has already been reported (Banks, 1965; Goz, 1967). The adenosine triphosphatase has optimum activity at pH 9–11 and pH 6.5. At a low concentration of Ca^{2+} (5mM) the activity at pH 6.5 was 30% of that at pH 9; at a higher concentration of Ca^{2+} (40mM) the activity at pH 6.5 was the same as that at pH 9. These properties are similar to those of myosin adenosine triphosphatase.

When the microsomal fraction was subjected to isopycnic density-gradient centrifugation in sucrose (0.7–1.7M) the particles containing the Ca^{2+} -stimulated adenosine triphosphatase activity could be distinguished from particles containing adenosine monophosphatase activity (presumably derived from the plasma membrane; see Wood, 1967) and from particles containing glucose 6-phosphatase activity. The distribution of the adenosine triphosphatase activity among the density-gradient fractions was, however, similar to that of thiamin pyrophosphatase, an enzyme that has been found in the Golgi apparatus of adrenal medulla by electron-microscopic histochemistry (Holtzman & Dominitz, 1968). These biochemical experiments raise the question whether the Ca^{2+} -stimulated adenosine triphosphatase is a component of the membranes of the Golgi apparatus.

To see whether this adenosine triphosphatase activity could also be demonstrated by a histochemical method, pieces of hamster adrenal medulla (fixed in 2.5% glutaraldehyde) were incubated in the same medium used for the biochemical experiments, i.e. ATP (2mM), calcium nitrate (40mM), tris (50mM) and succinic acid (50mM) titrated to pH 6.5 with sodium hydroxide, with the addition of lead nitrate (2.5mM). After incubation, the tissue was fixed in osmium tetroxide and prepared for electron microscopy. Reaction product was sometimes found in association with the plasma membrane of chromaffin cells and, more often, on the membranes of axons of the splanchnic nerve. However, in agreement with the biochemical findings, the reaction product was found most frequently between the membranes and in the vesicles of the Golgi apparatus.

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Stereochemical and Energetic Evaluation of the Phosphorylation of Histones in Chromatin

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It has been experimentally established (e.g. Stevely & Stocken, 1968) that chromosomal biosynthetic activities involve preliminary phosphorylation of the hydroxyl groups of serine/threonine. I have investigated relevant possible mechanisms using stereochemical and energetic considerations. Numerous correctly proportioned space-filling molecular models of histone–DNA (double helix) were constructed, similar to those previously reported (e.g. Lewin, 1967, 1969*a,b*, 1970*a,b*), in which the hydroxyl groups of serine/threonine were involved in hydrogen-bond donation to the hydrogen-bond acceptor groups already involved in hydrogen-bonded base-pairing (Watson & Crick, 1953; Pauling & Corey, 1956). The histone group's hydrogen-bond capacity to partially weaken the base-pairing hydrogen bonds enhances an increase in their length, thereby allowing the normally rigid double helix of DNA a 'bending latitude' of several degrees per each set of di-trinucleotides (Lewin, 1970*a*). Phosphorylation of the histone's hydroxyl groups has been computed to result in: (1) steric hindrance to the local adherence of histone to the base-pairing sites; (2) local repulsion away from the nearby DNA's phosphate group; (3) overall lowering of the isoelectric point of histone; (4) effective competitive attachment to a sufficiently nearby positively charged histone group initially attached to a DNA's phosphate group (because the newly phosphorylated group is likely to be double negatively charged, in contrast with the single negative charge in a DNA's phosphate group); (5) increased probability of rupture of any sufficiently near inter-histone's carboxylate–ammonium linkages by substitution of an intra-histone phosphate–ammonium linkage accompanied by liberation of the carboxylic acid groups.

All the above factors enhance local turning away of the relevant histone segment from the DNA's wide groove (and even rupturing local ribose phosphate ionic linkage with the positively charged group of the histone, thereby causing total local detachment) and exposing the originally masked area to initiation of biosynthetic processes. It is interesting that, taking into consideration the restrictions on the construction of the double-helix of the B-form of DNA (Lewin, 1969*b*, 1970*b*), the most likely groups to be unmasked in the wide groove are those of guanine, adenine and thymine.

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Some Aspects of the Effect of Deuterium Oxide (D₂O) Substitution for Water (H₂O) on the Helical Stability of Yeast Transfer Ribonucleic Acid

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Substitution of D₂O for H₂O in aqueous media has been shown to be capable of enhancing helical stability in DNA, polyribonucleotides and *Escherichia coli* ribosomes over certain pH ranges and at low ionic strength (Lewin, 1966, 1967a,b; Lewin & Williams, 1971). We have therefore examined spectrophotometrically the effect of the substitution on yeast tRNA prepared by the Holley (1963) method freed from protein contamination.

In solutions containing 0.033 g of tRNA/l and of ionic strength 10^{-3} (prepared and kept under nitrogen free from oxygen and preincubated for 17 h at 3°C) D₂O substitution resulted in an increase of several degrees Celsius in the T_m value ('melting' temperature) in the approximate pH/pD region 5.9, as well as in the alkaline pH region, but without any significant difference in the neutral pH region. Extinction comparisons at both pH/pH and pH/pD scale variations showed increased hypochromicity on D₂O substitution over most pH regions. The T_m differential decreased with increasing ionic strength and disappeared at 10^{-2} ionic strength and above. λ_{max} and λ_{min} varied somewhat with pH and ionic strength; the substitution caused a shift to a lower wavelength by approx. 0.5 nm in λ_{max} ; a parallel shift of approx. 0.5 nm was found in the trough region at about 230 nm. Our low-ionic-strength-D₂O pro-helix results can be collated with those of Mahler, Dutton & Mehrotra (1963), which showed only slight D₂O pro-helix effects with tRNA; they used standard $0.01 \times$ saline-citrate plus 1 mM-Mg^{2+} , which is a neutral pH medium of ionic strength approx. 10^{-2} , conditions that we

found to lower the D₂O pro-helix effect to almost non-significant proportions.

Our results and other relevant observations in the substitution of D₂O on yeast tRNA can be understood within the overall framework (suggested by Lewin, 1971b) that includes the following: (1) increased O-D...O bond strength as compared with that of O-H...O (Nemethy & Scheraga, 1964; Lewin, 1967a,b); (2) decreased differential of the O-D...O bond strength in relation to that of O-H...O with rise in ionic strength (Lewin, 1967a,b); (3) higher structural order of D₂O than that of H₂O (e.g. Nemethy & Scheraga, 1964), which results in enhanced extrusion of water involved in helix formation (Lewin, 1970, 1971a,b).

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Radioactive Labelling of Multiple-Drug-Resistance Factor in *Escherichia coli*

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Freifelder & Freifelder (1968) have described a technique for labelling specifically the DNA of the F-factor and F-merogenotes in *Escherichia coli* after transfer of the plasmids during conjugation. The method has been adapted by Silver & Falkow (1970) and in the present experiments for labelling the DNA of a fertility-de-repressed multiple-drug-resistance factor (R-factor) designated Rldrd19 (Meynell & Datta, 1966).

A strain of *E. coli* unable to utilize exogenous thymine was used as a donor of the de-repressed R-factor and mated with a thymineless recipient strain that is unable to excise u.v.-induced pyrimidine dimers in DNA and that had been previously killed by u.v. irradiation. During the first 30 min of mating [³H]thymine was incorporated into the trichloroacetic acid-insoluble fraction, in excess of that found when the donor strain, into which the

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