The Association of Clinical Pathologists: 86th general meeting

The 86th general meeting of the Association of Clinical Pathologists was held at Douglas, Isle of Man, on 22 and 23 April 1971. There were three sympoisa, 'The Isle of Man—a living laboratory', 'Erythoblastosis foetalis', and 'Chronic obstructive lung disease'. The guest lecture was given by Professor J. Landon on 'Radioimmuno—and related assays: their applicability to all branches of pathology'. The 'buzz groups' on this occasion were concerned with 'Realistic surveillance of infection', 'The Australia antigen' and 'Laboratory hazards'. The remaining of the very full programme was devoted to scientific communications, abstracts of which follow.

Recent Advances in Immunofluorescence Techniques

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Two recent advances in immunofluorescence techniques will be discussed.

The first concerns a marked improvement in optical filters, and the second the use of fibre optics for measuring fluorescence emission from single organisms as small as rickettsia. The optimum wavelength for exciting fluorescence in fluorescein is 495 nanometers because light of this wavelength is maximally absorbed by the dye which then emits light at 525 nanometers. Until recently it has not been possible to use a wavelength of 495 nanometers for this purpose because glass filters could not be made with a sufficiently sharp cut-off at 500 nanometers to allow separation of the exciting light at 495 nanometers from the emitted light at 525 nanometers. Hence it has been customary to use an ultraviolet or near ultraviolet light source to excite fluorescence, because light of the wavelength usually selected (365 nanometers) can be separated easily from the emitted light at 525 nanometers. Rygaard and Olsen have developed an all-dielectric interference filter by means of which it is now possible to use light at the optimum wavelength of 495 nanometers to excite fluorescence, resulting in several advantages which will be discussed.

The photometric equipment consists of a fibre optic probe forming part of the microscope eye-piece, connected, via a photomultiplier, to a photometer. Results of the application of this equipment to titration of a conjugate will be shown, and also measurements of rates of fading for single organisms under varying conditions.

Reaction of a Rabbit Antihuman Thymic Lymphocyte Serum with Leukaemic Blast Cells

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The antihuman thymic lymphocyte sera were raised in large white New Zealand rabbits by intravenous injection of thymic cells obtained at operation. One rabbit was sampled at six weeks and also at nine months of continuous immunization. The sera were inactivated at 56°C and adsorbed with equal volumes of washed human AB red cells. The sera were tested for cytophilic antibody by a modified Teraski technique. Both sera were active (20% kill) at a dilution of 1/80. They were used at a dilution of 1/20 by the indirect fluorescent technique on films and imprints of the material to be tested. Preparations from 75 cases of reticuloendothelial malignancy have been studied. Both the six weeks' and nine months' sera were strongly active against lymphocytes but continuing immunization rendered the serum active not only against normal lymphocytes, lymphoblasts, and plasma cells, but also against granulocytes and even megakaryocytes. The sera would not react with reticulocyte cells, splenic pulp cells, or nucleated red cells except in one case of Di Guglielmo's syndrome. Leukaemic blast cells showed only flecked fluorescence located over the nucleus but more differentiated cells showed cytoplasmic fluorescence as did the abnormal cells of lymphosarcoma. This was in contrast to the cells of reticulocyte cell sarcoma where the cells did not react. The plasma cells of myelomatosis uniformly showed good cytoplasmic fluorescence.

R Factors in Bacteria Causing Asymptomatic Urinary Infection in Antenatal Patients

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Drug resistance of bacteria infecting man has become increasingly prevalent over recent years, particularly amongst organisms which cause infection in hospitals. Resistance in the Enterobacteriaceae is commonly due to the presence of R factors, infective extrachromosomal DNA molecules. The presence of R factors in organisms colonizing the intestinal tract and also in organisms causing infections in hospitalized patients has been well documented. There have only been a few studies on the prevalence of R factors in bacteria causing infections outside hospitals. Because of the prevalence of R factors reported in livestock and human faeces it was of interest to see whether the R factors present in the normal intestine are causing disease in the general community.

Antenatal patients presenting at the Hammersmith Hospital since July 1969 were examined for asymptomatic bacteriuria and the causative organisms for R factors. Asymptomatic bacteriuria was found in 2% of patients and 25% of the causative organisms were resistant to at least one antibiotic. The resistant patterns found were unusual in that all but two of the isolates were resistant to only one drug, chiefly ampicillin (four cases) or sulphonamide (two cases). In no instance could transfer of resistance be shown. The survey is being continued to determine if strains collected so far are representative of the infecting organisms.

Coagulase-negative Staphylococci from the Blood of Neonates

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Obvious signs of infection are not usually present in newborn babies with non-specific symptoms who fail to thrive.

