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.

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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 pseudo-polycythaemia 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

for the unusually long stalks, repeated twisting of which would cause haemorrhage into the polyp and this, together with secondary inflammation, facilitates the passage of adenomatous epithelium through the muscularis mucosae into the submucosa. The haemosiderin-laden macrophages are the consequence of haemorrhage.

The recognition of pseudo-carcinomatous invasion is important in the differential diagnosis of benign and malignant polyps of the large bowel. Failure to distinguish it from carcinoma may lead to wrong treatment and false reports of the incidence and prognosis of cancer of the colon and rectum.

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False Polycythaemia

SYLVIA W. DAVIES, EVELINE GLYNNE-JONES, AND E. PATRICIA LEWIS (*Area Department of Pathology, Exeter*) Fifty-five patients were referred for investigation because they were suspected clinically of having either polycythaemia rubra vera or secondary erythraemia. Thirty had an increase in the red cell mass; 15 had polycythaemia rubra vera with enlargement of the spleen and abnormal haemopoiesis; 15 had secondary erythraemia which was due to hypoxia or renal disease; haemopoiesis was normal and in several there was increased erythropoietic activity of the plasma.

Twenty-five patients who were plethoric with high values for the blood haemoglobin and the packed cell volume had no abnormalities of the peripheral blood or bone marrow and there was no increase in the red cell mass above normal. The common feature to all was a decrease in the plasma volume. Twenty-one were hypertensive; the plasma volume was lowest in those who were receiving treatment.

These cases are presented in order to demonstrate that blood volume studies are essential in patients with plethora in order to differentiate the state of reduced plasma volume from polycythaemia rubra vera or secondary erythraemia.

SYMPOSIUM ON INFECTIOUS MONONUCLEOSIS

Virological Aspects

JOAN M. EDWARDS (*Virus Reference Laboratory, Central Public Health Laboratory, Colindale*) The Epstein-Barr (EB) virus, first isolated from Burkitt lymphoma, is a member of the herpesvirus group which are large ether-sensitive DNA viruses with an icosahedral core and an outer membrane. Eight proteins have been identified in the EB virus which has a density of 1·2 to 1·3, a molecular weight of 100×10^8d, and a DNA density of 1·72.

It is a cell-associated virus which cannot be grown in the usual tissue cultures nor has animal passage been achieved. However, progress has been made in the study of the relationship of the virus to infectious mononucleosis. It has been shown that leucocytes from individuals without EB antibody do not form continuous lymphoblastoid cell lines spontaneously on culture. Those from subjects who have at some time been infected with EB virus may do so. Leucocytes from EB antibody-negative individuals when exposed to various EB virus-containing cell line concentrates or to throat washing or swabs from cases of infectious mononucleosis will form continuous lymphoblastoid cell lines.

Antibodies arising at the time of development of infectious mononucleosis have been identified by complement fixation, gel diffusion, neutralization tests, and in an antigen-antibody complex blocking test using ferritin-tagged antibody. Several different immunofluorescence tests have been developed to detect and differentiate antibodies to early antigen, to membrane antigen, and to viral capsid antigen in various Burkitt lymphoma cell lines.

Diagnostic problems arise in infectious mononucleosis due to the inability to isolate virus by a routine laboratory technique and the persistence of most of the antibodies long after infection. The heterophil antibody test and the immunofluorescence test for the presence of specific EBV IgM are the most informative in the diagnosis of current infection.

Immunopathology

R. L. CARTER (*Chester Beatty Research Institute, London*) The best documented immune responses in infectious mononucleosis are those mediated by antibody. The antibodies encountered in this disease are (1) heterophil antibodies used in diagnostic serology, (2) antibodies directed against EB virus, and (3) auto-, iso-, and other heteroantibodies including anti-i, lymphocytotoxins, Wassermann antibody, rheumatoid factors, and anti-smooth muscle antibodies. When properly tested, the heterophil antibodies are unusually specific for infectious mononucleosis. They are predominantly or exclusively IgM and show little or no gammaM → gammaG conversion. The distinctive infectious mononucleosis sheep cell agglutinins have been induced in volunteers with sheep erythrocytes, and in Squirrel monkeys inoculated with EB 3 cells. Little is known of the immunochemistry of the relevant antigenic determinants. Most of the auto-, iso-, and other heteroantibodies that appear transiently during the acute phase of IM are also IgM or IgM/IgG complexes. Anti-i is the main cause of haemolysis in infectious mononucleosis. The other antibodies described have no known clinical significance though autoimmune antibodies may perhaps contribute to liver damage and the rare complications of thrombocytopenia and agranulocytosis. There is no certain evidence of immune complex disease in infectious mononucleosis.

Serum IgM levels are strikingly raised during the acute phase of infectious mononucleosis and they may remain elevated for months after apparent cure. The cells responsible for IgM synthesis are not yet clear: the circulating atypical lymphocytes are probably not involved but lymphoid cells in the bone marrow and lymph nodes may be. Plasma cells are not conspicuous in these tissues and, in the nodes, the main feature is hyperplasia of the (thymus-dependent) paracortex with many pyroninophilic blast cells.

The paracortical blast cells are almost certainly identical with the atypical lymphocytes in the blood, and it is probable that the circulating cells are T lymphocytes (based mainly on their capacity to form rosettes with sheep erythrocytes). Consequently, information is now urgently needed about cellular immune responses in infectious mononucleosis, in which T cells are implicated.

The circulating atypical lymphocytes appear to be antigenically different from normal blood lymphocytes, as shown in mixed lymphocyte tests and, more problematically, in continuous cultures. Cultured infectious mononucleosis leucocytes acquire (EBV-associated) neoantigens on their membranes and also a capacity to grow progressively as heterotransplants in animals. These and several