of various enzymes, such as NADPH-cytochrome *c* reductase and cytochrome *b*₅, which are associated with the endoplasmic reticulum, after the administration of phenobarbitone to rats. This suggests that polyribosomes do not lay down a membrane with a certain fixed spectrum of enzymes but that there is an addition and deletion of the proteins of the membrane. These studies *in vivo* are in accord with the work of Ragnotti, Lawford & Campbell (1969), who showed that various preparations of isolated polyribosomes from liver made NADPH-cytochrome *c* reductase and that the newly synthesized enzyme became associated with the membranes.

There have been many recent attempts to determine whether the membrane-bound polyribosomes of the adult liver cell effect the synthesis of proteins that are retained by the cell as well as those destined for export. A fraction rich in polyribosomes, which from electron microscopy seems to be free of fragments of the rough-surfaced endoplasmic reticulum, can be isolated from rat liver (Blobel & Potter, 1967). Since these polyribosomes are not apparently associated with membrane they are termed free polyribosomes. To determine whether the latter are functionally distinct from the membrane-bound polyribosomes the synthesis of specific proteins by the two fractions *in vitro* has been studied. Redman (1969) reports that the free polyribosomes synthesized 7–20 times as much ferritin, a protein retained by the cell, as did membrane-bound polyribosomes (see also Hicks, Drysdale & Munro, 1969). The failure of isolated free polyribosomes to effect the synthesis of albumin

has been reported by Takagi & Ogata (1968) and of glycoproteins by Hallinan, Murty & Grant (1968). One of the difficulties in this work is the satisfactory characterization of the newly synthesized protein (see e.g. Schreiber, Rotermund, Maeno, Weigand & Lesch, 1969). With this proviso it seems right to conclude that the adult liver cell contains two types of functionally active polyribosomes and that certain of the proteins retained by the cell are not synthesized by the membrane-bound polyribosomes.

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COMMUNICATIONS

Model Building Studies on the Structure of Deoxyribonucleohistone

By J. F. PARDON and B. M. RICHARDS. (*Searle Research Laboratories, Lane End Road, High Wycombe, Bucks., U.K.*)

Sequence studies on purified histone fractions are in progress in other laboratories. We have used the published sequence for fraction IV (DeLange, Fambrough, Smith & Bonner, 1969) as the basis for an atomic-space-filling model comprising a single molecule. Possible conformations of the molecule have been investigated for the histone molecule free from and also in complex with DNA.

A highly compact globular conformation is possible in which most of the hydrophobic residues readily lie in the centre of the molecule giving an overall diameter of approx. 50 Å. The distribution

of basic residues on the surface is such that this conformation is a most unlikely one for the histone in the nucleohistone complex. Similarly the fully extended chain is another unlikely conformation. The model readily permits 5–8 turns of α -helix, giving a proportion of 18–30%, which is not incompatible with experimental observations (Oriel, 1966; Bradbury, Crane-Robinson, Goldman, Rattle & Stevens, 1967).

In the complex between histone and DNA the following considerations have been taken into account.

- (1) The number of electrostatic bonds formed between basic residues of the histone molecule and charged phosphate groups of DNA is maximized.
- (2) All possible secondary structure within the histone molecule is permitted.
- (3) The histone is accommodated as far as possible in either groove of the DNA double helix.

(4) The DNA is assumed to be in the super-coil configuration (Pardon, Wilkins & Richards, 1967; J. F. Pardon & M. H. F. Wilkins, unpublished work).

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The Structure of Deoxyribonucleohistone and the Role of Individual Histone Proteins

By B. M. RICHARDS, J. F. PARDON and ELIZABETH HIRST. (*Searle Research Laboratories, Lane End Road, High Wycombe, Bucks., U.K.*)

In studies of the reconstruction of the nucleoprotein complex from separated histones and DNA the characteristic X-ray-diffraction pattern can be used as the criterion for the recovery of the correct molecular configuration. In this way we can test for the nativity of mixtures and individual histone fractions isolated by both physical and chemical means.

Histones produced by salt dissociation of nucleohistone are native by the stated criterion, but fractionation is virtually non-existent except for the separation of lysine-rich histone F1 (Ohlenbusch, Olivera, Tuan & Davidson, 1967). Similarly attempted fractionation by acid titration (Murray, Vidali & Neelin, 1968) is only partially successful, but nevertheless yields mixtures of native histones provided that they are sulphates precipitated with ethanol.

Individual histones fractionated by selective chemical separation or separation by column chromatography are being investigated for their ability to produce native material. Thus far the original mixture of five histones has been reduced to a mixture of three fractions that have undiminished ability to regenerate the super-coil configuration of nucleohistone (Pardon, Wilkins & Richards, 1967).

Progress towards a two-component nucleohistone is essential for the full elucidation of the interaction between any given histone molecule and DNA at the atomic level. Until this is achieved the application of crystallographic procedures remains severely limited.

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The Isolation of Rapidly Labelled Deoxyribonucleic Acid Fragments of Nuclear Origin from the Polyribosome Fraction of Cultured Embryonic Liver Cells

By R. WILLIAMSON. (*Beatson Institute for Cancer Research, Hill Street, Glasgow C.3, U.K.*)

During experiments designed to isolate low-molecular-weight RNA species, it was found that a large amount of DNA accumulates in the cytoplasm of cultured embryonic liver cells. Mouse 14½-day embryonic livers were disaggregated and cultured in the presence of [³²P]phosphate for 16 h at 37°C. The cells that do not stick to glass, predominantly erythroid in type, were isolated and lysed with a hypo-osmotic buffer. Total nucleic acid was isolated either from the postmitochondrial supernatant or from isolated polyribosomes; the results were apparently similar with regard to DNA in both cases.

Up to 20% of the total DNA was found in the cytoplasm. The base compositions of nuclear and cytoplasmic DNA were identical with each other and with established values for mouse cells. The nuclear and cytoplasmic DNA both had a buoyant density indistinguishable from that of native total embryonic DNA, demonstrating not only the identity of base composition but also that the cytoplasmic DNA is double-stranded.

Electrophoresis of the DNA on 2.4% polyacrylamide gels gave a regular series of bands roughly from the position of 5S to the top of the gel. At least ten bands can be detected by the eye. The four smallest DNA components were isolated by preparative polyacrylamide-gel electrophoresis, and their sedimentation coefficients were determined and molecular weights calculated. The DNA fragments appear to be multiples of a unit molecular weight approx. 150 000.

The specific radioactivity of the DNA from the cytoplasm was 1.3–2-fold higher than that of the nucleus, and the radioactivity was non-terminal, thus ruling out the introduction of [³²P]phosphate via a phosphokinase mechanism during degradation. The DNA of one band (molecular weight approx. 300 000) has been purified, depurinated and 'finger-printed' and is definitely not of unique base sequence.

It is thought that this DNA may be similar to that reported from a cultured embryonic muscle-cell system by Bell (1969). However, certain of the experimental results outlined here do not fully accord with his conclusions.