which would yield isethionic acid, but no sulphuric acid, on acid hydrolysis. Isethionic acid is stable under such conditions and might therefore be missed, especially as its barium salt is soluble in water. As *N*-sulphated mucopolysaccharides exist, a $\text{:C}\cdot\text{NH}\cdot\text{SO}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}$ link might be formed and, although ethionic acid (the sulphuric ester of isethionic acid) has not been detected in Nature, the linkages $\text{:C}\cdot\text{O}\cdot\text{SO}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\text{SO}_2\cdot\text{O}^-\text{K}^+$ or $\text{:C}\cdot\text{O}\cdot\text{SO}_2\cdot\text{O}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{SO}_2\cdot\text{O}^-\text{K}^+$ might conceivably occur. In a search for such compounds determination of the ratios $\text{K}/\text{total S}$, $\text{K}/\text{SO}_4^{2-}$ and $\text{SO}_4^{2-}/\text{S}$ would be useful.

An unusual sulpholipid containing a carbohydratesulphonic acid fragment, but no sulphate group, was found in photosynthetic plants, algae and bacteria (Benson, 1963).

It should, however, be stated that references to the biological occurrence of isethionic acid do not mention its formation by hydrolysis of a complex compound, and it appears to occur free in the organisms where it has been found. Nevertheless it seems that its mode of occurrence, free or combined, in various tissues should receive particular attention.

Support for such a suggestion is furnished by the isolation of 2-aminoethylphosphonic acid, $\text{HN}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{PO}(\text{OH})_2$, by hydrolysis of the lipids of rumen protozoa (Horiguchi & Kandatsu, 1959) and those of two sea-anemones (Kitteredge, Roberts & Simonsen, 1962; Quin, 1964), in one of which it occurs in lipid and in protein combination. Rouser, Kritchevsky, Heller & Lieber (1963) isolated a ceramide 2-aminoethylphosphonate from one of the anemones. With *Tetrahymena pyriformis* Rosenberg (1964), using ^{32}P orthophosphate in the culture medium, found that some aminoethylphosphonic acid entered macromolecular combination. The recognition of a C-P bond instead of the common C-O-P link [e.g. $\text{H}_2\text{N}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\text{PO}(\text{OH})_2$] suggests that a search for further examples of a C-SO₂·OH link in Nature might be rewarding.

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The Effects of Various Inhibitors on the Stimulation of Lipid Peroxidation Produced By Dimethylnitrosamine in Rat Liver Microsomes *in vitro*

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Dimethylnitrosamine increases lipid peroxidation, as measured by the thiobarbituric acid reaction, in a suspension of rat liver microsomes containing an NADPH-generating system. The experimental procedures used were similar to those described previously for use with carbon tetrachloride (Slater, 1968; Slater & Sawyer, 1969). Dimethylnitrosamine (1–3 μl) was added directly to the stock mixture (3ml) and incubations were for 30 min at 37°C.

Three inhibitors of the microsomal electron-transport chain were used at final concentrations known to strongly inhibit drug metabolism. SKF 525A and *p*-chloromercuribenzoate, at final concentrations of 100 μM and 94 μM respectively, decreased the stimulation of lipid peroxidation due to dimethylnitrosamine by 45 and 64% respectively. Inhibitions of aminopyrine demethylation by these two agents with the concentrations used were: SKF 525A, 126 μM, 59%; *p*-chloromercuribenzoate, 100 μM, 73% (Orrenius, 1965).

When the stock mixture was gassed for 5 min with carbon monoxide (or air for the control) a complete inhibition of the stimulation of lipid peroxidation due to dimethylnitrosamine was found. This procedure strongly inhibits drug metabolism utilizing the cytochrome *P*-450 pathway (Orrenius, 1965).

The inhibition by *p*-chloromercuribenzoate, SKF 525A and carbon monoxide of the stimulation of lipid peroxidation due to dimethylnitrosamine contrasts with the increases produced by these agents in the stimulation of lipid peroxidation due to carbon tetrachloride (Slater & Sawyer, 1969). These results suggest that carbon tetrachloride and dimethylnitrosamine interact with the microsomal electron-transport chain to stimulate lipid peroxidation at different sites: dimethylnitrosamine reacting in the vicinity of cytochrome *P*-450 and carbon tetrachloride reacting near the NADPH-flavoprotein site.

The effects of the phenothiazine drug promethazine on the stimulation of lipid peroxidation due to dimethylnitrosamine and carbon tetrachloride were also studied. Promethazine at a final concentration of 0.1 μM had little or no effect on dimethylnitrosamine-stimulated lipid peroxidation. It had, however, a marked inhibitory effect on the increased lipid peroxidation due to carbon tetrachloride, as reported by Slater (1968).

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 &