glucose 6-phosphatase (endoplasmic-reticulum marker) activity. By using male rats starved overnight, a variety of conditions have been tried in attempts to improve the purity of the plasma-membrane vesicles separated by zonal centrifugation. Best results have been obtained by using a novel technique involving exposure of sonicated microsomes for 20 min at pH 6.8 to Pb^{2+} ions at a concentration of about 0.5 mM. No substrate need be added (cf. Leskes & Siekevitz, 1969). Sonication is then repeated. The Pb^{2+} ions may bind specifically to any glucose 6-phosphatase-containing membranes, thereby increasing their equilibrium density; the second sonication may help avoid aggregation of the vesicles due to the presence of Pb^{2+} . Subsequent centrifugation of a fraction prepared in this way, in a BXIV zonal rotor at 47000 rev./min for 4 h without bivalent cations, gives a sharp peak of glucose 6-phosphatase activity with a median density of 1.23; this peak may contain fully half of the 5'-nucleotidase activity and over 95% of the microsomal protein. There remains a second peak of 5'-nucleotidase at density 1.14, now without detectable glucose 6-phosphatase activity. This peak of plasma-membrane vesicles shows better than tenfold enrichment in 5'-nucleotidase specific activity as compared with homogenate.

As an adjunct to separatory work, pelleted membrane-containing fractions are being examined for 5'-nucleotidase and glucose 6-phosphatase by a technique analogous to that of Marchesi & Palade (1967). The resuspended material is incubated with buffered substrate, usually for 15 min, in the presence of Pb^{2+} at a non-inhibitory concentration, 3.8 mM. Finally the reaction is stopped by adding fixative, buffered osmium tetroxide. Centrifugation gives a pellet from which ultrathin sections are cut after dehydration and embedding.

Electron micrographs show fair morphological preservation and selectivity in the location of lead phosphate deposits within mixed populations of vesicles. With an enriched plasma-membrane fraction obtained by zonal centrifugation as outlined above virtually all the vesicles stained for 5'-nucleotidase and none for glucose 6-phosphatase, in agreement with the biochemical results.

Thanks are expressed to Mr M. Dobrota and Mr R. H. Hinton for help and advice. Financial support came from the British Empire Cancer Campaign for Research, the Science Research Council and the Tunstall Laboratory of Shell Research Ltd.

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The Circular Dichroism of Deoxyribonucleic Acid and Deoxyribonucleohistone and the Effect of Thermal Denaturation

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Circular-dichroic spectra of DNH,* DNA and samples of DNH partially depleted of histone in NaCl solutions were measured. In addition, the changes in the circular-dichroic spectra obtained on thermal denaturation of the above samples are compared with the changes observed in extinction.

In DNH the protein circular-dichroic spectrum is superimposed on that of the DNA, giving a maximum at 227 nm, shoulders at 245 and 220 nm and a trough at 210 nm. Protein difference curves were calculated and an attempt was made to analyse the results in terms of the secondary structure in the histone fractions when they are bound to DNA.

The magnitude of the maximum at 277 nm in the circular-dichroic spectra, $\Delta\epsilon_{277}$, was smaller in DNH than in DNA; in 2M-NaCl, however, where the histone is completely dissociated from the DNA, the values were identical. $\Delta\epsilon_{277}$ decreased when the F1 fraction had been removed in 0.7M-NaCl, but increased to a value comparable with that of DNA in DNH samples that had been partially depleted of histone in 1.0M-NaCl. The difference in $\Delta\epsilon_{277}$ between DNH and DNA largely disappeared at alkaline pH and in 6M-urea the dichroism of DNH exceeded that of DNA. The intrinsic viscosities of DNH in these solvents were, however, very similar to the value for native DNH (Henson & Walker, 1969). This implies that the differences in $\Delta\epsilon_{277}$ between DNH and DNA are not directly related to supercoiling of the nucleohistone, as has been suggested by Tuan & Bonner (1969). The effect of thermal denaturation on $\Delta\epsilon_{277}$ was measured and compared with extinction (E_{260}) thermal denaturation curves. $\Delta\epsilon_{277}$ for DNA increased gradually from room temperature to 56°C, where the E_{260} remained constant, and then decreased as the E_{260} increased. The E_{260} 'melting' curve for DNA was monophasic, as was also that of the DNH sample depleted in 0.7M-NaCl. The $\Delta\epsilon_{277}$ denaturation curve of this sample was also like that of DNA. The curves for DNH and the sample depleted in 0.7M-NaCl were different, however, in that $\Delta\epsilon_{277}$ continued to increase sharply after the E_{260} had started to increase and reached values far higher than those achieved by DNA. The

