failure. The average discrepancy between the two methods in 12 cases was 50 ml. A low value of 27.5 ml/kg was obtained in one normal subject using ^{125}I HSA compared with a value of 50 ml/kg using $^{99\text{m}}\text{Tc}$ HSA.

The 30 μCi dose of $^{99\text{m}}\text{Tc}$ HSA used produces a smaller radiation dose to the whole body including the blood and to the thyroid and would be suitable for sequential plasma volume measurements and measurements in pregnant women.

Reference

Beazley, J. M., Matthews, J. C., Leaver, E. P., and Smith, G. M. (1968). Placental localisation using $^{99\text{m}}\text{Tc}$ labelled serum albumin. *J. Obstet. Gynaec. Brit. Cwlth*, 75, 470.

Effects of Natural Oestrogens on Blood Clotting—a Double-Blind Cross-over Trial

L. POLLER, J. M. THOMSON, AND J. COOPE (Department of Haematology, Withington Hospital, Manchester) A double-blind cross-over study on the effect of natural oestrogen on blood clotting and platelet aggregation has been performed on a group of 30 women. It had been claimed that natural oestrogen did not have the harmful effect of synthetic oestrogen on blood coagulation.

The women were randomly divided into two groups, the first group received natural oestrogen (Premarin) for three months and then for a further three months received a placebo, whereas the second group received the placebo first.

Significant acceleration of the prothrombin time and factor VII and X assays occurred with natural oestrogen administration but the intrinsic tests were not accelerated at the three-month stage. The changes are similar to those which occur after three months' synthetic oestrogen/progestogen oral contraceptive administration.

Endocrine Assessment of Threatened Abortion

R. E. REWELL (Department of Pathology, The United Liverpool Hospitals, Liverpool) At the end of pregnancy human chorionic gonadotrophin (HCG) and human per centile lactogen (HPL) in the blood fall sharply. Since the half-life in the circulation of HPL is much shorter than that of HCG, it is the former that would be expected to be more useful in assessing whether or not a threatened abortion will in fact take place. Several small series have confirmed this, eg, Genazani *et al* (1969), though their

patients threatened to abort for much longer than happens in Liverpool before this became inevitable.

Blood levels of HPL and HCG were measured on admission to hospital for threatened abortion. Women admitted for therapeutic abortion were used as controls. A highly significant difference was found between the mean levels of both hormones for women who in fact aborted and for those whose pregnancy continued: the differences between those whose pregnancy continued and the controls were not significant. Further, it is possible to calculate the changes of abortion taking place from the level of either hormone, but unexpectedly HCG gives a more accurate figure (analysis of results by Mr M. C. K. Tweedie). Using both levels a still more accurate assessment emerges.

This work was partly supported by a Research Grant from the former United Liverpool Hospitals.

Reference

Genazani, A. R., Aubert, M. L., Casolif M., Fioretti, P., and Felber, J.-P. (1969). *Lancet*, 2, 1385-1387.

An EM Study of Human Thymus

W. JONES WILLIAMS, D. L. JONES, AND K. THOMAS (Pathology Department, Welsh National School of Medicine, Cardiff) In a fine structure study of human, normal, hyperplastic, and tumour thymus, we demonstrate similar cell types though they are present in varying proportions. The mixed thymoma differed only in showing increased numbers of mitosis and predominance of large lymphocytes.

We describe, for the first time, in postnatal human thymus, the presence of nuclear pockets in lymphocyte nuclei. We also found that both lymphocytes and epithelial cell nuclei contain nuclear bodies. It is likely that both are features of actively metabolic cells.

Three varieties of lymphocytes are present—small, large, and 'activated'. In addition, occasional plasma cells suggest the presence of B type lymphocytes.

We consider that epithelial cells have a functional as well as a structural rôle. There appears to be a continuity between mucoprotein-containing epithelial cell cytoplasm and extracellular material, which is taken up by macrophages, all in close contact with lymphocytes.

Some mucoprotein-containing macrophages are converted into foam cells. These features are most prominent in the

thymoma. The possible significance of these features in relation to humoral epithelial/lymphocyte interaction will be discussed.

