consisting of 5 male and 5 female rats. The first group received 2-PAM.Cl (30 mg/kg i.m.). The second group was pretreated with thiamine HCl (10 mg/kg i.m.) followed by 2-PAM.Cl (30 mg/kg i.m.) with a time interval of 15 min. Blood from both the groups was taken from orbital plexus at 3, 15, 30, 45, 120, 150 and 180 min in heparinized tubes. Blood oxime levels were determined by the spectrophotometric method of Creasey and Green[8]. The biological half-life of 2-PAM.Cl in the blood was calculated from the regression equation. Student's t-test was used for calculation of significance[9].

*Results and discussion.* The oxime was found in the blood within 3 min after i.m. injection. The blood level of the oxime reached its peak at 30 min in both sexes. On administration of thiamine HCl, the peak was at 45 min in the female and retention was also more than in the male. Without thiamine HCl, the male rat showed more retention than the female till 180 min. The biological half-life of 2-PAM.Cl was also more in the male than in the female and vice versa on pretreatment with thiamine HCl (table).

Studies in the intact animal and man indicated that 2-PAM.Cl and other pyridinium ions were rapidly excreted into the urine[3–4], thus it was felt that one possible way of prolonging the blood concentration of 2-PAM.Cl was to block its secretion by the tubule cells. It is well-established