- Lewin, S. (1969a). In *Protides of the Biological Fluids*, vol. 16, p. 51. Oxford: Pergamon Press Ltd.
- Lewin, S. (1969b). *J. theor. Biol.* **23**, 279.
- Lewin, S. (1970a). *J. theor. Biol.* **24**, 481.
- Lewin, S. (1970b). *J. theor. Biol.* **29**, 1.
- Pauling, L. & Corey, R. B. (1956). *Archs Biochem. Biophys.* **65**, 164.
- Stevely, W. S. & Stocken, L. A. (1968). *Biochem. J.* **110**, 187.
- Watson, J. D. & Crick, F. H. C. (1953). *Nature, Lond.*, **171**, 737.

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 × saline-citrate plus 1 mM-Mg²⁺, 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).

- Holley, R. W. (1963). *Biochem. biophys. Res. Commun.* **10**, 186.
- Lewin, S. (1966). *Archs Biochem. Biophys.* **115**, 62.
- Lewin, S. (1967a). *J. theor. Biol.* **17**, 181.
- Lewin, S. (1967b). *Studia biophys.* **4**, 29.
- Lewin, S. (1970). *J. theor. Biol.* **26**, 481.
- Lewin, S. (1971a). *Biochem. J.* **122**, 20P.
- Lewin, S. (1971b). *Displacement of Water in Biochemical Reactions*. New York and London: Academic Press (in the Press).
- Lewin, S. & Williams, B. A. (1971). *Archs Biochem. Biophys.* (in the Press).
- Mahler, H. R., Dutton, G. & Mehrotra, B. D. (1963). *Biochim. biophys. Acta*, **68**, 199.
- Nemethy, G. & Scheraga, H. A. (1964). *J. chem. Phys.* **41**, 680.

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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R-factor had not been introduced, was incubated under the same conditions with the irradiated recipient. Since the donor strain is unable to utilize thymine and the chromosomal DNA of the recipient is unable to replicate, the incorporation excess is ascribed to the synthesis of the DNA of the R-factor after transfer to recipient.

Although incorporation of [^3H]thymine during incubation of the irradiated recipient strain with the donor strain lacking the R-factor was less than that observed during transfer of the R-factor, some incorporation did occur. In contrast, much less incorporation occurred when the two strains were incubated separately with [^3H]thymine. It was found that incubation of the irradiated recipient strain with [^3H]thymine gave rise to [^3H]dTMP in the medium. Moreover, this material, when added to a culture of the donor strain, was incorporated efficiently in trichloroacetic acid-insoluble material. It is believed that the conversion of thymine into dTMP followed by incorporation of this compound into the DNA of the donor bacterial accounts for the incorporation of radioactivity into DNA in these experiments in the absence of transfer of R-factor.

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Freifelder, D. & Freifelder, D. R. (1968). *J. molec. Biol.* **32**, 15.

Meynell, E. & Datta, N. (1966). *Genet. Res.* **7**, 134.

Silver, R. P. & Flakow, S. (1970). *J. Bact.* **104**, 331.

Genetic Expression of Sex-Factor after Transfer into Irradiated Cells of *Escherichia coli* *uvrA*⁻

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It has been demonstrated that, after transfer of the episome into cells of *Escherichia coli* previously killed by u.v. irradiation, synthesis of DNA of the F-merogenote (Freifelder & Freifelder, 1968) and of the R-factor Rldrd19 (Silver & Falkow, 1970; Barker, Hardman & Twose, 1971) takes place even though chromosomal DNA synthesis is suppressed in the recipient cells. Experiments have shown (N. Hardman & T. M. Twose, unpublished work) that cells subjected to doses of approx. 7000–8000 erg/mm² are non-viable and exhibit no measurable synthesis of protein or increase in cell mass. The present experiments are concerned with the ability of irradiated cells to support the normal functions of sex-factors.

The F'-lac episome was transferred to a suitable mutant of *E. coli*, which was then subjected to appropriate conditions for induction of the lac operon. It was found that induction of β -galactosidase activity was completely blocked by u.v. irradiation of the recipient with a dose of 7400 erg/mm² before introduction of the episome. At lower doses partial inhibition of the induction was observed. Analogous experiments to investigate the possible formation of enzymes following transfer of R-factor present special difficulties and have not yet been carried out.

The presence of sex-factor in a viable cell confers the ability to act as donor in conjugation with a second recipient. U.v. irradiation of recipient cells with a dose of 7400 erg/mm² before acceptance of either of the episomes Rldrd19 and F'-lac completely prevented the ability subsequently to act as donor in conjugation. With lower doses of irradiation partial inhibition was observed. It is concluded that the episomes do enter the u.v.-irradiated cells, since synthesis of DNA takes place after incubation of mixed cultures under conditions suitable for conjugation (Barker *et al.* 1971), but that expression of episomal genes probably does not take place.

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Barker, G. R., Hardman, N. & Twose, T. M. (1971). *Biochem. J.* **122**, 48P.

Freifelder, D. & Freifelder, D. R. (1968). *J. molec. Biol.* **32**, 15.

Silver, R. P. & Falkow, S. (1970). *J. Bact.* **104**, 331.

Protein Content of Myelin

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Determination of the protein content of membrane preparations is difficult because of the large amounts of lipids not readily dissociable from the proteins. Systematic study of the problem has been made by several methods to find a valid procedure for comparison of protein contents of central-nervous-system myelins from different animal species and of membranes from different tissues.

Removal of lipids by a variety of solvents from freeze-dried rat central-nervous-system myelin and gravimetric measurement of the protein residue yields results dependent on solvent (Winder & Gent, 1970a), but suggests that protein does not exceed 20% (w/w).

By taking the nitrogen content of myelin to be