Gaucher's Disease with Biclinal Gammopathy

MORVEN MACDONALD, MARGARET MCCATHIE, M. J. W. FAED, R. PRINGLE, H. B. GOODALL, J. S. BECK, G. R. TUDHOPE, P. E. G. MITCHELL, A. J. J. WOOD, W. GUTHRIE, AND D. SHAW (Pathology Department, Ninewells Hospital, Dundee) The association between Gaucher's disease and monoclonal gammopathy is well documented (Pratt *et al*, 1968). The present case appears to be the first in which Gaucher's disease is associated with biclinal gammopathy. A woman aged 48 complained mainly of tiredness and was found to have pinguiculae, splenomegaly, raised serum acid phosphatase, and low β -glucosidase activity in cultured skin fibroblasts. The blood showed pancytopenia with dimorphic red cells. The marrow showed typical Gaucher cells and atypical degenerate foamy forms; excess of two types of plasma cells, large and small; transitional megaloblasts and giant metamyelocytes. Serum proteins (9 g per 100 ml) included two abnormal bands on electrophoresis, one IgC and one IgA.

Anaemia was partly corrected by oral iron and folic acid, but hypersplenism persisted, and thrombocytopenia and leucopenia necessitated splenectomy. Two weeks after operation the serum IgG was at the preoperative level but IgA was halved.

With fluorescent anti-IgG and anti-IgA sera two populations of plasma cells were identified—one predominantly in marrow, producing IgG; the other in marrow and spleen, producing IgA.

Galactocerebroside, one of the lipids in reticulo-endothelial cells in Gaucher's disease, is known to be strongly antigenic because of its ability to produce plasma cell proliferation when injected into mice. Could leakage of this or other lipids from the foamy, degenerate Gaucher cells have stimulated the production of the two clones of plasma cells in this patient?

Reference

Pratt, P. W., *et al* (1968). *Blood*, 31, 633.

Dissociation of Carboxyhaemoglobin in the Cadaver

H. M. RICE (Department of Pathology, General Hospital, Nottingham) Many

pathologists have experience of unexpectedly low carboxyhaemoglobin saturation levels in fatal carbon-monoxide poisoning. The textbooks give 60% as the minimum lethal concentration for healthy subjects. Why then should apparently fit persons, below the age of 70, die with saturation levels below 50%? A series of 300 included 24 such cases, with values from 40 to 49% (palladium chloride analysis accurate to about 5%).

An explanation was provided by two cases in which subclavian blood gave unbelievably low readings, but femoral samples showed higher saturation levels. Vigorous artificial respiration had been given to both. An infant from a fire was also given intravenous saline at hospital where artificial respiration was continued for an hour. Subclavian blood showed only 15% saturation and femoral blood 31%, though hypostasis was characteristically pink. In a male suicide of 57 the values were 32 to 52%, respectively.

Enquiries revealed that the local ambulance services give artificial respiration, with oxygen or Entonox, to all persons found dead, unless obviously inappropriate.

Since blood with a high carboxyhaemoglobin content does not clot, vigorous artificial respiration moves blood into and out of the lungs of the cadaver. Dissociation of carboxyhaemoglobin occurs, assisted by high oxygen concentrations in the respired gas. Some mixing of the blood in veins near the lungs results, lowering their carboxyhaemoglobin content. Femoral blood from the thigh is much less affected. Circulation of the infant's blood was probably assisted by the intravenous infusion and by the prolongation of artificial respiration.

When artificial respiration has been tried, carboxyhaemoglobin saturation levels will be falsely low in blood from sites near the lungs (heart, subclavian vein). Blood for analysis should be taken from more distant sites such as the thigh.

Cryofibrinogenaemia and Activation of the Coagulation/Lysis Systems in Accidental Hypothermia of the Elderly

H. B. GOODALL, A. S. TODD, D. MACLEAN, R. HENDERSON, AND J. F. KING (*Department of Pathology, Ninewells Hospital, Dundee*) This work investigates the paradox of ischaemic lesions in hypothermia despite reduced demands for oxygen when tissues are cooled. Tests for cold-precipitable proteins (cryoproteins) and activation

of coagulation/fibrinolysis systems were made in 33 elderly patients with accidental hypothermia.

