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is assumed to be in the super-coil configuration (Pardon, Wilkins & Richards, 1967; J. F. Pardon & M. H. F. Wilkins, unpublished work).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

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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

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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.

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The Effects of Chloramphenicol on the Synthesis of Bacterial Ribonucleic Acids

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It has been known for some time that amino acids play an important role in regulating the rate of biosynthesis of bacterial RNA (Pardee & Prestidge, 1956; Gros & Gros, 1958; Aronson & Spiegelman, 1961a,b). High concentrations of chloramphenicol (up to 0.2 g/l) inhibit protein synthesis severely and spare the requirement for amino acids as stimulants of RNA synthesis (Aronson & Spiegelman, 1961a; Kurland & Maaløe, 1962). Further, when bacteria are grown in an enriched medium containing amino acids the overall rate of RNA synthesis is virtually unaffected by adding chloramphenicol, whereas it is almost doubled when the antibiotic is added to cultures growing in glucose-salts medium (Fraenkel & Neidhardt, 1961; Kurland & Maaløe, 1962).

Using the technique of DNA-RNA hybridization (Gillespie & Spiegelman, 1965; Pigott & Midgley, 1968), I have studied the antibiotic-induced changes in the rate of mRNA synthesis in *Escherichia coli* M.R.E. 600 growing in broth, glucose-salts or sodium lactate-salts media at 37°C. On comparing the kinetics and relative rates of incorporation of [5-³H]uracil into mRNA, rRNA and tRNA, after addition of chloramphenicol, with those holding for all states of steady exponential growth (Gray & Midgley, 1969), I found the following.

(1) mRNA is stabilized in the presence of chloramphenicol and accumulates as about 6% of the total cellular RNA (Midgley, 1963; Friesen, 1966). Though also RNA turnover can be demonstrated in antibiotic-inhibited cells, and the pools of nucleotides are of normal size, the kinetics of labelling all RNA fractions gives incorporation curves that extrapolate to the origin.

(2) Whereas in exponentially growing cells in any medium and at all temperatures exogenous uracil enters mRNA and stable RNA pools in a 1:2 (weight/unit time) ratio, only 14% of the RNA formed in the presence of chloramphenicol has the hybridization characteristics of mRNA. Thus the synthesis of mRNA in bacteria is not rigidly coordinated with that of rRNA + tRNA.

(3) The kinetics of labelling mRNA in broth cultures suggests that, when chloramphenicol is

added, about half the mRNA present eventually decays, and the remainder persists throughout chloramphenicol inhibition.

(4) It appears that, in antibiotic-inhibited cultures, new steady states of synthesis of the various RNA fractions are set up that are unlike those found in normally growing cultures. The question whether these changes are brought about by alterations in the rate of RNA transcription by DNA-dependent RNA polymerase or in the rates of initiation of new chains is considered by Midgley (1970).

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The Rate of Transcription of 23S Ribosomal Ribonucleic Acid in Normal and Chloramphenicol-Inhibited Bacteria

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Gray (1970) has shown that, when exponentially growing and dividing cultures of *Escherichia coli* M.R.E. 600 are inhibited by the addition of chloramphenicol, a rapid change takes place in the relative rates of production of various RNA species. Further, when cultures growing in simple defined media such as lactate-salts or glucose-salts are incubated with the antibiotic, the overall rate of RNA synthesis is rapidly increased (Fraenkel & Neidhardt, 1961; Kurland & Maaløe, 1962). In broth media, where amino acids are in plentiful supply, there is virtually no change in the rate of RNA synthesis in the presence of chloramphenicol, although mRNA is stabilized and accumulates (Friesen, 1966).

I have studied the kinetics of synthesis of the 23S rRNA of *E. coli* at 37°C in each of three media, in the presence or the absence of 0.2 g of chloramphenicol/l. My object was to discover if the alterations in