J. clin Path. (1967), **20**, 918

The Association of Clinical Pathologists: 79th general meeting

The 79th general meeting of the Association of Clinical Pathologists was held at the Middlesex Hospital Medical School, London, on 21-23 September 1967. The programme included the Foundation Lecture by Professor J. N. Cumings on 'Trace metals in the brain and in Wilson's disease', the Presidential Address by Dr. A. J. McCall on 'Embolic cerebral infarction', and a symposium on 'Leucocytes', for which the speakers were invited. There was a seminar on 'New drugs and the clinical pathologist', also addressed by invited speakers, who included representatives of certain pharmaceutical firms. Abstracts of the other papers follow.

THE ERYTHROBLAST CRISIS AS AN INDICATOR OF RESPONSE TO THERAPY IN MEGALOBLASTIC ANAEMIA

H. G. H. RICHARDS AND I. SLATER The authors describe the use of buffy coat films (from sequestrene anticoagulated blood) for identification and counting of nucleated erythrocytes (erythroblasts) in 25 cases of megaloblastic anaemia due to vitamin B_{12} or folic acid deficiency. The erythroblasts were expressed as the number of cells encountered while observing 500 leucocytes. Pre-treatment erythroblast counts were nil in four cases, but varied from 1 to 14 in 21 cases. Following appropriate treatment for the deficiency a usually short-lived outpouring of erythroblasts occurred, reaching its peak most frequently at the third day after the commencement of treatment, *i.e.*, two days or more before the reticulocyte peak. This 'erythroblast crisis' appears to be specific for vitamin B_{12} or folic acid therapy in cases of deficiency, since it does not arise in iron-deficiency anaemia treated with parenteral iron; neither does it occur when normal subjects are given these haematinic vitamins. The erythroblast peaks varied between 322 and 3/500 leucocytes. In 12 cases it exceeded 24. The higher peaks generally occurred in the younger patients, and those with the lowest haemoglobins, highest pre-treatment serum iron levels, but lowest serum B_{12} and folic acid levels.

The erythroblast crisis is recommended as a very simple, probably specific and additional or alternative method of assessing early response to appropriate treatment in cases of megaloblastic anaemia.

HAEMOGLOBIN CONCENTRATION, P.C.V., AND M.C.H.C. IN WOMEN USING AN INTRA-UTERINE DEVICE OR TAKING ORAL CONTRACEPTIVES

J. ALAGHBAND ZADEH, C. D. KARABUS, and J. FIELDING (Paddington General Hospital) Iron balance is precarious in adult women. As many as 50% have iron deficiency either as anaemia or sideropaenia. Menstrual blood loss is an important factor in iron balance. Two new methods of contraception now widely adopted are said to alter menstrual loss. They may therefore have significant effects on haematological status. Haemoglobin, P.C.V., and M.C.H.C. were estimated in 219 women attending a F.P.A. clinic: (1) a control group, 53 women attending the clinic for the first time; (2) 72 women fitted with an intra-uterine contraceptive device (I.U.D.); (3) 94 women taking oral contraceptive pills of combined formulation. Six types of pill were in use.

The validity of the control group was ascertained by showing no difference in haematological status between those of them subsequently advised an I.U.D. and those advised the contraceptive pill.

The I.U.D. has marked effects, with mean haemoglobin concentration falling from $13{\cdot}4 \pm 0{\cdot}1$ g. per 100 ml. in the controls, to $12{\cdot}8 \pm 0{\cdot}12$ g. ($P < 0{\cdot}01$). The fall in P.C.V. reached greater degrees of significance; the M.C.H.C. also diminished. After six months, no women had haemoglobin concentrations above 14 g. per 100 ml. while in 60% it was less than 13 g. per 100 ml.; in other words, virtually all were iron depleted. These effects need to be considered when giving contraceptive advice, or in a programme of population control in less developed countries, where anaemia is common.

