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Increased Concentrations of Malonaldehyde in the Livers of Rats Treated with Carbon Tetrachloride

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The administration of CCl_4 to rats results in numerous biochemical changes in the liver within the first hour after dosing. These changes are particularly evident in the endoplasmic reticulum and have been suggested to be, at least partially, dependent on lipid peroxidation, which, once initiated by free-radical metabolites of the CCl_4 , is an autocatalytic process (Recknagel & Ghoshal, 1966; Slater, 1966).

CCl_4 has been shown to stimulate the NADPH-dependent peroxidation of rat liver microsomal suspensions (Comporti *et al.*, 1965; Ghoshal & Recknagel, 1965; Slater & Sawyer, 1971). CCl_4 has also been shown to initiate the peroxidation of 'microsomal' lipids *in vivo* within 5 min of its administration, as evidenced by diene conjugation absorption (Rao & Recknagel, 1968). However, attempts to detect malonaldehyde (which is an extensively used index of lipid peroxidation) in the livers of rats treated with CCl_4 have failed (Priest *et al.*, 1962; Recknagel & Ghoshal, 1965). In retrospect this is not surprising, since malonaldehyde has been found to be metabolized *in vivo* (Placer *et al.*, 1965). We have overcome this problem by homogenizing a small piece of liver in cold 20% (w/v) trichloroacetic acid in a top-drive Waring Blendor as soon as possible after the animal was killed. The supernatant from the centrifuged homogenate was incubated (at 100°C for 10 min) with thiobarbituric acid. The pink chromogen, which is indicative of malonaldehyde, was extracted into butanol and its E_{535} determined.

The concentrations of malonaldehyde found in rat livers 2 h after the administration of liquid paraffin or a mixture of CCl_4 and liquid paraffin (1:1, v/v) were 1.39 ± 0.11 and 2.97 ± 0.31 nmol/g of liver respectively ($P < 0.001$). The administration of promethazine, an antioxidant that protects CCl_4 -treated rats against the onset of necrosis, decreased the concentrations of malonaldehyde in the livers of untreated rats or of rats that had been dosed with liquid paraffin or with the mixture of CCl_4 and liquid paraffin. However, promethazine did not completely inhibit the stimulation of lipid peroxidation caused

by CCl_4 , as assessed 2 h after dosage with the hepatotoxic agent.

The finding that CCl_4 stimulates lipid peroxidation *in vivo* is consistent with, and lends further support to, the suggestions that CCl_4 stimulates free-radical-motivated reactions in rat liver shortly after administration (Recknagel, 1967; Slater, 1972).

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The Absorption of Aliphatic Carbamates by Everted Gut Sacs of Rat

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The everted-gut-sac technique (Wilson & Wiseman, 1954) has been used to study the absorption of a series of carbamates of general structure R-O-CO-NH_2 , where R is an aliphatic group ranging from methyl to *n*-heptyl. These compounds show a wide range of lipophilicity and are not ionized nor hydrolysed in aqueous solution.

Cannulated everted sacs of rat were incubated at 37°C in an oxygenated Krebs-Henseleit medium containing Tween 80 (5%, w/v) and one of the carbamates (10–50 mM). Samples (20 μl) were removed from the interior of the sac every 10 min for 1 h and assayed by g.l.c. on a Porapac P column. No metabolism was evident, recoveries averaging 97%. The results obtained, expressed as % of carbamate of the initial mucosal fluid absorbed/h per cm^2 were as follows: methyl, 2.81; ethyl, 3.37; *n*-propyl, 5.07; *n*-butyl, 6.60; *n*-pentyl, 3.75; *n*-hexyl, 2.25; *n*-heptyl, 0.67. It was found that the presence of Tween 80 did

not affect the absorption rate of the highly water-soluble carbamates. This is in agreement with previous work (Levy & Perala, 1970). It was shown that the Tween 80 maintained all carbamates in solution throughout the experiment. The amount of carbamate remaining in the tissue after the incubation was also determined and a similar trend was observed.

The same pattern was established for the absorption of these carbamates through skin.

In the gut-sac system butyl carbamate showed optimal lipophilicity for absorption. Contrary to the hypothesis of previous investigators (Schanker, 1960), any further increase in compound lipophilicity resulted in a decrease in absorption. The validity of these findings *in vivo* remains to be elucidated.

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The Effect of Increasing Concentrations of Potassium Chloride on Rat Liver Lysosomes

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In muscle, and possibly in other tissues, phosphorylated compounds provide the bulk of the negative charge that balances intracellular cations (Harris, 1960) and, during freezing, these salts will increase in concentration within the cell irrespective of whether ice forms intracellularly or extracellularly. As lysosomes are known to be damaged by freezing and thawing (Sellinger *et al.*, 1960), and we decided to study the effect of increasing concentrations of salt on these organelles. However, the intracellular anionic composition of most cells is unknown, and since determinations of the composition are likely to be affected by the time required to arrest metabolic reactions (Wollenberger *et al.*, 1960; Lowry *et al.*, 1964) we opted to study the effect of increasing concentrations of KCl.

The latency of acid phosphatase and β -glucuronidase in homogenates of rat liver was found to decrease progressively on exposure to concentrations of KCl between 0.13 and 1.5M in spite of the fact that the concentration of sucrose was maintained at 0.25M. These latencies were determined solely with Triton X-100, and the response could have been due to disruption of the lysosomes or to an alteration in the permeability of the lysosomal membrane that would allow access of substrate to enzymes still within the lysosomal membrane. However, we were also able to demonstrate the decrease in latency when centrifuga-

tion (300000g-min) was used to determine free acid phosphatase, and it appears that lysosomes are actually disrupted by exposure to high concentrations of salt.

The early work on rat liver lysosomes showed that they were freely permeable to salts such as NaCl and KCl (Berthet *et al.*, 1951), but our own studies and those of Lloyd (1971) indicate that these salts penetrate the rat liver lysosomal membrane relatively slowly. Consequently the disruptive effect of high concentrations of KCl on rat liver lysosomes could be due to transient osmotic pressure gradients that will arise when the concentration of KCl is lowered, but we cannot exclude a direct effect on the lysosomal membrane.

Cryoprotective agents lower the concentration of salt that is in equilibrium with ice at any given temperature (see Farrant & Woolgar, 1970), and they could therefore protect cells against damage during freezing by preventing the disruption of lysosomes. However, compounds such as glycerol and dimethyl sulphoxide have also been shown to have a deleterious effect on lysosomes (Lee, 1971, 1972), and this could be one of the mechanisms of toxicity of cryoprotective compounds.

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Lysosomal Localization of Enzyme-Containing Liposomes Injected into Rats

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Liposomes with entrapped material have been proposed as carriers for enzyme or drug administration (Gregoriadis *et al.*, 1971; Gregoriadis & Ryman, 1971, 1972). Our experiments with liposomes containing ^{131}I -labelled albumin or *Aspergillus niger* [^3H]amyloglucosidase have shown that injection of such liposomes into rats results in their