brandenburg, stanley, and anatum, showed no reduction in viable count compared with MacConkey’s medium. The growth of salmonellae was somewhat slower than on ordinary MacConkey agar but most produced colonies of 1 mm diameter at 22 hours and only five required incubation for 36 hours. Six of the 41 Salmonella species tested failed to grow (typhi, paratyphi A and C, cholera-suis, gallinarum and pullorum) but all are rare causes of food poisoning in Britain. All the faecal flora was prevented from growing apart from occasional strains of lactose-fermenters and some paracons. Inocula of one million organisms of Pseudomonas pyocyanea, E. coli, Proteus mirabilis, vulgaris and morgani, Klebsiella aerogenes, and Shigella sonnei were completely suppressed. The new medium was simple and reproducible from batch to batch.

An outbreak of S. enteritidis food poisoning amongst nursing staff allowed a comparison of the results from the new medium with those from deoxycholate-citrate agar (DCA). Each sample of faeces from 640 people was examined in two laboratories, one employing selenite F and the new medium and another making orthodox use of first class DCA and selenite F. The total number of salmonellae isolated from the new medium was 36 compared with only 22 from DCA.

The new medium appeared to be a distinct advance on any other for the investigation of salmonella food poisoning.

AN APPRECIATION OF THE BRUCELLIN SKIN TEST

C. H. L. HOWELLS (Wolverhampton) The paper describes an epidemiological and serological survey of brucellosis on volunteers. Each had venepuncture and received 0·1 ml of brucellin (Olin, 1935) intradermally, using a disposable tuberculin syringe and needle. Details of age, occupation, consumption of unpasteurized milk, contact with cattle, and any history of brucellosis were noted. Participants suffering from brucellosis were excluded. Reactions were measured 48 hours later (positive tests = 10 mm erythema). Second venepuncture was done three weeks later to determine whether there had been any antibody stimulation. Agglutinations in phenol saline and mercaptoethanol, Coombs test, and complement-fixation tests were performed at Colindale, Truro, Northallerton, and Wolverhampton.

Proportion of positive reactions was greater in farming areas and increased with age. Consumption of unpasteurized milk and contact with cattle were associated factors. Four to seven per cent of healthy young adults might have been exposed to Brucella organisms in the past, as positive skin reactions persisted after circulating antibody disappeared. Serological tests were more valuable than skin tests in assessing level of infection in community. In the survey, antibody was detected more easily with agglutination and Coombs tests than with mercaptoethanol and complement-fixation tests. This finding was expected in healthy volunteers since the latter tests indicate active infection and the former measure residual antibody.

It was concluded that the brucellin test is a poor indication of disease since many with positive tests did not possess antibody and some with negative tests did. Positive tests in farming areas were of little diagnostic significance since many of the healthy population were positive. Stimulation of antibody by brucellin was a further disadvantage. Caution therefore is needed in interpreting serological tests in anyone previously skin tested.

Bruccelin appeared to be less useful than serological methods in epidemiology, while in diagnosis its use might be entirely misleading.

AN ASSESSMENT OF SERUM 57CO CYANOCOBALAMIN AS AN INDEX OF VITAMIN B12 ABSORPTION

D. DONALDSON AND P. T. LASCELLES (London) A technique has been developed for assaying accurately the low levels of 57CO cyanocobalamin obtained in the serum during the conventional Schilling test. Emphasis is placed on the length of time (approximately two hours) required for obtaining reliable data in counts that are on only slightly above the background. The maximum radioactivity was found by serum tolerance tests to occur at eight hours. Correlation between the eight-hour serum levels and 24-hr urinary excretion was studied in 96 patients and the data subsequently were subjected to mathematical analysis.

Patients studied in the survey include those with pernicious anaemia, and without neurological involvement, malabsorption and postgastrectomy states, undiagnosed peripheral neuropathies, patients with hypopituitarism and myxoedema, and folate-deficient epileptic patients on treatment.

By serum counting it was possible clearly to demarcate patients with pernicious anaemia from the normal controls, but pernicious anaemia (part two, with intrinsic factor), malabsorption syndrome and postgastrectomy patients, and folate-deficient epileptic patients on treatment fell into an intermediate range which could not be differentiated from the normals.

A further group, namely, eight hypopituitary and myxoedematous patients on replacement therapy had significantly higher serum and urine levels than the normal controls. No obvious explanation was apparent.

The value of counting serum radioactivity in Schilling tests is shown to be of particular value where urine collection is inaccurate, where there may be contamination of urine with extraneous radioactivity, and where there is the possibility that other isotopes have been given to the patient.

INVESTIGATION OF THE CELLULAR DEFECT IN VITAMIN B12 DEFICIENCY

D. G. CHALMERS (Cambridge) By flash labelling with tritiated thymidine freshly aspirated human bone marrow, it is possible to determine, in individual cells, their morphological category, their content of DNA, and whether they were in DNA synthesis at the time of aspiration.

Previous investigations of pernicious anaemia have shown a failure of DNA synthesis in a proportion of cells in this condition, and a pile up of cells in G1.

