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ASSOCIATION OF CLINICAL PATHOLOGISTS: 43rd SCIENTIFIC MEETING

The forty-third scientific meeting of the Association of Clinical Pathologists was held in the Royal Pavilion, Brighton, from January 26 to 28, 1950, under the Presidency of Dr. Norah Schuster.

At the first session papers were read by J. Ungar (Greenford), R. Cruickshank (London), A. Macdonald (Liverpool), and G. Stewart Smith (Exeter). Summaries of these papers follow.

Comparison of Penicillin Levels in Blood and Inflamed Tissues

Dr. UNGAR said that experiments with rabbits, in which artificial muscular oedema was caused by injection of turpentine, showed that after a single intramuscular injection of penicillin, the latter could be detected in the tissue exudate for considerably longer periods than in the blood. Levels of penicillin were demonstrable in concentrations and after lengths of time sufficient for effective antibacterial action, but no penicillin was detected in the organs of rabbits three or five hours after injection of a single dose of penicillin. This selective accumulation of penicillin in tissue exudate was one of the important factors responsible for the therapeutic effect of single massive doses of penicillin, and gave further justification for this method of treatment, involving less frequent injections. The accumulation of penicillin in the exudate was noticed with aqueous solutions of either crystalline or amorphous penicillin, of penicillin in oil–beeswax suspensions, or of procaine penicillin suspensions. Tissue was shown injected with penicillins G, F, and K.

The Present Position of the Newer Antibiotics

Professor ROBERT CRUICKSHANK spoke about the present position of the newer antibiotics. Besides the infections scheduled by the Ministry of Health as being suitable for treatment with streptomycin and/or chloramphenicol, the Medical Research Council's Antibiotics Trials (Non-tuberculous Infections) Committee was testing these drugs in a number of other infections, including whooping-cough, in different centres in London and the provinces.

With the increasing number of antibiotics now becoming available, the clinician was more often seeking the help of the clinical pathologist not only in regard to the drug sensitivity of the pathogen, but also on the choice of drug and the dosage for particular infections. Factors to be considered in this connexion were the efficiency of the antibiotic, *in vivo* as well as *in vitro*; the risk of toxic effects; and the ease and cost of administration. The variety of causative agents in such conditions as pneumonia, meningitis, and urinary infections called for careful laboratory investigation to establish the drug of choice. More work was needed on the value of combined therapy. Certain drugs have a synergistic action, and the combination of another bacteriostatic drug with streptomycin may inhibit the emergence of streptomycin-resistant pathogens as has happened in the treatment of pulmonary tuberculosis with streptomycin and P.A.S. He advocated the use of massive dosage of penicillin in subacute bacterial endocarditis and in staphylococcal septicaemia, even when the organism was apparently penicillin-resistant. He showed the *in vitro* sensitivity of a wide range of bacterial pathogens to aureomycin, chloramphenicol, and polymyxin, discussed the relative merits of these new drugs, and indicated the methods which might be used for testing bacterial sensitivity and for assay of the drug-levels in blood and other body fluids.

Laboratory Diagnosis of *Toxoplasma* Infection

Dr. MACDONALD spoke of the value of the various laboratory procedures used in the diagnosis of toxoplasmosis, and said that the complement fixation test was probably the most convenient for the ordinary laboratory. Sabin and Feldman's test for cytoplasm-modifying antibody was extremely sensitive, but required the use of living parasites. The soluble egg antigen used for complement fixation tests maintained its potency if kept in the frozen state. In Liverpool they had found good general agreement between the results of different types of test. Examinations of maternal and cord blood showed that transference of maternal antibody occurred so that serological diagnosis had to be made with caution in very young infants. Dr. Macdonald also discussed his serological findings in children with various clinical signs. While he had obtained evidence that infection with *Toxoplasma* was not so rare as had been thought, it was well to remember that toxoplasmosis was only one of a number of conditions which could produce very similar clinical manifestations.

Pulmonary Tuberculosis as a Necropsy Hazard

Dr. STEWART SMITH briefly reviewed the literature in the American medical press which indicated that in three different universities in America and in one in Sweden it had been noted that medical students were more liable to develop tuberculosis than those in other faculties, and that a significant increase in cases occurred while the students were attending necropsies, there a pre-clinical subject. Thus, possible sources of infection were narrowed down. Experimental evidence showed that tubercle bacilli were disseminated from fresh tuberculous material and could be grown from towels, instruments, and gloves for 24 hours after a necropsy. A rigorous review of necropsy-room hygiene had in each case reduced the risk.