* Abbreviation: DNH, deoxyribonucleohistone.

temperature at which $\Delta\epsilon_{277}$ finally started to fall coincided with the beginning of the second step in the biphasic E_{260} 'melting' curve (Henson & Walker, 1969).

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Molecular Models for Macromolecules: a New Concept for Teaching and Research

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X-ray crystallography is providing a rapidly increasing stream of accurate data for the construction of macromolecules of biological origin and simultaneously the chemist and the kinetic enzymologist are locating and studying active sites and clefts in such molecules. Models built so far have been based on skeletal or space-filled units, each of which represents an atom, but valuable as these are to those interested in a particular model or structure they are of little or no use for general purposes. The disadvantages of this type of model are the extreme cost, the tremendous amount of building time involved, the relatively large amount of superstructure necessary to support the model such that the model itself is often lost within its superstructure, and the size and weight, which make transport a problem such that the model often cannot easily be rotated even on its own axis.

For many workers and certainly for all students the amount of data inherent in such models is self-defeating as time is not available for the study of such models. We have therefore designed a new type of model with the intention of overcoming all these problems. The models to be described are extremely light in weight, flexible, free of visible superstructure and all have been built by students in 2-3 days (Smith & Smith, 1969; Smith, 1969; Smith, Smith & Roberts, 1970). The model 'bits' correspond to groups of atoms such as the various R groups of amino acids, nucleic acid bases etc., and are size- and colour-coded to draw out immediate similarities and differences in an easily recognizable manner. 'Backbones' of the proteins and nucleic acids are constructed from flexible tubing incorporating repeating units representing the amino acid or sugar phosphate monomer. Through the tube runs a plastic-coated wire, which helps to retain the model shape built from the X-ray data, and having built a section of 'backbone' the different R groups are inserted in holes placed circumferentially on each monomer unit to indicate the general direction in which the particular group points. The whole model is supported on a frame. The final

models readily show tertiary structures, including helical and non-helical regions, substrate 'pockets', active sites, codon-anticodon interactions etc., and the direct student participation in model building serves as a valuable aid to learning. Models of myoglobin, lysozyme, ribonuclease S, DNA and various tRNA species will be shown.

Because the 'backbone' can be readily cut and rejoined it is possible to demonstrate a variety of other chemical and genetic reactions. Thus a wall-board to illustrate the various enzymic and chemical digests of a protein will illustrate the current techniques useful in determining primary structure, and similar boards can illustrate genetic cross-reactions, transcription etc.

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Transport Pathway for Glycine Betaine in Rat Kidney Cortex *in vitro*

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Inhibition studies established that betaine (NNN-trimethylglycine) shares a transport pathway in the hamster small intestine in common with N-methylglycine (sarcosine) and NN-dimethylglycine. L-Proline and L-hydroxyproline had a greater affinity for the betaine carrier than for the neutral amino acid carrier (Hagihira, Wilson & Lin, 1962).

Uptake of ^{14}C -labelled compounds was studied with kidney-cortex slices from female albino Wistar rats. After washing in appropriate buffer solution (Robinson, 1949) kidney slices were incubated with test compound in buffer at 37°C in O_2 for 30 min. Uptake was related to the intracellular fluid and extracellular fluid of rat kidney cortex as determined with inulin (Rosenberg, Downing & Segal, 1962).

Recovery of ^{14}C -labelled betaine was >95% from the incubation media and 95% from the tissues. Hence there were negligible losses due to oxidation to CO_2 and incorporation into protein.

Betaine accumulated in kidney-cortex slices to give a concentration ratio (c.p.s. per ml of intracellular fluid/c.p.s. per ml of extracellular fluid) of 2.36 ± 0.09 and therefore did not occur by passive (physical) diffusion. 2,4-Dinitrophenol inhibited

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