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.

Thanks are due to Mr T. Davey, B.Sc., for technical assistance, to Dr A. A. G. Lewis and Dr R. R. de-Mowbray for helpful discussions and involvement in the insulin application, to Dr G. Carnachan and Novo Industri, Copenhagen, Denmark for the supply of pure insulins and to Dr H. Caplin for discussions and the supply of sera.

Lewin, S. (1969). *Postgrad. med. J.* **45**, 729.

Lewin, S. (1970). *Biochem. J.* **117**, 41P.

Countercurrent Studies of Porcine Liver Porphyrins

By S. G. SMITH, R. V. BELCHER and R. MAHLER.
(*Tenovus Institute for Cancer Research, Department of Metabolic Medicine, The Welsh National School of Medicine, Heath, Cardiff CF4 4XX, U.K.*)

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.

We thank the Abbotshill Trust for generous financial support.

French, J. M. & Thonger, E. (1966). *Clin. Sci.* **31**, 337.

Smith, S. G., Belcher, R. V. & Mahler, R. (1969). *Biochem. J.* **114**, 88P.

Two Techniques for the Study of Hepatic Membrane-Containing Fractions

By A. A. EL-AASER, K. A. NORRIS and E. REID.
(*Wolfson Bioanalytical Centre, University of Surrey, Guildford, Surrey, U.K.*)

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