Blood was drawn from antecubital veins and cultures were made from 163 babies who failed to thrive, from 40 babies jaundiced without known cause, and from 55 normal babies. Ninety-six blood cultures were made from placental veins at delivery.

After six weeks there was no growth in specimens from normal babies or from placental veins. There were 20 positive cultures from 163 babies who failed to thrive (12%) and five positive cultures in 40 jaundiced babies (12.5%). The bacteria isolated were Gram-positive, catalase-positive, coagulase-negative staphylococci.

The relationship of this finding to the clinical findings is discussed.

Experience in Estimation of Placental Function in Late Pregnancy

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The small, premature foetus is notoriously at risk peri-natally. An established clinical rule that a birth weight of less than 2,100g and a period of gestation of less...
than 36 weeks, separately or together, are of serious import has been confirmed and amplified by recent studies. During the last six weeks of gestation the foetus grows rapidly, but the placenta does not. The most important measure of placent al function is thus the continuing growth of the foetus. Purely placent al parameters would be useful if their variation were to precede demonstrable foetal damage. A number have been studied.

For several years we have used output of oestriol in 24-hr urine. This substance is finally elaborated by trophoblastic enzymes from dehydroepiandrosterone produced in the foetal suprarenal. Examples of the use of this to foretell slow growth of the foetus will be given and some pitfalls discussed, also its significance in conditions inimical to the foetus such as maternal hypertension or pre-eclamptic toxemia.

More recently we have tried to use the output of oxytocinase (aminopeptidase) produced by trophoblast alone. A series is presented to confirm norms for this and to compare it with oestriol output in complicated pregnancies. The mean level of oxytocinase output varies widely from case to case, so it is even more important to establish a trend over several weeks than for oestriol. Since foetal tissues are not involved, early trouble in the foetus is not revealed, but what can be interpreted as early placental failure has been demonstrated. As in all similar recent studies this is often unconfirmed since, if in doubt, the obstetrician may terminate pregnancy as soon as the foetus is mature enough to risk this and the biochemistry is only one factor in making his decision.

Trypsin and Chymotrypsin Determinations in Human Duodenal Aspirate under the Influence of Continuous Secretin-pancreozymin Stimulation as an Aid in the Diagnosis of Pancreatic Disease

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The output of trypsin and chymotrypsin was measured in the duodenal aspirate of 46 patients free of known pancreatic disease or with normal pancreatic function as defined by volume, bicarbonate, and amylase response. The data from this group were compared with those obtained in 16 patients with objectively defined pancreatic disease and three patients with gastric ulcer and defective pancreatic function. In all, 150 tests were conducted, most subjects having their enzyme output measured under different regimes of stimulation, all hormones being given by continuous intravenous infusion and results expressed as milligrams enzyme per 30 minutes.

With all pancreozymin tests, 0-25 units secretin/kg/hour was used to promote background flow. Insufficient data were obtained to allow evaluation of dose rates of 8 units pancreozymin/kg/hour and 2 units secretin/kg/hour. Some overlap between the two groups was found when 4 units pancreozymin/kg/hour was used, but separation was almost complete when the dose of pancreozymin was raised to 16 units/kg/hour. Better discrimination was achieved with trypsin than with chymotrypsin, although the occasional case in the second group gave low values for the latter only with some forms of stimulation.

It is recommended that for routine purposes trypsin be measured during infusion of 0-25 units secretin and 4 units pancreozymin/kg/hour. Under these conditions an output > 100 mg/30 minutes indicates normal pancreatic function and < 50 mg abnormal pancreatic function. Where intermediate values are obtained, the dose of pancreozymin should be increased to 16 units/kg, and it may be worth while to measure chymotrypsin output as well in order to define the status of the patient more precisely.

The Effect of Instant Cirrhosis on Rat Serum Proteins

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Cirrhosis of the liver is known to be accompanied by marked alterations in the levels of serum proteins. Only in experimental cirrhosis, however, is there a ready opportunity to study serum protein levels before, during, and after the production of cirrhosis.

Cirrhosis was produced in rats using the method of McLean, McLean, and Sutton (1969). Serum samples were obtained at weekly intervals before, during, and after treatment with carbon tetra-chloride and sodium phenobarbitalone, and also from animals treated with either preparation alone. Using a radial immunodiffusion technique the levels of four serum proteins, namely, albumin, slow a1, globulin, transferrin, and y-globulin, were determined and the concentrations expressed as a percentage of a pooled normal rat serum sample. All animals were necropsied and macroscopic and microscopic proof of the presence or absence of cirrhosis was obtained. Striking alterations in the levels of all four serum proteins were observed. The significance of these findings was discussed.