involved in histone-DNA associations. The types of bond include ionic linkages, hydrogen bonds and hydrophobic bonds.

(1) Ionic linkages. The ionic linkages between the histone guanidinium (arginine) and ϵ -ammonium (lysine) and the phosphate groups of DNA are the strongest; this group association has been computed to result in decreased local hydration (Lewin, 1970a,b).

(2) Hydrogen bonds. Hydrogen-bonding between the histones and the DNA can involve both the former and the latter in hydrogen donation and in hydrogen acceptance. Because of the positively charged state of the histones they will tend to act primarily as hydrogen donors, though partial hydrogen-acceptance activity has to be allowed for.

(2a) Histone hydrogen donation to DNA. This takes place mainly in the wide groove of the DNA double helix and involves the hydroxyl groups of serine and threonine in donation to (i) the ternary N groups, (ii) the C=O groups involved already in Watson-Crick hydrogen-bonding, and (iii) to the lone-pairs of electrons of the amino groups already involved in Watson-Crick hydrogen-bonding.

(2b) Histone hydrogen acceptance. The Watson-Crick hydrogen-bonded amino groups can act as hydrogen donors to the C=O groups and to the carboxylate groups of the glutamate or aspartate residues of the polypeptide chains.

The overall hydrogen-bonding has been computed to result in partial weakening of the Watson-Crick hydrogen bonds. This effect is counterbalanced by the binding strength of the paired histones (Lewin, 1970c), which can be ionically linked by ϵ -ammonium carboxylate groups; the latter binding bridges the two DNA strands and thus supports double-strandedness. The overall result has been computed to be the 'bending allowance' as a result of which the DNA double helix is less rigid on histone attachment; i.e. the structure tends towards an overall less helical double-strandedness.

(3) Hydrophobic bonds. Weak hydrophobic associations can take place between hydrophobic groups of histones and hydrophobic loci on the DNA (such as the methyl group of thymine).

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Kinetic Studies with Pig Brain Aldehyde Dehydrogenase

By R. J. S. DUNCAN and K. F. TIPTON. (*Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, U.K.*)

The aldehyde dehydrogenase of brain is important in the oxidation of aldehydes derived from the catechol- and indolethyl-amines.

Aldehydes from 3,5-dihydroxyphenethylamine and tyramine are predominantly oxidized, whereas those from adrenaline and octopamine are reduced in brain (Breese, Chase & Kopin, 1969; Rutledge & Jonason, 1967).

The specificity and kinetics of an aldehyde dehydrogenase purified from acetone-dried powders of whole pig brain have been investigated by using a fluorimetric assay dependent on NADH production. The pH optimum of the reaction is about 9.8, but at this pH certain aldehydes react with NAD⁺ (Burton & Kaplan, 1953), so all assays were carried out at pH 7.2, at which the interfering reactions are not detectable.

Michaelis constants for a series of aliphatic aldehydes decrease with increasing chain length and range from 10^{-4} M for formaldehyde to 2.3×10^{-7} M for decylaldehyde. Indolylacetaldehyde is a substrate, as is the aldehyde from tyramine, both with Michaelis constants less than 2×10^{-7} M. These results are in broad agreement with those of Erwin & Deitrich (1966). Benzaldehyde and *p*-nitrobenzaldehyde are also substrates.

Double-reciprocal plots obtained by varying propionaldehyde concentration at a series of fixed NAD⁺ concentrations, and vice versa, are linear in the concentration ranges $40\text{--}7\text{ }\mu\text{M}$ for NAD⁺ and $2.95\text{--}0.3\text{ }\mu\text{M}$ for aldehyde, and meet at a point close to the reciprocal-of-substrate-concentration axis. Secondary plots are also linear.

Reciprocal plots obtained in the presence of a series of fixed NADH concentrations show that NADH is a competitive inhibitor with respect to NAD⁺ and a mixed inhibitor with respect to propionaldehyde, except at high concentrations of NAD⁺, when NADH is not inhibitory. The acid products are not inhibitory, and the reaction is irreversible. These results are consistent with a catalytic mechanism in which NAD⁺ is bound first, followed by the aldehyde. After the reaction has taken place the acid is postulated to leave the enzyme irreversibly. Such a mechanism has been proposed for certain aldehyde dehydrogenases by Jakoby (1963). The monoamine oxidase inhibitors 1-methyl-2-phenethylhydrazine (J.B.516) and Phenethylhydrazine sulphate (nardil) both inhibit aldehyde dehydrogenase in a complex time-

dependent fashion, in addition to forming hydrazones with the aldehyde substrates. Iproniazid (*N*-isonicotinoyl-*N*-isopropylhydrazine) is not inhibitory.

