poietin's role in the regulation of erythropoiesis and its disturbance in disease depends on accurate measurement of the hormone. Estimation of erythropoietin has relied on measuring an effect *in vitro* or *in vivo*. The *in-vivo* assays have used animals made more sensitive to erythropoietin by starvation, hypophysectomy, or by exposure to hypoxia. The response to erythropoietin has been measured by the incorporation of radioactive iron into haemoglobin following the injection of the test substance. Comparison of a dose-response curve for the test substance with the curve obtained from an international standard of erythropoietin in the hypoxic polycythaemic mouse has been one of the most successful methods of assay (Cotes and Bangham, 1961).

Complexity, the time needed for assay, and the use of large amounts of test substance in the *in-vivo* assay has stimulated the search for a suitable *in-vitro* assay. Immunochemical techniques have not been generally accepted because of the difficulty of obtaining pure erythropoietin with which to raise an antibody. The incorporation of radioactive iron into the haem of marrow cells in culture has been used (Ward, 1967). The order of sensitivity was the same as that of the *in-vivo* methods. More recently a more sensitive assay using foetal mouse liver cells has been introduced (Wardle, Baker, Malpas, and Wrigley, 1973).

The estimation of erythropoietin cannot be said to have a place yet in the routine investigation of most anaemias or polycythaemias. The presence or absence of erythropoietin production does provide a basis for a classification of the polycythaemias and in those associated with renal, cerebellar, liver, or uterine tumours the demonstration of erythropoietin is of great theoretical interest. Failure of erythropoietin production in anaemias such as those due to renal failure may not only be of theoretical interest now but of practical value in the future. Studies, using new techniques of assay and tissue culture, of the erythropoietin responsive cell will, it is hoped, lead to a more fundamental understanding of these anaemias.

References

Cotes, P. M., and Bangham, D. R. (1961). *Nature (Lond.)*, **191**, 1065.

Ward, H. P. (1967). *Proc. Soc. exp. Biol. (N.Y.)*, **125**, 370

Wardle, D. F. H., Baker, I., Malpas, J. S., and Wrigley, P. F. M. (1973). *Brit. J. Haemat.*, **24**, 49.

Red Cell Volume and its Normal Limits

S. M. LEWIS (*Department of Haematology, Royal Postgraduate Medical School, London*) Measurement of red cell volume (RCV) is essential in order to distinguish true polycythaemia from the pseudopolycythaemia caused by decreased plasma volume; also to assess the severity of polycythaemia when planning treatment and to study the course of the disease. Total blood volume is usually maintained within narrow limits and there is a fairly reliable correlation between RCV and venous PCV in normal subjects and in anaemia. In polycythaemia, plasma volume increases and PCV give a misleadingly low estimate of the red cell volume. ^{51}Cr is the most commonly used isotope label for measuring red cell volume. To ensure the reliability of the method a number of factors must be taken into account. These include (1) delayed mixing time, (2) early elution of the label, (3) variations in venous PCV and the presence of trapped plasma, and (4) variable ratio of venous/whole body haematocrit.

The commonest method of reporting blood volume is in terms of body weight. This is liable to be unsatisfactory in very thin or very fat subjects. However, predictions from formulae based on height and weight are only slightly better and give a 95% confidence limit of $\pm 15\%$. Thus, in practice expressing results in ml/kg body weight is adequate for routine procedures. Using this method, normal RCV values are usually taken as 30 ml/kg (2 SD = ± 5 ml) in adult males and 25 ml/kg (2 SD = ± 5 ml) in adult females. Total blood volume can be calculated from RCV by using PCV corrected for body haematocrit. It is, however, more reliable, as a rule, to calculate total volume as the sum of simultaneous measurements of RCV and plasma volume.

A recent development for measurement of RCV has been the introduction of short-lived isotopes such as ^{11}CO and $^{99}Tc^{m}$. These have the advantage of reducing radiation dose and allowing serial measurements in a short-time period. Results with $^{99}Tc^{m}$ are comparable to those obtained with ^{51}Cr.

Pseudocarcinomatous Invasion in Adenomatous Polyps of the Colon and Rectum

B. C. MORSON, T. MUTO, AND H. J. R. BUSSEY (*St. Mark's Hospital, London*) The histology of pseudo-carcinomatous invasion in adenomatous polyps of the colon and rectum is described and the appearances are contrasted with those seen in malignant polyps.

The most essential feature for the diagnosis of malignancy in adenomatous polyps of the colon and rectum is the spread of adenocarcinoma across the line of the muscularis mucosae. Whatever the terminology given to neoplastic changes superficial to this line, once cancer cells have reached the submucosal layer they undoubtedly have the potential for metastasis as well as further spread in continuity.

In the polyps with pseudo-carcinomatous invasion gland-like structures in the submucosa were lined by epithelium which showed the same degree of dysplasia as in the head of the polyp and histological continuity across the line of the muscularis mucosae could be established. The submucosal glands were, however, surrounded by lamina propria without any desmoplastic reaction to the epithelial cells which is usual in invasive carcinoma. Cystic change was very pronounced in many of the pseudo-carcinomatous glands, and this is unusual and less pronounced in true invasive carcinoma. Much retention of mucus within glands with atrophy of lining epithelium was a feature of some cases and the distinction from early mucinous or colloid carcinoma is thus particularly important. In pseudo-carcinomatous invasion the submucosal glandular tissue was well circumscribed without the characteristics of malignant infiltration.

In 48 out of 56 polyps showing pseudo-carcinomatous invasion there were deposits of pigment with the staining characteristics of haemosiderin around the submucosal glands. In some cases this was massive and a very obvious feature of the histology.

Having recognized the existence of pseudo-carcinomatous invasion in adenomatous polyps, the problem of its pathogenesis has to be considered. In our view the most likely explanation is that the epithelium in the submucosa is misplaced through the muscularis mucosae as a result of haemorrhage due to repeated twisting of the stalk. As evidence in support of this theory we would point out that most of the polyps were found in the sigmoid colon which is the part of the large intestine showing the most powerful muscular activity. This accounts