In women taking the contraceptive pill, the mean haemoglobin increased to $13{\cdot}7 \pm 0{\cdot}10$ ($0{\cdot}05 < P < 0{\cdot}1$). The increase in P.C.V. is more significant ($P < 0{\cdot}02$). There were no significant changes in M.C.H.C. The increase in haemoglobin and P.C.V. occurs mainly in the first six months. In 40% of women, haemoglobin concentration was greater than 14 g. per 100 ml. compared with 19% in the control group ($P < 0{\cdot}01$). There was no difference in the proportion with haemoglobin less than 13 g. per 100 ml. Some formulations, especially those containing norethysterone acetate as progestogen, appear to have greater effects than others in raising haemoglobin concentration. Diminished blood loss alone does not account for all the changes observed; it is possible that haemoconcentration occurs in some subjects; if this is so, the resulting increased viscosity may have some bearing on thrombotic phenomena.

CAUSES OF THE GROSSLY ELEVATED E.S.R.

RICHARD W. PAYNE (Victoria Infirmary, Glasgow) This small study was undertaken to ascertain the validity of

the widely-held belief that the finding of a grossly elevated E.S.R. commonly has a sinister significance.

During a 15-month period in a district general hospital, 5,931 E.S.R.s were performed, and in 100 patients the result was more than 100 mm. in one hour.

Of these 100 patients, 53 were suffering from an infective condition, 33 a 'collagen disorder', and 14 a malignant condition.

In the 53 patients with infective conditions, 26 had renal tract infections (of whom 18 had a history suggestive of a previous attack) and 15 had respiratory tract infections (12 simple bacterial, two viral, and one tuberculous). It is of interest that there were six diabetics in this group.

In the 33 patients with 'collagen disorders', 17 had rheumatoid arthritis.

In the 14 patients with malignant conditions, nine had widespread carcinomatosis, three had myelomatosis, and two a reticulosis.

It is therefore concluded that the belief that the grossly elevated E.S.R. commonly has a sinister significance is erroneous, it should again be stressed that more than half the patients studied had an infective condition; or alternatively more than half the patients had an infection of the respiratory or renal tract or had rheumatoid arthritis.

TREATMENT OF VON WILLEBRAND'S DISEASE WITH POOL'S CRYOGLOBULIN ANTI-HAEMOPHILIC GLOBULIN CONCENTRATE

J. R. O'BRIEN (From the Portsmouth and Isle of Wight Area Pathological Service, Portsmouth) Five adult patients with typical von Willebrand's disease from two families were studied on 12 occasions. Transfusion with reconstructed dried plasma produced the expected delayed rise in the patient's plasma anti-haemophilic globulin (A.H.G.) level. On nine occasions cryoglobulin concentrate was given and the A.H.G. levels were followed. The expected immediate rise occurred but the evidence strongly suggests that no delayed rise occurred. These findings, if they can be confirmed, indicate that the plasma factor necessary in von Willebrand's disease to produce the delayed rise in plasma A.H.G. is not a cryoglobulin; thus it differs radically from A.H.G. itself.

In spite of the high levels of plasma A.H.G. achieved by this treatment platelet loss on passage through a column of glass beads and the Borchgrevink bleeding time were not significantly improved.

A HOSPITAL STUDY OF TRANSFERABLE DRUG RESISTANCE

ELLEN C. MOORHOUSE (Dublin) *Escherichia coli* strains isolated from the faeces of 22 children on admission and while in hospital were tested for resistance to five common antibiotics. The children, with one exception, were under 2 years of age. All resistant strains isolated were tested for resistance transfer.

Fifteen of the 22 children excreted resistant *E. coli* strains on admission, and transferable drug resistance was found in all 15 strains. Nine of these children had been patients in other hospitals before admission, the other six were admitted from their own homes with no previous history of hospitalization. Within four days of their admission to hospital, four of these 15 children excreted either fully sensitive *E. coli* strains, or strains resistant to one drug only.

The *E. coli* strains isolated from the other seven children on admission were sensitive to all five drugs, but within a week multiple resistant *E. coli* strains were isolated from their faeces. The resistance in these strains was transferable. A small investigation was done to determine if the appearance of these R-factor strains could be the result of hospital cross infection. Faecal swabs from children in wards occupied by three of these seven children were examined. The results obtained confirmed the impression that there was cross infection in the wards with R-factor *E. coli* strains.

The epidemiological and clinical importance of transferable drug resistance was discussed, with particular reference to the problems associated with R-factor bacteria within a hospital.