Ethanol in concentrations up to 500mM is not inhibitory towards pig brain aldehyde dehydrogenase, although the thiol reagents iodoacetic acid and iodoacetamide are inhibitory.

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Subcellular Distribution of Rat Liver L-Alanine-Glyoxylate Aminotransferase

By K. SNELL, E. V. ROWSELL and J. A. CARNIE.
(Department of Biochemistry, University of Manchester Institute of Science and Technology, Manchester M60 1QD, U.K.)

Rat liver was homogenized in 0.4M-sucrose at 0–5°C. Centrifugation at 7.5×10^6 g-min gave supernatants with 88–97% of lactate dehydrogenase (values are percentages of activity in original homogenate), <5% of glutamate dehydrogenase, glucose 6-phosphatase, uricase or acid phosphatase and 19–25% of L-alanine-glyoxylate aminotransferase (assayed as described by Rowsell, Snell, Carnie & Al-Tai, 1969). Of the particle-marker enzymes (as indicated above) more than 95% were recovered in the sedimented particles with 75–82% of the aminotransferase.

For subfractionation of the particles differential centrifugation procedures were then applied, based on methods used by Appelmans, Wattiaux & de Duve (1955). Preparations of nuclei contained 80–90% of the DNA, 8–10% of the glutamate dehydrogenase or acid phosphatase, 5% of the uricase or glucose 6-phosphatase, 4% of the lactate dehydrogenase and 10% of the aminotransferase. Microsomal preparations contained 48% of the glucose 6-phosphatase, 56% of the arylsulphatase C, 11% of the lactate dehydrogenase, no measurable glutamate dehydrogenase and 7% of the aminotransferase. Crude mitochondrial preparations contained 90% of the glutamate dehydrogenase, 50% of the acid phosphatase, 40% of the uricase, 10% of the lactate dehydrogenase and 75% of the

aminotransferase. It was concluded that one could narrow the field for investigation to mitochondria, lysosomes and peroxisomes.

Lysosomes were prepared by flotation after intraperitoneal injection of rats with Triton WR1339 (Leighton *et al.* 1968). Of the acid phosphatase, 26–30% was recovered with <3% of the glutamate dehydrogenase, uricase or aminotransferase.

For application to a density gradient a particle fraction was prepared by differential centrifugation, designed to discard nuclei and microsomes. In a representative experiment percentage recoveries of aminotransferase, glutamate dehydrogenase, rhodanese, lactate dehydrogenase, acid phosphatase and uricase were respectively 70, 65, 68, 6, 70 and 82. A resuspension of these particles in 48% (w/v) sucrose (density 1.18g/ml) was layered over a discontinuous sucrose gradient (1.18–1.25g/ml) and after centrifugation at 2.9×10^6 g-min tube contents were recovered as four separate density-range fractions. In the topmost fraction percentage recoveries (compared with activities in the layered particles) for aminotransferase, rhodanese, uricase and acid phosphatase were respectively 73, 75, 12 and 32; corresponding values for the bottom fraction were 2, 2, 42 and 15. Altogether the profile for the aminotransferase closely matched that of rhodanese but clearly differed from those observed for uricase or acid phosphatase.

We conclude that a large part, at least, of the particulate alanine-glyoxylate aminotransferase is mitochondrial.

The aminotransferases in the cytosol and in solubilized particles are different; K_m values for glyoxylate are respectively 1.2 and 0.24 mM and pH-activity curves showed marked differences.

Triton WR1339 was a gift from Hilton Davis Chemicals Ltd., Newcastle upon Tyne, U.K.

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A Prostaglandin Isomerase in Cat Plasma

By R. L. JONES (introduced by E. W. HORTON).
(Department of Pharmacology, University of Edinburgh, Edinburgh EH8 9JZ, U.K.)

Prostaglandin A_1 [15-hydroxy-9-oxoprost-10,13-(*trans*)-dienoic acid] is a natriuretic agent in the dog