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, 1968*a*,*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 $105000 g_{av.}$ 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