PATHOLOGY OF LIVER ALLOTRANSPLANTATION IN THE PIG

A. C. HUNT (Department of Pathology, University of Bristol) In a series of 41 pigs, in which the Department of Surgery of the University of Bristol had transplanted livers from unrelated donor pigs, 16 survived the immediate post-operative period. None received any immunosuppressive therapy. In the first week there was a moderate degree of infiltration of the portal tracts and interlobular septs by pyroninophilic mononuclear cells and variable amounts of centrilobular and focal liver cell necrosis. Two pigs died on the eighth and tenth days and no extrahepatic cause of death was found. There was extensive hepatic necrosis and cellular infiltration in one, but less severe changes in the other. Three pigs survived for 21 to 33 days and died of gastrointestinal ulceration. Histological evidence of liver rejection was minimal. A fourth pig is still alive 15 months after transplantation. Its liver function is normal and subcapsular biopsies at six months and one year show slight increase in fibrous tissue.

The result of this investigation suggest that the transplanted liver is less readily rejected than some other organs, a finding compatible with other evidence that liver is relatively less immunogenic than, for example, the kidney or spleen.

RHEUMATOID ARTHRITIS WITH PHENYLBUTAZONE-INDUCED THROMBOTIC MICROANGIOPATHY

D. L. GARDNER (Kennedy Institute, London) and D. THOMPSON (Pathology Department, University of Edinburgh) A woman of 69 was recognized to have had rheumatoid arthritis for two years. After aspirin had been proved ineffective, phenylbutazone was begun. Seven days later bruising was noted; all drugs were stopped. Thereafter, there was progressive anaemia and thrombocytopenia. Five days after the onset of bruising a blood count showed platelets 10,000/c.mm., haemoglobin 9·5 g./100 ml., E.S.R. 53 mm./hr., reticulocytes

7·0%; direct and indirect Coombs tests negative; no atypical haemagglutinins; Rose-Waaler, latex fixation, and antinuclear factor tests negative; no L.E. cells. Two days after beginning chlorpromazine, jaundice developed (serum bilirubin 2·4 mg/100 ml., alkaline phosphatase 13 K.A. units, S.G.P.T. and S.G.O.T. each less than 20 units). There was no clinical response to the transfusion of packed red cells and to prednisolone.

Necropsy showed severe rheumatoid arthritis, especially of the left elbow joint, a small spleen, many intramyocardial petechiae, and bleeding into the gastrointestinal tract. The characteristic microthrombi of Moschowitz's syndrome were present in the rheumatoid synovia, in the thyroid, which showed an active thyroiditis, in the bone marrow, and in all viscera. The marrow was highly cellular and contained numerous megakaryocytes: similar cells were lodged in pulmonary and portal capillaries.

In a survey of the 203 cases of thrombotic microangiopathy reported up to early 1966 no other case has been encountered in which histologically proven rheumatoid arthiritis and thrombotic microangiopathy coexisted; in only one other recorded case has microangiopathy been associated with the therapeutic use of phenylbutazone. Thyroiditis and thrombotic microangiopathy have not previously been described in association.

BASEMENT MEMBRANE HOMEOSTASIS IN THE GLOMERULAR CAPILLARIES OF DIABETICS

J. T. IRELAND, B. K. PATNAIK, and L. J. P. DUNCAN (Southern General Hospital, Glasgow, and University of Edinburgh) Renal tissue obtained by percutaneous biopsy from patients having longstanding insulin-dependent diabetes mellitus with either minimal retinopathy (five cases) or advancing proliferative retinopathy (six cases) and from 10 patients having diabetes secondary to pancreatic disease, has been compared with that of nine healthy controls by examination by light and electron microscopy. Besides statistical analysis of the peripheral glomerular capillary basement membrane thickness, the ultrastructure of the epithelial, endothelial, and mesangial cells of the capillaries has been compared in the various clinical groups. Significant thickening of the basement membrane was found in all but one of the longstanding idiopathic diabetics and in seven of those having pancreatic diabetes, and was invariably associated with increased epithelial cytoplasm containing prominent mitochondria, R.N.A.-studded endoplasmic reticulum, and Golgi zones. In patients having proliferative retinopathy the endothelial and mesangial cells were frequently atrophic, and there was greater basement membrane thickening than in patients having minimal retinopathy or those having diabetes secondary to pancreatic disease.

