[6-¹⁴C]orotic acid *in vivo* for 24h. Cross-over experiments between fed and starved rats in which 1 vol. of labelled postmitochondrial supernatant was mixed with 9 vol. of unlabelled postmitochondrial supernatant indicated that the yield of free polyribosomes depended on whether the labelled free polyribosomes were derived from fed or starved animals, and was little affected by the source of the membranes.

Profile analysis indicated that liver from fed rats contains polyribosomes of larger average aggregate size than does liver from starved rats. It is postulated that the exaggerated delay in the sedimentation of free polyribosomes of liver from fed rats is due to reversible entanglement of large polyribosomes with non-sedimenting membranous components.

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Amino Acid Incorporation into Protein of Neuronal and Neuropil Fractions *in vitro*

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The density-gradient centrifugation method of Rose (1967) produces cell fractions from cerebral cortex that are metabolically active, designated: A, myelin, undisrupted tissue; B, 'neuropil', neuroglia, sections of dendrites and axons, nerve endings; C, 'neuronal', cell bodies with various lengths of processes attached; D, blood cells, nuclei; S, initial cell suspension. When the washed-cell fractions were incubated at 37°C in a phosphate-buffered medium (Elliott & Henderson, 1948) containing 10mM-glucose with a labelled amino acid (e.g. [³H]lysine), all incorporated radioactivity into trichloroacetic acid-insoluble material over a $\frac{1}{2}$ -3h period. The suspension S showed a rate of incorporation less than that of slices (Folbergrova, 1966), comparable with that of chopped-tissue suspensions (Austin & Morgan, 1967) and considerably greater than that of cell-free homogenates (Peterson & McKean, 1969). This incorporation was roughly linear for about 1h, falling off thereafter. The C layer showed a rate of incorporation from [³H]lysine 30% higher than the B layer, calculated

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on the basis of the total protein content of the suspension. This can be compared with the results of Blomstrand & Hamberger (1969), who report a greater difference in the same direction for incorporation *in vivo* followed by a basically similar cell separation.

When a mixture of 19 amino acids and taurine (each 1mM) was included in the medium, an inhibition of incorporation was observed with the initial suspension (20%) and the B layer (35%), but not with the C layer. The most likely explanation for the inhibition, which is similar in magnitude to that reported for cortex slice by Folbergrova (1966) and for homogenate by Peterson & McKean (1969), with individual amino acids, is inhibition of transport across a membrane barrier by competition of different amino acids for a common transport mechanism. The absence of inhibition from the neuronal fraction could indicate a less intact membrane, or a difference in the kinetics of amino acid uptake or utilization. The latter is more likely, since both neuronal and neuropil fractions are known to be able to accumulate ions (Rose & Sinha, 1969).

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Speculations on the Biological Occurrence of Isethionic Acid

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Isethionic acid (2-hydroxyethanesulphonic acid) has been isolated from heart tissue of dogs and rats, from normal human urine, from the axoplasm of the giant nerve fibres of a squid, from the brain of the honey-bee, from taurine in homogenates of rat brain and from the urine of rats after taurine administration (for references see Jacobsen, Collins & Smith, 1967). The possibility that it may occur in some lesser-known polysaccharides, mucopolysaccharides and lipids, giving products analogous to the ordinary sulphated derivatives, is attractive. In such compounds the group :C·O·SO₂·OH might be partially replaced by :C·O·SO₂·CH₂·CH₂·OH,

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).