albumin region after antigen addition was taken as evidence for the existence of carrier proteins; a smaller extent, or disappearance, of bands in the γ -globulin region accompanied by the formation of new bands elsewhere was considered evidence for antibody reactivity.

These principles were applied to insulin reactivity with human sera. Insulin addition to the sera of diabetic and normal patients resulted in a differential. In the former both considerable weakening of the bands in the overall albumin region and decrease in their number in the presence of γ -globulins, as well as the development of new bands, were displayed; in normal sera only considerable weakening of the bands in the overall albumin region was noted, but no weakening of the γ -globulin bands was observed. These findings are compatible with the existence of insulin carrier proteins in both normal and diabetic patients, and with the interpretation that some diabetic patients possess insulin antibodies.

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Countercurrent Studies of Porcine Liver Porphyrins

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During investigations of the ether-soluble porphyrin of various tissues (Smith, Belcher & Mahler, 1969) a group of porphyrins was found in extracts from fresh pig liver that differed from those present in bile, blood, meconium and bone marrow. Examination of countercurrent fractions derived from these extracts showed the presence of coproporphyrin and protoporphyrin together with three porphyrin fractions with Soret maxima at 420, 415 and 423nm in 5% HCl (described below as the 'S420', 'S415' and 'S423' porphyrins).

Six livers from pigs killed at the abattoir and three livers from pigs that had been starved for 2 days and then anaesthetized were examined. The 'S415' and 'S420' fractions were found in seven of the livers. In two of the abattoir livers, which were not fresh, only small amounts of the 'S420' fraction was found together with 2-carboxyl and 3-carboxyl porphyrins of the type found in bile (Smith *et al.*

1969). The three livers from starved anaesthetized pigs and one of the abattoir pig livers showed all three atypical fractions. The countercurrent tube positions of the fractions with the system described by French & Thonger (1966) were as follows: 'S420', tubes 19–32; 'S415', tubes 34–48; 'S423', tubes 69–81.

These porphyrins, which were characterized by a 'smoky' red-brown fluorescence, were stable for at least 56h in 5% HCl, and exhibited sharp Soret maxima typical of porphyrins. Sufficient 'S415' and 'S420' material was collected to demonstrate that these porphyrin fractions had four-banded aetioporphyria-type spectra in visible light.

The 'S415' and 'S420' fractions both separated into two bands, occupying 2-carboxyl and 3-carboxyl positions on lutidine chromatograms, the separated bands having the same Soret wavelength as the parent fraction.

The 'S423' fraction ran as a single band in the 2-carboxyl position.

Preliminary experiments indicate that the 'S415', 'S420' and 'S423' fractions are not protein-bound conjugates and that it is not possible to incorporate zinc into the nucleus.

It was also noted that the coproporphyrin/protoporphyrin ratio in the extracts from the abattoir pig livers was always less than 1, whereas the extracts from starved anaesthetized pigs gave ratios greater than 1.

The 'S420' fraction was also found in a human liver removed at autopsy from a road-accident casualty.

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Two Techniques for the Study of Hepatic Membrane-Containing Fractions

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For membrane fragments as found in liver homogenates, it would be useful to have techniques for separating plasma-membrane vesicles from endoplasmic-reticulum vesicles and for ascertaining the location of marker enzymes within a population of vesicles. Exploratory work is now reported.

Earlier work on the zonal centrifugation of postlysosomal supernatant (El-Aaser *et al.* 1966; Hinton *et al.* 1967) achieved a partial separation of 5'-nucleotidase (plasma-membrane marker) from

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.

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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.