The results found with promethazine provide further evidence that dimethylnitrosamine and carbon tetrachloride react with different sites in rat liver endoplasmic reticulum to produce a stimulation of lipid peroxidation.

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Effects of Aliphatic Aldehydes on Protein Synthesis in Cell-Free Systems

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Aliphatic aldehydes have been shown to depress protein synthesis in normal and neoplastic tissues *in vitro* (Guidotti, Fonnesu & Ciaranfi, 1964), possibly by a mechanism involving their condensation with the intracellular cysteine to form thiazolidine-4-carboxylic acids (Guidotti, Loreti & Ciaranfi, 1965). These effects have been confirmed by using bacteria (Együd & Szent-Györgyi, 1966) and cultured mammalian cells (Gregg, 1968). In experiments with cell-free systems we found that several aliphatic aldehydes unexpectedly inhibited the translation of synthetic mRNA coding for cysteine-devoid polypeptide chains. Here we report the results of experiments designed to investigate the effects of aliphatic aldehydes on the attachment of mRNA to ribosomes.

Rat liver polyribosomes were obtained by the procedure of Wettstein, Staehelin & Noll (1963). Ribosomes were derived by incubating the polyribosomes as reported by Williamson (1969) and the monomeric fraction was collected from a sucrose density gradient. Incubation conditions suitable for protein synthesis were similar to those described by Munro, Jackson & Korner (1964).

When 0.1 mM-DL-glyceraldehyde, 0.1 mM-methylglyoxal, 0.1 mM- $\alpha\beta$ -dihydroxybutyraldehyde or 0.5 mM-propionaldehyde was added simultaneously with poly U to a ribosome cell-free system, a definite inhibition of the polyphenylalanine biosynthesis occurred; this inhibition disappeared when one of the aldehydes was added 5 min after the poly U-directed phenylalanine incorporation had started. Comparable concentrations of aldehyde did not affect the endogenous messenger-directed incorporation of labelled phenylalanine, leucine and

lysine into protein by the polyribosome fraction. When U-¹⁴C-labelled $\alpha\beta$ -dihydroxybutyraldehyde was incubated (20 min to 4 h) with equal amounts of polyribosomes and ribosomes, the radioactivity recovered bound to the monomeric fraction was higher than that bound to the polyribosome fraction at all the time-intervals studied. A 20 min preincubation of monomeric ribosomes with unlabelled $\alpha\beta$ -dihydroxybutyraldehyde decreased the extent of attachment of ³H-labelled poly U to ribosomes adsorbed on nitrocellulose filters, as measured by a flow system (Moore, 1966).

These results indicate that aliphatic aldehydes impair the attachment of mRNA to ribosomes and suggest that this effect may be involved in the inhibition of protein biosynthesis by these compounds.

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Protein Synthesis in Mammalian Epidermis: Are Polyribosomes Involved in Fibrous-Protein Biosynthesis?

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During the growth of fibrous protein tissue, fibrous protein molecules must be synthesized and assembled into specific protein aggregates. The processes of biosynthesis and assembly may be concurrent or consecutive.

Epidermis has been separated from the dermis of slivers of sheep skin by treatment with trypsin. It has been incubated in a tissue culture medium containing radioactively labelled algal-protein hydrolysate or leucine, washed and then disrupted in a Dounce tissue grinder. On sucrose density gradients the cell-free and nuclei-free tissue homogenates show (i) a radioactive supernatant layer, (ii) a 74S peak, which is attributed to single ribosomes and is not radioactively labelled, (iii) no evidence of polyribosomes containing, say, one to ten ribosomes, and (iv) a radioactive pellet from which ultrasonic irradiation releases single ribosomes (Priestley &

Speakman, 1966). These results may indicate that very large polyribosomes, or several polyribosomes, are involved in the biosynthesis of epidermal fibrous assemblies, as in collagen synthesis (Manner, Kretsinger, Gould & Rich, 1967; Speakman, 1968).

However, in experiments designed to discover whether there is a soluble precursor of epidermal protein, it has proved difficult to distinguish between incorporation of radioactive leucine into protein and non-specific adsorption of the label on protein. Trichloroacetic acid precipitation of epidermal protein gives a precipitate that cannot be redissolved. Isopycnic density-gradient centrifugation of a supernatant from the tissue homogenate, in a CsCl solution containing unlabelled leucine, shows an absorption peak at 280 nm at protein density. Much non-specific label is displaced from the peak, but some label, which may represent incorporated leucine, remains associated with it.

The incorporation of leucine into epidermal protein that is insoluble at neutrality but soluble at pH 2.5 is also being studied.

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The Site of the Inhibitory Action of Salicylate on Protein Biosynthesis *in vitro*

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Salicylate, in concentrations of 0.3 mM and above, inhibits the incorporation of radioactive leucine into the protein of cell-free systems prepared from rat liver (Dawkins, Gould & Smith, 1966; Reunanen, Hänninen & Hartiala, 1967). This action of the drug is independent of its uncoupling action on oxidative phosphorylation, since it occurred in microsomal preparations supplied with an external source of energy. We have therefore studied the effects of salicylate on the two main stages of protein biosynthesis *in vitro*.

The transfer of the radioactive amino acid from leucyl-tRNA to a polyribosome fraction prepared from rat liver is not affected by 10 mM-salicylate. Aminoacyl-tRNA synthetase activity was studied by the ATP-pyrophosphate exchange reaction and by the formation of aminoacyl-tRNA species from labelled amino acids. Salicylate, in concentrations of 0.6 mM and above, inhibits the ATP-pyrophosphate exchange reaction promoted by either an equimolar mixture of 20 amino acids or by individual

amino acids including leucine. However, the formation of the aminoacyl-tRNA species is differently affected by salicylate. The most sensitive amino acids are glutamate, aspartate and histidine. High concentrations of the drug (10 mM) inhibit the incorporation of cysteine, methionine, threonine and tryptophan, but not of leucine and the other amino acids.

These results show that salicylate interferes with the biosynthesis of proteins *in vitro* by preferentially inhibiting the formation of certain aminoacyl-tRNA species. They also suggest that the ATP-pyrophosphate exchange reaction does not accurately reflect the first stage in the incorporation of amino acids into proteins *in vitro* (Loftfield & Eigner, 1969).

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Protein and Ribonucleic Acid Synthesis *in vitro*: Calculation of the Bivalent Cation Requirement and the Extent of Complex-Formation in Incubation Media

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Staehelin (1969) has recently drawn attention to the possibility that the use of excess quantities of compounds such as phosphoenolpyruvate in protein-synthesizing systems *in vitro* can complex Mg^{2+} ions to such an extent that dissociation of polyribosomes occurs. It is a frequent practice in studies of amino acid incorporation by ribosomes *in vitro* to measure the optimum magnesium concentration of the system. This value is always the total [Mg] and as such gives little indication of the concentration of free Mg^{2+} , which is presumably the crucial entity affecting the activity of the preparation. Thus with 5 mM total magnesium, 1 mM-ATP and 10 mM-phosphoenolpyruvate, calculation suggests that $[Mg^{2+}]$ is likely to be about 0.8 mM. Knowledge of the concentration of free Mg^{2+} should enable more meaningful comparisons to be made of optimum magnesium requirements of systems developed by various workers (usually coming out at about 0.5–3 mM for translation of endogenous messenger), the actual concentration of Mg^{2+} at which use of synthetic messenger is most efficient (4–5 mM) and the $[Mg^{2+}]$ at which misreading of the code occurs (from 3.5 mM upwards). Recent