in chylomicrons remained well below 1% of the total ^{14}C infused.

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The Use of Fatty Acids by Muscle during Exercise

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Muscle tissue may obtain long-chain fatty acids for oxidation from the following sources: (1) plasma lipid transport system: chylomicrons, lipoproteins, free fatty acids; (2) intracellular lipid pools: triglycerides, phospholipids, other (?); (3) local extracellular lipid pools: adipose tissue between muscle fibres. Changes in these different lipid pools during exercise will be reviewed. Quantitative, as well as qualitative, aspects will be covered. Thus in 1965 ten healthy subjects walked 50 km. a day for 10 days. Their daily caloric intake was only about 200 cal. It can be estimated that they spent around 4500 cal./day (3000 for walking and 1500 for basal metabolism) corresponding to 500 g. of fatty acids or 700 g. of adipose tissue. They lost about 0.75 kg./day (Carlson & Fröberg, 1967). The concentration of free fatty acids in the plasma increased to $1.4 \mu\text{moles/ml}$. If we assume the fractional turnover rate of free fatty acids to be about 0.33/min. ($t_{1/2}$ about 2 min.) the value for the concentration of free fatty acids corresponds to a transport of 500 g. of fatty acids from adipose tissue/24 hr. These values support the hypothesis that, in the starving state, free fatty acids are the major transported energy substrate.

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COLLOQUIUM ON 'INTERACTIONS OF NUCLEAR MACROMOLECULES'

Conformations of Nucleohistones and Histones

By E. M. BRADBURY. (*Biophysics Laboratory, Department of Physics, College of Technology, Portsmouth*)

Because of the close association of histones with DNA in the cells of higher organisms, it has been suggested that histones are involved in genetic regulation and much effort has been expended in attempting to demonstrate this role. However, histones are a major component of chromosomes and they could be involved in a purely structural role in controlling the conformational changes that chromosomes undergo during mitosis. Unfortunately there is little precise physical information on the molecular structure of chromosomes and their components and the whole process of chromosomal coiling is not understood.

X-ray-diffraction studies of nucleoproteins have

been disappointing because of the lack of detail in the diffraction patterns. However, a feature of the diffraction patterns that is not present in the diffraction patterns of either the DNA or the total isolated histone is a series of low-angle diffraction rings at 110, 55, 35, 27 and 22 Å (Wilkins, Zubay & Wilson, 1959; Luzzati & Nicolaieff, 1959). From the values of these spacings and their partial meridional orientation, Wilkins (1964) suggested that nucleohistone was in the form of a supercoil of 120 Å pitch and 100 Å diameter. Evidence for this coiled form of nucleohistone has been provided by the experiments of Pardon, Wilkins & Richards (1967) in which a stretched nucleohistone fibre was shown to have lost the low-angle diffraction rings characteristic of the superstructure; the rings, however, reappeared on relaxing the fibre and this cycle could be repeated. It is clear from this experiment that there is a folded structure for

nucleohistone that can be extended under tension. Many of the hydrodynamic properties of nucleohistone and the changes that occur on dissociation of the histone can also be correlated with a change from a more compact form of nucleohistone to an extended DNA molecule.

There is little doubt that there is a superstructure for nucleoproteins, and if the supercoil is accepted as a working hypothesis it is of interest to determine which of the histone fractions are involved in coiling of the DNA. In collaboration with K. Murray, B. M. Richards & J. F. Pardon (unpublished work) we have studied several series of partial nucleoproteins from chicken erythrocytes (these are the nucleoprotein complexes that remain after exposure of nucleohistone to increasing salt molarity) and it was found that the series of low-angle diffraction rings disappeared in the range 1.0–1.3M-NaCl when up to 80% of the histone had been dissociated. Since most of the lysine-rich histones are dissociated before this salt range, it is clear that they are not involved in the formation of the supercoil and that they have some other function, structural or otherwise, in chromosomes. It is not possible to decide which, or whether all, of the other histone fractions are involved in the formation of the supercoil, since all of these are being dissociated over the range of salt molarities where the supercoil breaks down. Spectroscopic studies of the same series of partial nucleoproteins (Bradbury, Crane-Robinson, Rattle & Stephens, 1967) show that the lysine-rich histones contain a very low helix content whereas the helix content of the other fractions is in the range $33 \pm 5\%$.

The histones from calf thymus can be fractionated into five main fractions F1, F2a₁, F2a₂, F2b and F3, using the notation of Johns and Phillips (see Butler, Johns & Phillips, 1968), and although chicken erythrocyte nucleohistone contains an additional lysine-rich fraction (Neelin, 1964) it appears now that there is not the large number of different histones as was thought earlier in histone chemistry. The same histone fractions from many different tissues of higher organisms have been shown to be very similar and this similarity has been demonstrated dramatically by the sequences of histone F2a₁ from calf thymus and peas (De Lange, Fambrough, Smith & Bonner, 1968, 1969) in which there appear to be only two conservative replacements. This conservation of sequence is unique in protein studies and implies that the function of histones involves the conformation, and possibly conformational changes, of the whole molecule and not specific regions of the protein as is required, for example, by the active sites of enzymes. Since the conformation of the histones is determined by the ionic environment provided by DNA, it is of interest to investigate whether

