survey. Seventy-three of the organisms were urinary-tract isolates, 14 respiratory tract isolates, and 27 were isolated from other sites. Two-thirds of the 97 patients studied in detail had been in hospital for more than seven days and three-quarters had been on antibiotic therapy before *Serratia marcescens* was first isolated. Details of antibiotics used before the first isolation of *Serratia marcescens*, and of the *in vitro* antibiotic susceptibility of the *Serratia marcescens* isolates would be presented.

Factors Affecting Transfer of Antibiotic Resistance between Gram-negative Bacteria in the Human Intestine

J. D. ANDERSON (*University of Bristol*) In the absence of chemotherapy, no transfer of bacterial antibiotic resistance transfer (R) factors could be detected in the faeces of four subjects who swallowed potential donor and recipient organisms even though the plasmids concerned could be freely transferred in broth, both to the ingested potential recipients and to a variety of faecal coliforms. The faeces of these subjects contained such large populations of the relevant organisms that one would have expected transfer to occur if the bacteria had been in a broth medium (Anderson, Gillespie, and Richmond, 1973). Reasons for the discrepancy between results obtained *in vivo* and *in vitro* were therefore investigated.

R factor transfer between donor and recipient strains of *Escherichia coli* was found to be completely inhibited in nutrient broth by dense suspensions of *Bacteroides fragilis*. Comparable amounts of inert bacterial matter (formolized suspensions of *E. coli* or *B. fragilis*), populations of *Streptococcus faecalis*, or bile salts were only moderately inhibitory. Strict anaerobiosis had no effect upon R factor transfer. Population densities of organisms used in these studies were similar to those found in faeces.

The presence of *Bacteroides fragilis* thus provides a satisfactory explanation for the almost total inhibition of conjugation in the human gastrointestinal tract in the absence of antibiotics. Other factors inhibiting conjugation to a lesser degree may reinforce the effect of *B. fragilis*.

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Serum Amylase and Related Enzymes in Diabetic Ketoacidosis

D. M. GOLDBERG, R. J. SPOONER, AND A. H. KNIGHT (*Royal Hospital, Sheffield*) In previous studies we have confirmed the high incidence of hyperamylasaemia in diabetic ketoacidosis and have shown that this is not related to acute pancreatitis, renal failure, macroamylasaemia, or hepato-biliary disease (Knight, Williams, Ellis, and Goldberg, 1973; Knight, Williams, Spooner, and Goldberg, 1973) nor does it appear to influence the prognosis in individual cases.

It has recently been proposed that the source of the amylase in such subjects is the hepatocyte, and that amylase is released from its endoplasmic reticulum as a consequence of attack by lysosomal enzymes (Belfiore, Napoli, and Lo Vecchio, 1972; Belfiore and Napoli, 1973). This proposal rested on the demonstration that raised levels of lysosomal enzymes were found in the serum of subjects with diabetic ketoacidosis and hyperamylasaemia and followed a similar time-course to the latter.

The following lines of evidence exclude the hepatocyte as the source of hyperamylasaemia in diabetic ketoacidosis and cast doubt on the role of lysosomes in its release from other tissues.

1 Sequential determinations of serum enzyme activities in 10 consecutive patients revealed that whereas beta-glucuronidase was elevated at some time in all patients, amylase was raised in only eight and acid phosphatase in only four.

2 A low correlation was found between amylase and beta-glucuronidase, and between amylase and acid phosphatase in the above, whether peak activities or activities of all samples were considered.

3 Measurement of the same enzymes in 24 cases of acute viral hepatitis showed that whereas raised beta-glucuronidase activities were found in 20, amylase was raised in only three, and acid phosphatase in but a single case. Again, correlation between amylase and beta-glucuronidase was poor.

4 Analysis of six samples of normal postmortem human liver revealed that, in contrast to acid phosphatase and beta-glucuronidase, its amylase content was negligible, especially when care was taken to remove all pooled blood. In fact the concentration of amylase in human liver is far below that of human serum, even when steps are taken to ensure solubilization of lysosomal and microsomal enzymes whereas the hepatic content of acid phosphatase and beta-glucuronidase are respectively approximately 30-fold and 6000-fold, the upper normal limit for serum.

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Aspects of EB Virus Infection

R. N. P. SUTTON (*King's College Hospital Medical School, London, introduced by* H. A. SISSONS) The association of the EB virus with Burkitt's lymphoma, infectious mononucleosis, nasopharyngeal carcinoma, and possibly with some other conditions (notably Hodgkin's disease) is now well recognized. Asymptomatic infection with this virus is also frequent and most of the population have acquired antibodies by early adult life.

Although the isolation of EB virus from nasopharyngeal secretions is possible in acute infectious mononucleosis, this procedure is not practicable at the moment as a routine measure and evidence of infection depends upon the demonstration of rising antibody titres. A variety of such antibodies may be demonstrated, including antibodies to virus capsid antigen, membrane, and complement-fixing antibodies. We have observed that antibodies to EB virus capsid antigen develop more rapidly than those to EB soluble complement-fixing antigen and this discrepancy could form the basis for a relatively simple diagnostic test for the presence of recent infection.

Infections with EB virus also result in the development of autoimmune antibodies and in the impairment of cell-mediated immunity. In our report, we describe some of these phenomena in active infectious mononucleosis and also in asymptomatic infections.

SYMPOSIUM IN POLYCYTHAEMIA

The Assay of Erythropoietin

J. S. MALPAS (*Department of Medical Oncology, St Bartholomew's Hospital, London*) Investigation of erythro-

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 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