Cryofibrinogen precipitates in plasma when cooled, redissolves on warming, and is thereafter clottable with thrombin, but a simple screening procedure is to separate heparinized plasma at 37°C, chill in a Wintrobe tube at 4°C for 48 hours, spin at 3000 rev/min for 30 minutes, and read the percentage of precipitate—the plasma (or heparin) cryocrit. Of the 33 hypothermic patients, 15 were in the normal range (up to 1.5%), 5 equivocal (1.5 to 2.0%), and 13 raised (over 2.0%). Serum cryocrits, reflecting cryoglobulin, were all normal; thus raised plasma cryocrits indicate excess cryofibrinogen. The cryofibrinogen level appears to be related to prognosis. Only 1 of 20 patients with normal or equivocal levels died before normal temperature was restored, but 6 of 13 patients with raised plasma cryocrit died hypothermic. The mean plasma cryocrit for those surviving hypothermia (2.8%) is significantly lower than for those dying hypothermic (9.9%)— $P < 0.025$. The long-term overall mortality is 30% with normal cryocrit, 40% with equivocal cryocrit, and 62% with raised cryocrit. These findings are probably related to serious underlying disorders, especially infections. Hyperviscosity from gelling of cryofibrinogen at low temperature may impair the microcirculation. In addition, many patients show evidence of activation of the coagulation/lysis systems. Damage to vascular endothelium may play a part in this complex process.

Molecular Weight Markers for Polyacrylamide Gel Electrophoresis

J. T. WHICHER AND D. BAREFORD (*Department of Chemical Pathology, Bristol Royal Infirmary*) Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis is a method widely used for determining the molecular weight of proteins. When a protein combines with SDS, a negatively charged micelle is formed, the electrophoretic mobility of which on polyacrylamide gel depends entirely upon the molecular size of the protein.

This method of separating proteins according to their molecular weight has been useful in determining whether proteinuria is glomerular, tubular or due to Bence Jones protein.

The successful use of the method

depends on the availability of suitable purified proteins of known molecular weight to act as markers.

A method is described of preparing proteins with a known molecular weight for use as markers in this electrophoretic system. Polymers of light chains extracted from the urine of myeloma patients were prepared by forming peptide bonds with carbodiimides. The polymers spanning molecular weight range 22 500–112 500 are stable in pH 5.0 buffer for several months at 4°C and may also be stored deep frozen. The advantage of such molecular weight marker is that it obviates the need for a number of purified proteins of different molecular weight. The starting material is widely available and the polymers are easily made and store well.

Leucocyte Enzyme Assay as a Means of Diagnosis

BRENDA E. RYMAN (*Department of Biochemistry, Charing Cross Hospital Medical School, London W6 8RF*) In the last 20 years the use of leucocyte enzyme assay to aid diagnosis has become increasingly important. Possibly the greatest disadvantage of this technique is that as yet, it does not form part of the normal armamentarium of hospital laboratories. Various methods of isolating leucocytes are available; it will be appreciated, however, that different methods give preparations containing varying proportions of the individual cells that make up the so-called 'white cell' population. Such considerations may influence the quantitative results obtained.

In the field of the hereditary metabolic diseases the use of leucocytes in some cases obviates the need for surgical biopsies under anaesthesia. It must, however, be stressed that in certain genetic disorders such as white cell studies are not diagnostically useful and, in fact, may give controversial results. Several groups of genetically inherited disorders are amenable to diagnosis via the use of employing leucocytes, and these include some of the disorders associated with amino acid metabolism (eg, maple syrup urine diseases, hypervalinaemia, argininosuccinic aciduria), carbohydrate metabolism (eg, galactosaemia, glycogen storage diseases types II, III, IV, and VI, fructose 1,6-diphosphatase deficiency, mannoseidosis, fucosidosis) and lipid metabolism (Gaucher's, Niemann Pick's, Tay Sachs,