Dr. Stewart Smith said that during the past 25 years he knew of six necropsy-room attendants who had developed pulmonary tuberculosis, and three of them had died from the disease. The speaker then outlined measures which could be undertaken if the experience of other members of the Association confirmed that there was an appreciable hazard of pulmonary tuberculosis associated with necropsies. These were (1) a review of autopsy-room hygiene, (2) Mantoux tests and periodic radiological examination of staff, (3) scheduling tuberculosis as an industrial hazard of necropsy work, and (4) limitation of the number of necropsies on frank tuberculous cases.

At the morning session on January 27, the President, Dr. Norah Schuster, took the chair, and papers were read by A. Richardson-Jones (Portsmouth), C. D. Anderson and G. B. Roberts (Glasgow), D. F. Cappell and J. R. Anderson (Glasgow), and A. Spriggs (Oxford). These are summarized below.

Dextran as a Potentiator of Incomplete Anti-Rh Antibodies

Dr. RICHARDSON-JONES said that dextran had shown itself to possess a potentiating action, but in the state in which it was commercially available was unsatisfactory for routine laboratory use because it caused gross rouleaux formation of the test cells and failed to potentiate all incomplete antibodies. He outlined the factors which he considered to contribute to rouleaux formation with dextran : the age of the test cell and the resistance to rouleaux possessed by unwashed cells.

The failure of commercial dextran to potentiate all incomplete sera was thought to be related to its polydisperse constitution. The potentiating property was due to a relatively narrow range of molecular size, and furthermore it seemed as though certain other molecules had an inhibitory effect. In an attempt to relate potentiating power to molecular size he had undertaken the acetone fractionation of commercial dextran. Although this phase of the work was incomplete, he had succeeded in producing a fraction which potentiated all the incomplete anti-sera with which it had been tested. It caused no rouleaux, and gave antibody titres closely comparable with those obtained by albumin potentiation. He felt that this fraction offered a substitute for bovine albumin in cross matching for transfusion and in testing the serum of pregnant women for anti-Rh antibodies.

Dextran was chemically stable and cheap.

Acute Monocytic Leukaemia Terminating with Multiple Chloromata

Drs. ANDERSON and ROBERTS reported a case of monocytic leukaemia in a young girl who had had a leukaemic blood picture and a highly cellular leukaemic marrow.

The patient, after treatment with urethane, developed staphylococcal pneumonia, successfully treated with penicillin. Subsequently, the white cell count in the peripheral blood fell to normal and mature cells reappeared. The patient was discharged, but three weeks later lumps appeared in both breasts. One lump was removed and found to be a chloroma, the histological features of which were very similar to those seen in the bone marrow on admission. Shortly afterwards the patient developed paraplegia and died. Localized chloromatous tumours were found in breast and meninges, both on the surface of the brain and spinal cord. There was no evidence of a diffuse leukaemic process. The bone marrow was normal, and the spleen showed old healed infarcts. The termination of the diffuse leukaemic process had been regarded as an apparent cure. The development of frank leukaemia in chloroma was considered to be a frequent terminal event, but the subsequent development of chloromata in apparently cured leukaemia was unusual.

Mothball Anaemia

Professor D. F. CAPPELL and Dr. J. R. ANDERSON presented a case of megalocytic anaemia with hyperplastic marrow of megaloblastic type. The patient was a girl of 17 whose history included mental disturbances and persistent gastro-intestinal upset. On admission to hospital she was critically ill with a profound anaemia. The findings in examinations of the peripheral blood and sternal marrow were indistinguishable from true pernicious anaemia in relapse. The gastric contents contained free HCl. In spite of slow drip blood transfusion and liver therapy the patient died after three days in hospital.

Necropsy showed the general changes of severe megalocytic anaemia, but without evidence of atrophy of the gastric mucosa. The ileum showed an unusual inflammatory lesion, most severe in its mid-portion where the mucosa was partially necrotic, and incorporated in a thick firmly adherent membrane which resembled fibrin, but was shown microscopically to consist largely of inspissated mucin. Search for a history of possible consumption of drugs revealed that the girl had been in the habit of sucking naphthalene mothballs. Some of the reported cases of acute haemolytic anaemia resultant upon ingestion of naphthalene were mentioned; chronic naphthalene poisoning was noted to be rare, and no record of a chronic anaemia or of a fatal case of chronic poisoning could be found. The metabolism of naphthalene in experimental animals was briefly described.

It was concluded that the case was a true megalocytic anaemia, such as results from deficiency of the haemopoietic principle. None of the usual causes of such an anaemia appeared applicable, and it was suggested that either (1) the prolonged absorption of naphthalene had produced a chronic haemolytic anaemia, which, in association with impairment of absorption consequent upon the intestinal lesion, might have resulted in exhaustion of the haemopoietic principle, or (2) naphthalene may have some more direct inhibitory or destructive action upon the haemopoietic principle or its component factors.