In the knowledge that RNA synthesis, as measured by
incorporation of radioactive precursors, is unimpaired, we investigated protein synthesis using labelled leucine. A proportion of cells throughout the cell cycle showed no evidence of uptake.

It is postulated that this may be due, not to the failure of DNA synthesis, but to a separate defect resulting from inability of B₁₂ coenzymes to methylate both transfer and messenger RNA, and that RNA, although synthesized, is ineffective until methylation occurs. Such an action has not been described in man although there is biochemical evidence to support it.

INHIBITION OF FACTOR XIII BY HEPARIN

J. GREEN (Perth) In human citrated plasma clotted by thrombin, comparison, at equivalent clotting times, of the weight of fibrin formed and the fraction soluble in 6 M urea shows that in the presence of heparin, the total weight of fibrin formed is less, and the rate and the extent of cross-linkage, as measured by the weight of urea-insoluble fibrin, is decreased. There is some variation between plasmas, but all samples studied at pH 7-0 with minimal dilution have shown complete inhibition of cross-linkage at heparin levels of 8 units/ml and clotting times longer than 30 seconds. Dilution of plasma at constant ionic strength results in cross linkage occurring at lower thrombin/heparin ratios.

The inhibitory effect of heparin can be reversed by protamine sulphate after clotting has occurred.

In fibrinogen preparations of increasing purity, inhibition of cross-linkage by heparin is increasingly difficult to demonstrate, suggesting that other factors may be involved.

THE POSSIBLE PHYSIOLOGICAL SIGNIFICANCE OF THE MICROANGIOPATHIC RED CELL FORM

C. WARDROP AND H. E. HUTCHISON (Glasgow) Irregularly-contracted erythrocytes (burr cells) are a recognized sign of red cell damage, often associated with vascular disease, and this may sometimes lead to a frank haemolytic state—the microangiopathic haemolytic anaemia syndrome.

Recent experimental work by Dacie et al (Bull, Rubenberg, Dacie, and Brain, 1967; Rubenberg, Bull, Regoez, Dacie, and Brain, 1967, has provided further information on the pathogenesis of such haemolysis and cases consistent with their suggestions are described.

Other clinical associations of irregularly-contracted red cells exist which the Dacie hypothesis would not readily explain. Some of these associations and their possible pathogenesis are discussed in the light of current concepts of the fibrinolytic system.

REFERENCES


RENAI BIOPSY: AN ASSESSMENT OF ROUTINE ELECTRON MICROSCOPY

J. R. TIGHE, A. E. CLARK, A. J. EISINGER, AND N. F. JONES

(St. Thomas' Hospital, London) Renal biopsies have been routinely examined by electron microscopy during the past two years. Of 103 biopsies, 83 from 79 patients were suitable for this study. Dissecting microscope examination enabled the adequacy of the biopsy to be assessed and suitable pieces selected for electron microscopy. The whole biopsy was also examined by light microscopy. Processing for electron microscopy takes four days.

The investigation proved to be of most value in cases of the nephrotic syndrome. Of eight patients diagnosed on light microscopy as minimal change glomerulonephritis, three were subsequently reclassified on electron microscopy as membranous glomerulonephritis. Of these, two failed to respond to steroid therapy. In contrast, four of the remaining five patients with minimal change glomerulonephritis responded to treatment with steroids and the fifth has not been followed long enough to assess response. Only one of 11 patients with membranous glomerulonephritis is known to have had a complete remission with treatment. In patients with less severe proteinuria electron microscopy revealed abnormalities, such as foot process disease, which were not apparent in the light microscope.

Electron microscopy is of much value in the diagnosis of minimal deposits of amyloid when the results of light microscopy are equivocal.

The characteristic basement membrane deposits of streptococcal proliferative glomerulonephritis are confirmed.

Routine electron microscopy of renal biopsies, particularly in the nephrotic syndrome, is desirable, but because of the considerable cost, these facilities may well have to be based on regional centres.

HEPATIC AND RENAL DAMAGE WITH PARACETAMOL OVERDOSAGE

R. A. G. BROWN (Dundee) N-acetyl para amino phenol was discovered in 1889, recognized as a metabolite of phenacetin with good analgesic properties in 1949, and introduced into British clinical medicine in 1956.

Since then reported toxic effects have been few and the sales of various paracetamol-containing compounds have increased to an estimated 680 million in 1967.

During 1967, five patients admitted to Dundee Royal Infirmary were cases of paracetamol self poisoning. In three, there was histological evidence of hepatic damage, varying from centrilobular fatty change and cloudy swelling to frank centrilobular necrosis, along with an increase in lipofuscin pigmentation and increased mitotic activity signifying regeneration. The remaining two showed clinical evidence of hepatic derangement. In one, a fatal case, there was renal damage. The capsular spaces contained a foamy lace-like material—presumably proteinous—which spilt over into the proximal convoluted tubules and there was evidence of distal tubular degeneration with focal necrosis. The renal papillae appeared intact.

Paracetamol, reputedly the analgesic of choice for occasional use, is freely available, but in overdosage it is potentially lethal.
Investigation of the cellular defect in vitamin B12 deficiency.

D G Chalmers

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