On the basis of these findings the maintenance of basement membrane homeostasis is discussed in relation to the effects of the metabolic disturbance, the inherited diathesis and the anterior pituitary on the functions of the epithelial, endothelial and mesangial cells.

HISTOCOMPATIBILITY TESTING FOR RENAL TRANSPLANTATION

S. D. NELSON (Royal Victoria Hospital, Belfast) It is generally accepted that the chances of long-term survival after renal transplantation are greater if donor and recipient are closely matched for histocompatibility antigens. The evaluation of histocompatibility tests in clinical transplantation practice is made difficult by the fact that the postoperative course is subject to modification by immunosuppressive agents given to the patient, and by many other factors, such as sensitization of the patient to transplantation antigens present in preoperatively transfused blood. In general, however, a relatively compatible kidney will provoke only a mild reaction which can be controlled by the immunosuppressive regimes currently available. Successful prediction of compatibility will be reflected in the long-term results of transplantation, and especially in the histological features of the transplanted kidney beyond one year after operation.

A more immediate, although less conclusive assessment of the value of histocompatibility tests may be obtained from the correlation between the predictions of the tests and the clinical features of the postoperative course during the six to nine months after transplantation. Using the Spearman ranking correlation test, an attempt was made to relate the predictions of two histocompatibility tests, namely, the normal lymphocyte transfer (N.L.T.) test and the mixed lymphocyte culture (M.L.C.) test with the outcome of renal transplantation carried out at Massachusetts General Hospital. The correlation between the clinical courses and the N.L.T. predictions in 10 cases was found to be significant at the 0·05 level, whereas the M.L.C. predictions did not correlate significantly with the nine month outcome of transplantation.

COT DEATH

R. I. K. ELLIOTT (Hove) Sudden unexplained death in the postneonatal age group is a perennial problem; in 1903 it was ascribed to overlaying, in Laennec's day to 'suffocating catarrh'. Whereas infantile mortality from other causes has decreased remarkably in the last 60 years, death from this cause has not. Statistics show that the incidence is strongly influenced by maternal care and by infection; but the event itself is determined by the limited mobility of the infant, which makes it incapable of extricating itself from difficult situations; by limited respiratory capacity; and by the danger of inhalation. Probably no single factor is entirely responsible for cot death; suffocation is commoner than suspected, and so is inhalation of stomach contents. Overwhelming infection is probably a myth; instead of searching for a mysterious cause, pathologists would do better to concentrate on prevention, by campaigning for the instruction of mothers during the neonatal phase in commonsense methods of eliminating avoidable risks, such as soft pillows, loose bedding, and clothing hung on or over the cot; and the importance of adequate 'winding' after feeds.

RADIOACTIVE ISOTOPES IN ORGAN SCANNING

C. B. CAMERON (Royal Marsden Hospital, London) The usefulness of the technique of organ scanning using radioisotopes makes it certain to spread beyond the range of specialized units. When this happens it will be attached to existing service departments such as diagnostic radiology or clinical pathology. The experience of such a unit in a clinical pathology department is presented. The range of isotopes used and scanning apparatus available was outlined, and some illustrative liver, brain, thyroid, and pancreas scans were shown. Questions such as radiation hazard, capital and running costs, and administrative problems were touched on.

SERUM ZINC IN HEALTH AND DISEASE

I. J. T. DAVIES, M. MUSA, and T. L. DORMANDY (Whittington Hospital, London) Zinc is essential for the development and growth of plants and animals. Many key enzymes contain zinc: the ones of clinical importance are carbonic anhydrase, alkaline phosphatase, and alcohol dehydrogenase. Zinc deficiency syndromes are well recognized in plants and animals and also occur in man. Zinc salts accelerate the healing of surgical wounds and radioactive zinc preferentially accumulates in healing skin.

The tissues which contain the highest concentrations of zinc are the eye, pancreas, male genital tract, epidermis, and blood.

The serum zinc is frequently low in alcoholics and this may be responsible for the vitamin A-resistant night blindness of some alcoholics.