The Morphology of Cells in Pleural and Peritoneal Exudates

Dr. SPRIGGS showed a number of colour photomicrographs illustrating the morphology of the cells of pleural and peritoneal effusions. He described the technique of preparing the smears, a thin, quick-drying film being essential to success. The smears were stained by May–Grünewald–Giemsa and other methods; for instance, mucus secretion had been demonstrated in malignant cells of carcinomatous ascites by the periodic-acid–Schiff technique (following salivary digestion to exclude glycogen).

Dr. Spriggs said that 255 fluids from 175 cases had been examined. Of 30 effusions proved malignant by biopsy of a growth, laparotomy, or necropsy, 20 contained recognizable malignant cells.

The morphology of the endothelial cells was illustrated for comparison. Although frequently showing multinucleate forms and

mitotic figures, the round or oval nuclei showed little variation. Abundant mitotic figures had been found in those effusions where plasma cells were frequent. Slides were also shown illustrating the degeneration of neutrophils in sterile and in infected fluids, the phagocytic properties of macrophages, and the occurrence of unusual cell types, such as "Mott cells," promyelocytes, and megaloblasts.

The second group of papers read on January 27 were by N. F. Maclagan and Barbara Lunnon (London), M. A. Bundock and J. N. Cumings (London), and C. Dent (London), and are summarized.

After lunch two films, by courtesy of the Central Office of Information, were shown: (1) *Tracing the Spread of Infection in Pemphigus Neonatorum*, and (2) *Another Case of Food Poisoning*.

Flocculation Tests

Professor MACLAGAN and Miss BARBARA LUNNON gave an account of their results with the zinc sulphate turbidity test which had been correlated with the serum globulin level and with other flocculation tests in a series of 100 cases in which the diagnosis was reasonably certain on clinical grounds.

They found a less close correlation with serum globulin than that described by Kunkel, but a very close correlation with the serum colloidal gold test. The zinc sulphate test was also roughly correlated with the thymol test, but there were considerable differences in the number of cases. It appeared that the zinc sulphate test was probably more sensitive in chronic hepatitis than the thymol test, but on the other hand it was more likely to give positive results in non-hepatic conditions. The thymol test appeared to be more sensitive in acute hepatitis and gave occasional false positive results in cases of lipaemia.

While the zinc sulphate and thymol tests were frequently in agreement, useful information was sometimes obtained only by one of these tests in particular cases. It was therefore recommended that for diagnostic purposes the best results would be obtained by combining the two tests.

Methods for Estimating the Lange Reaction in Cerebrospinal Fluids

Drs. BUNDOCK and CUMINGS gave an account of a comparison they had made between three methods of performing the Lange colloidal gold test on cerebrospinal fluids from various diseases, but with special reference to neurosyphilis. The techniques used were (1) the standard citrate gold method with saline dilutions of C.S.F.; (2) Maclagan's six tube method with buffered dilutions of C.S.F.; and (3) Lange's method of 1939 in which gum ghatti is used to stop the reaction of a citrate gold with buffered dilutions of C.S.F., and in which specially prepared standards are used for colour matching.

Reasonably good agreement between all three methods was demonstrated in normal fluids and in those fluids showing only minimal chemical abnormalities. The first method was shown to give a few midzone curves, but these gave rise to no difficulty in interpretation when considered with the other findings. It was shown to parallel the clinical findings in general paralysis and tabes better than the third method, and both the first and third methods better than the second. Tables showing the results found in other diseases were also shown, and some variation between the results in the three methods was demonstrated.

The third method was considered to be technically rather difficult, and although useful for a laboratory dealing with many C.S.F.s daily, it was not recommended for routine use in an ordinary clinical laboratory. Drs. Bundock and Cumings recommended the first method, the standard technique, which gave most help to the clinician and was reasonably accurate and relatively simple to perform.

The Technique and Scope of Paper Partition Chromotography

Dr. DENT began by describing the discovery in 1944 by Consden, Gordon, and Martin of a revolutionary method of qualitative analysis. Only filter paper, a few solvents, and a minimum of apparatus were required, and although originally devised for amino-acids in fairly pure solutions, it was now finding applications with many other types of compounds,

for instance, sugars. Furthermore it could be used with very crude mixtures of partly unknown composition such as urine, plasma, sweat, cerebrospinal fluid, tissue extracts, etc.