increasing the ionic strength of aqueous solutions of histone fractions causes conformational changes and whether or not the changes involve specific regions of the polypeptide chain. Optical-rotatory-dispersion studies (Bradbury, Crane-Robinson, Goldman, Rattle & Stephens, 1967) show that in 1M-NaCl the histone fractions, except for F1, can assume up to 30% helix content, which is very similar to that found in native nucleohistone. Nuclear-magnetic-resonance studies of the same solutions show that the mobilities of apolar regions of the polypeptide chain are restricted and it is reasonable to suggest that these residues are involved in helical segments, the mobilities of which may be further restricted by specific aggregation between them. This observation can be accommodated by the sequence of histone F2a₁ and the distribution of amino acids in peptides of histone F1 (Bustin, Rall, Stellwagen & Cole, 1969) in which the distribution of amino acids is very irregular (this had long been suspected from the peptide analyses by Phillips; see Butler *et al.* 1968). The ratio of basic to acidic residues of the N-terminal 0–51 residues of histone F2a₁ is 1.5, whereas for the C-terminal 51–102 residues it is 1.5; for histone F1 the situation is reversed in that the 100 residues at the N-terminal end have a basic/acidic ratio of 1.2 whereas for the C-terminal end it is 1.5. Also, whereas the N-terminal end of histone F1 is rich in proline, a helix destabilizer, the C-terminal end of histone F2a₁ is very rich in glycine, which in high proportions would also act as a helix destabilizer. It is highly unlikely that these regions would be involved in helix formation and this is confirmed from n.m.r. studies (E. M. Bradbury, M. Boublik & C. Crane-Robinson, unpublished work). The other regions of the polypeptide chain have an amino acid composition similar to that of globular proteins and these regions should include any helical segments that are formed. The distribution of amino acids suggests that the N-terminal end of histone F2a₁ and the C-terminal end of histone F1 are 'sticky' and are the likely sites of interaction with DNA.

It is highly probable that one of the main roles of histones is structural and that they are involved in the process of chromosomal coiling in which DNA molecules of great length are packed into a chromosome the order of micrometres. It is difficult to accept that this process, which is reversible, results from a haphazard interaction of histones with DNA, and it may be suggested that each stage of condensation is precisely controlled by the interactions of histones with DNA, possibly by conformational changes in the histones themselves and by the interactions of histones with one another. Such a process probably demands precision of interactions that involve the whole of the histone molecule, and

it may be for this reason that few changes can be tolerated in the sequences of histones.

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Histone Changes and Cell Division

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Four changes have so far been found to affect the microstructure of histones: acetylation, methylation, phosphorylation and oxidoreduction. In synchronized or partially synchronized cell systems, the first of these increases with the onset of new RNA synthesis (Pogo, Pogo, Allfrey & Mirsky, 1968), whereas the peak of methylation occurs just before mitosis, when DNA synthesis has declined (Tidwell, Allfrey & Mirsky, 1968).

Phosphorylation and oxidoreduction changes will be considered here. Phosphorylation may occur on all the histones so far described (Langan, 1969), but the increased phosphate content of histone F1 during DNA synthesis is the most firmly established. This increase has been found in regenerating liver, kidney (Ord & Stocken, 1968; Stevely & Stocken, 1968) and pancreas (P. J. Fitzgerald, M. G. Ord & L. A. Stocken, unpublished work), and conversely a decline in phosphate content occurs after birth in rat liver (Ord & Stocken, 1969) and during maturation of fish sperm (Ingles & Dixon, 1967).

Variations in phosphate content have been found for other histones remaining after the selective removal of histone F1. In regenerating rat liver an increase occurs during the first 2 hr. after partial hepatectomy, when new messenger RNA is being formed. The amount is even further increased during the period of DNA synthesis (Ord & Stocken, 1969). This process entails enzyme(s) different from that required for the phosphorylation of histone F1.

Oxidoreduction changes in the thiol groups of histones have been studied in fertilized sea-urchin eggs and in phytohaemagglutinin-stimulated lymphocyte cultures (M. E. Cross & M. G. Ord, unpublished work). Correlations between an increasing proportion of thiol to disulphide and increasing information transfer from DNA will be examined and also the importance of thiol histones in structural changes in cell nuclei. Possible enzyme changes leading to the alterations in histone micro-groups will be considered and the suggestion that alterations in micro-structure are required as permissive states for information transfer discussed.

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Formation and Processing of Ribonucleic Acid in Subnuclear Components of *Chironomus tentans*

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Recent progress in the field of intranuclear RNA metabolism (for a review see Darnell, 1968) has to a large extent been due to the successful development of methodology for isolation of nuclei and certain gross nuclear fractions like nucleoli and nucleoplasm. It would be highly desirable, however, to achieve more extensive separations. Nucleoli are often of different genetic origin and may in certain cases also fulfill different functions: nucleoplasm contains not only chromatin, functionally highly heterogeneous, but also nuclear sap, differing from the chromosomes in metabolic properties.

It may be easiest to obtain various subnuclear components if they can be identified under the microscope and individually isolated under control