The serum zinc has been measured using an atomic absorption spectrometer in over a thousand cases. A normal range of 76 to 125 μg./100 ml. has been established; there is no variation due to age. Serum zinc falls after oral and intravenous glucose; this is not due to haemodilution. The serum zinc is lowered in alcoholic cirrhosis and carcinoma of the bronchus, but is normal in acute hepatitis, cardiovascular disease, diabetes mellitus, gastrointestinal haemorrhage, and other carcinomata.

A NEW METHOD OF ESTIMATING BLOOD AMMONIA AND ITS APPLICATION IN CLINICAL DIAGNOSIS

HELEN MCCULLOUGH (Department of Clinical Biochemistry, Royal Victoria Hospital, Belfast, N. Ireland) The clinical application of blood ammonia determinations has been restricted in the past because of the difficulties and errors involved in the estimation and the necessity for arterial sampling.

A method is described which allows the determination to be carried out rapidly on a routine basis in any laboratory. The method may be applied to 100 I.U. samples of blood from a thumb or heel prick. These capillary levels approximate closely to arterial levels and are unaffected by muscular exercise or tissue uptake of ammonia.

The metabolism of ammonia is reviewed and raised peripheral levels may be expected where gross ammonia formation in the gut occurs thereby overloading the normal or defective liver, in the presence of a defective urea cycle in the liver due to parenchymal damage or hereditary enzyme defects, and in the presence of portal-systemic collateral circulation.

The mechanism of ammonia intoxication is discussed together with the clinical significance of blood ammonia levels. The potential clinical uses of the determination and the ammonia tolerance test include the prevention and treatment of hepatic encephalopathy and the detection and treatment of liver disease, portal hypertension, and oesophageal varices.

THE ANTIBIOTIC ACTIVITY OF BACTERIA IN APPENDICITIS AND PERITONITIS

W. A. GILLESPIE and K. G. BARBER (Bristol) Bacteriological study of acute appendicitis showed that infections were always mixed. The predominant organisms were *Esch. coli*, aerobic and anaerobic streptococci and Bacteroides *spp*. Several others were present occasionally. Most streptococci were sensitive to penicillin, ampicillin, cephaloridine, chloramphenicol, tetracycline, and moderately sensitive to streptomycin and gentamycin. Most *Esch. coli* were sensitive to all except penicillin. Bacteroides were always sensitive to chloramphenicol, nearly always to tetracycline; a few were sensitive to ampicillin and cephaloridine.

Antibiotics for acute general peritonitis complicating appendicitis must be chosen before sensitivity tests can be done. Probably the best choice is parenteral therapy with streptomycin and either chloramphenicol or tetracycline. Chloramphenicol may be slightly better than tetracycline; the danger of marrow damage is less than the danger of the infection.

RESISTANCE OF STAPHYLOCOCCUS AUREUS TO ERYTHROMYCIN AND RELATED ANTIBIOTICS

J. H. HEWITT and M. T. PARKER (Cross-Infection Reference Laboratory, Colindale, London) The results of erythromycin and oleandomycin sensitivity tests on 2,527 strains of *Staph. aureus* consecutively isolated from the lesions of patients in eight London hospitals in the years 1963-66 are reported.

Three types of resistance were encountered, and are related to the types described in earlier published reports. Their recognition by disc tests, and by agar and broth dilution tests, is described.

By far the commonest type, which was found in 26% of all strains examined in 1966, was the so-called inducible or dissociated resistance. In disc tests, there was a slight or moderate narrowing of the zone of inhibition around the erythromycin disc and little or no evidence of resistance to oleandomycin, but after a brief exposure to subinhibitory concentrations of erythromycin the cultures showed very high resistance to erythromycin and a variable but often moderately high resistance to oleandomycin. Under these conditions they remained sensitive to lincomycin.

Other types of resistance encountered were (1) a very high-level non-inducible resistance to erythromycin, oleandomycin, and lincomycin, and (2) a moderate non-inducible resistance to erythromycin and oleando-

mycin. Strains with either of these resistances formed less than 1% of those examined in any year.

Inducible resistance was found in strains with a wide variety of phage-typing patterns, many of which were prevalent in individual hospitals for months or years. Strains with the non-inducible types of resistance were widespread in distribution, but were seldom responsible for groups of epidemiologically related clinical infections.

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J Clin Pathol 1967 20: 918-922
doi: 10.1136/jcp.20.6.918

Notes