polyribosomal patterns show a further shift towards the lighter forms also in reversibly damaged liver cells: protein synthesis stays at the low rate reached at the end of ischaemia.

The return of blood flow in a formerly ischaemic tissue seems to be a critical stage for the state of polyribosomes in the liver cell.

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Ribosome Tetramers from Cold-Treated Chick Embryos

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Byers (1967) has described a structure occurring in cold-treated chick embryo cells that is composed of ribosomes arranged in a crystal-like lattice. The smallest repeating unit of the lattice is a group of four ribosomes. From the work of Humphreys & Bell (1967) it was concluded that these tetramers were stable to the processes of homogenizing and centrifuging as applied to the preparation of ribosomes for analysis on sucrose density gradients. It seemed probable that further investigation of them would provide information about ribosome structure and function.

Ribosomes were prepared from cold-treated chick embryos by the method of Blobel & Potter (1967) and analysed on sucrose density gradients in the Spinco SW 40 rotor, by either 10–40% (w/v) sucrose or the isokinetics described by Noll (1967). A ribonuclease-stable peak has been found in the region of the tetramer, but the overall pattern of peaks found in the TKM buffer of Blobel & Potter (1967) is complex, with a number of minor peaks in addition to the easily recognizable monomer, dimer etc. The tetramer region has been resolved into two peaks with sedimentation coefficients 183S and 194S.

Lowering of the Mg^{2+} concentration caused a reversible dissociation of the higher aggregates into monomers and subunits. Increasing Mg^{2+} (or other bivalent cation) concentration caused non-specific aggregation. Ca^{2+} can replace Mg^{2+} , but in some circumstances an increased tendency to dimer formation was noticed. Mn^{2+} also caused dimer formation.

Increasing K^+ concentration caused disaggregation and at high concentration (0.35M) extensive

subunit formation. The 'tetramer' peak, however, was not lost, i.e. a peak equivalent in size to the tetramer remained, but with a stepwise alteration to positions with lower sedimentation coefficient.

Results of attempts to fractionate the ribosome peaks and data on amino acid-incorporating activity will be reported.

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Factors Affecting Separation of Free Polyribosomes from Membrane-Bound Polyribosomes

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Accumulating evidence suggests that free and membrane-bound polyribosomes from rat liver synthesize different non-overlapping classes of proteins (e.g. Hallinan, Murty & Grant, 1968a,b; Takagi & Ogata, 1968; Redman, 1969). A necessary prerequisite for such investigations is of course the separation of the two polyribosome classes from each other. A standard method used in this study consists of centrifuging postmitochondrial supernatant at 105000g_{av} through steps of 1.5M- on 2.0M-sucrose; free polyribosomes form a pellet at the bottom of the tube while most membranes, having a lower density, remain in the 1.5M-sucrose layer. It has been shown (Blobel & Potter, 1967; D. Lowe, T. Hallinan & E. Reid, unpublished work) that centrifugation time is very important. After only 4–5h (the centrifugation time used by many authors) sedimentation of free polyribosomes is incomplete. This phenomenon has now been further investigated.

Rats were either fed *ad libitum* or starved overnight. Polyribosome pellets obtained after centrifugation for various times were analysed for RNA content. Not only was the 4–4½h free polyribosome yield a small percentage of the 20–21h yield (which was similar for fed or starved rats), but this percentage was much smaller for fed (average 5% of the 21h value) than for starved rats (average 28%). However, this low yield at 4–5h could be increased tenfold by using diluted postmitochondrial supernatant, suggesting interference by concentration-dependent interaction of polyribosomes with non-sedimenting components.

In other experiments RNA was labelled with

[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,