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,

and Fabry's diseases, as well as meta-chromatic leucodystrophy).

Leucocyte studies of families have done much to increase our knowledge of the genetics of the inherited metabolic disorders and in many cases the heterozygous state can be clearly delineated using these readily available cells. Direct enzyme assay on the isolated cells is usually satisfactory, although in some cases the white cell of choice may be the stimulated lymphocyte. Accumulation of material within leucocytes (Pompe's disease, cystic fibrosis) may aid diagnosis, as do functional tests on leucocytes, such as the loss of ability of these cells to phagocytose and kill bacteria or to take up nitroterazolum blue as is seen in chronic granulomatous disease.

Leucocyte assay (not necessarily enzyme orientated) may also have potential use in early recognition of graft rejection, and radioactive leucocytes may prove useful probes in seeking out septic foci in the body. Preliminary reports suggest that pyruvate kinase levels in the leucocyte of the mother may be a useful diagnostic tool to monitor fetal malnutrition. Histochemical examination of enzyme activity in the leucocyte has been suggested to be of value in the differential diagnosis of certain leukaemias.

Leucocyte Changes in Cirrhosis

G. E. LEVIN AND D. N. BARON (*Department of Chemical Pathology, The Royal Free Hospital, London*) Patients with cirrhosis and ascites commonly develop electrolyte disturbances, of which hypokalaemia and hyponatraemia are the most frequently encountered. Few attempts have been made to investigate the intracellular water and electrolyte status of such patients, who are often on a low salt diet and taking potassium losing diuretics.

Viable leucocytes were isolated from venous blood by the method of Baron and Ahmed (*Clin. Sci.*, 37, 205, 1969) and the intracellular sodium, potassium, and water contents measured. Nineteen measurements were made on 13 patients with cirrhosis and ascites. These results were compared with those obtained from 35 healthy control subjects. There was no evidence of any leucocyte potassium deficiency when the results were expressed in terms of the cell dry weight. There was a significant increase in the cell water to solid ratio, and this was associated with a considerable fall in the cell potassium concentration in the cell water. It was

observed that cell water values were within normal limits in all five of the severely hyponatraemic individuals and that in such patients the intracellular sodium and potassium content was considerably reduced in comparison with the other cirrhotic subjects.

Studies of Leucocyte Electrolytes in Renal Failure and Hypertension

N. F. JONES (*St Thomas' Hospital, London*) There are considerable difficulties in the study of intracellular electrolytes but it is clearly desirable that we should know much more about the intracellular compartment in disease. The leucocyte is a suitable model for such studies, having the methodological advantages of an 'isolated cell preparation'. Compared with erythrocytes, leucocytes are less uniquely specialized and react more quickly to changes in the extracellular fluid. In this report I describe studies of leucocyte electrolyte composition in chronic renal failure and in hypertension.

Leucocytes from patients with advanced renal failure were swollen cells with an increased water content. The latter correlated better with the cell sodium content, which was elevated, than with cell potassium content in these patients, whereas the converse is true in normal subjects. The cell content of potassium and its concentration in cell water were reduced in uraemia. These findings implied abnormalities of leucocyte cation transport in uraemia, and, in confirmation, we found that the rate constant for active (ouabain-sensitive) sodium efflux from this cell was markedly reduced in chronic renal failure. Passive fluxes of sodium across the cell membrane remained normal.

Observations on patients receiving maintenance haemodialysis and in others with acute renal failure indicate that the abnormality described above may develop rapidly and are also rapidly improved, but not totally corrected, by dialysis.

Examination of the results obtained in uraemia suggested a possible relationship between leucocyte sodium content and the blood pressure. Measurements were therefore made on leucocytes from patients with untreated essential hypertension and normal renal function. Control normotensive subjects were carefully matched for age, sex, and race. Leucocyte sodium and water contents were elevated in essential hypertension, and the rate constant for active sodium

efflux was reduced. Whether these findings are a consequence of the hypertensive state or reflect a fundamental abnormality in cell sodium regulation in this condition is the subject of continuing research.

Cytochemistry of Leucocytes

D. CATOVSKY (*MRC Leukaemia Unit, Royal Postgraduate Medical School, London*) Cytochemical tests applied to leucocytes may help in the diagnosis and classification of leukaemia, particularly the acute types (AL). It is convenient to divide the cytochemical reactions into those which help to characterize myeloid cells and those useful for the study of lymphoid cells.

Myeloid cells: some enzymes are regarded as specific markers of myeloid differentiation.

1. Myeloperoxidase: localized in the early granules of granulocytes and monocytes. The enzyme present in neutrophils is cyanide-sensitive, while part of the one present in eosinophils is cyanide-resistant. Results with the peroxidase reaction often parallel those obtained with the Sudan Black B stain for phospholipids. The reaction is usually positive in acute myeloid leukaemia and is negative in the lymphoblastic and undifferentiated types of AL.

2. Chloroacetate esterase: probably localized in the same granules as myeloperoxidase; leukaemic eosinophils may be positive while normal ones are not.

3. Non-specific esterase: a peptidase specific to monocytic cells, particularly when the reaction is inhibited by the presence of NaF.

4. Lysozyme: a cytobacterial method could easily be applied for the study of this enzyme in leucocytes.

(3) and (4) are useful in the differential diagnosis of AL; leukaemic monocytes are, as a rule, positive; myeloblasts and lymphoblasts are negative.

5. Alkaline phosphatase: present in mature neutrophils but probably not, as in the rabbit, in their secondary granules. Useful in the differential diagnosis between myeloproliferative disorders and leukaemoid reactions. Its changes in disease may only reflect changes in the life span of neutrophils.

Lymphoid cells: no specific enzymes of lymphocytes have been found. However, a high content in acid phosphatase demonstrated in lysosomal granules and in the membranes of the Golgi apparatus