There were various techniques. In the most simple form strips (2 × 50 cm.) of filter paper were used, the urine (or other fluid to be analysed) being placed near one end, allowed to dry, the end being then dipped into a vessel containing the solvent (usually water-saturated phenol). When the solvent had soaked along the strip a suitable distance it was dried off in an oven. The strip was then sprayed with ninhydrin solution and heated to 100° C. for a few minutes. The amino-acids could now be seen in the form of purple bands spread along the strip where the solvent had passed, and could be provisionally identified from the position they had taken along the strip. A more accurate identification was obtained if large squares (18 × 22 in.) of filter paper were used, two solvents being successively soaked along the paper in directions at right angles to each other. A rapid, mass production method using smaller squares had now been developed. The papers were held together in a frame and almost any number could be run simultaneously. Dr. Dent thought that this should be the most suitable method for routine use.

The method was, first, an ultra-micro method, only microgram quantities of materials being required. As a result, only a few drops of, for instance, urine, were necessary for a fairly complete analysis. Secondly, it was eminently suitable for the study of the metabolism of radioactive materials, since the presence of these on the paper chromatograms could be revealed by pressing the paper against an *x*-ray plate. Thirdly, although it was essentially a qualitative method, it was nevertheless likely to be developed further so that accurate quantitative work could be done.

It must be emphasized, however, that its most spectacular value at the moment was as a purely research weapon. With regard to its ordinary medical applications it was so far limited to the diagnosis of a few rare metabolic disorders in the amino-acid field, and to the identification of sugars and porphyrins. There was no further need to search for cystine and tyrosine crystals in urine. Dr. Dent nevertheless believed that at the present pace of development many other substances, such as steroids, would soon come within the scope of the method and that it was therefore only a matter of time before most laboratories would find it necessary to have some sort of paper chromatographic apparatus.

The Fanconi Syndrome and Other Problems of Amino-acid Metabolism

Dr. DENT stated that in 1936 Fanconi described three cases of an unusual form of "renal rickets." They were all in young children who presented with gastro-intestinal disorders, dwarfism, and the typical bony deformities and *x*-ray signs of florid rickets. The bone condition appeared to be unaffected by the usual doses of vitamin D. In addition, the cases showed a chronic acidosis, proteinuria, renal glycosuria, and an inability to pass an acid urine. However, unlike the findings in classical renal rickets the blood urea and NPN were normal or only slightly raised and the serum inorganic phosphate was low. Other curious urinary features were a high titration for organic acids, a high ammonia coefficient, and some evidence that crystals resembling leucine could be obtained on concentration of the urine.

Similar cases were described about the same time by Debré and by de Toni and later workers showed that the disease was a true entity. More recently an apparently identical condition, in a much more chronic form, had been found in adults, which presented, usually in middle age, as a typical osteomalacia. There was some difficulty in interpreting the literature dealing with a related condition, cystinosis, in which large cystine deposits had been found in the reticulo-endothelial system.

Dr. Dent had been able to collect records and urine specimens from at least 18 cases of the Fanconi syndrome. Chromatographic analysis readily confirmed that there was a gross amino-aciduria involving most of the common amino-acids. Chemical and chromatographic analysis of the blood in several cases showed, however, that the plasma amino-acids were quite normal in their concentrations. The excretion must therefore by definition have been the result of a low renal threshold due to defective tubular reabsorption mechanisms. Other tubular functions were also defective, namely the glucose and phosphate reabsorption, the urine acidification mechanism, and, in some cases, there was poor water reabsorption, which showed clinically as a pitressin-resistant polyuria.

The excretion of large amounts of many amino-acids in the urine was, according to the

previous literature, an excessively rare occurrence, only occurring with regularity in acute yellow atrophy of the liver in which the onset of the amino-aciduria coincided with, and presumably resulted from, a large rise in the blood levels of amino-acids, an "overflow mechanism." Dr. Dent had confirmed chromatographically these hypotheses in five cases of acute yellow atrophy. Further work had now shown that the "overflow mechanism" occurred also in phenylketonuria, where, however, only one amino-acid, phenylalanine, was involved, and when a rapid intravenous transfusion of protein hydrolysate was given, large quantities appearing in the urine. The renal mechanism was the more common, occurring not only in the Fanconi syndrome but also in hepatolenticular degeneration and in cystinuria. There were also interesting variations, relatable to tubule function, in the patterns of excretion of amino-acids in normal people. Most of these renal mechanisms seemed to be under direct genetic control, but acquired tubule damage could cause a similar picture as, for instance, after lead or uranium poisoning. Dr. Dent thought that the term "error of metabolism" when applied as an explanation of amino-aciduria, should be restricted to mechanisms of the "overflow" type where the body tissues as a whole seemed to be unable to metabolize certain substances at their normal reaction rates. He had studied seven cases of cystinuria and had found no clinical or chemical evidence to suggest that this was anything more than a renal cystinuria. It seemed no more justifiable to describe it as an "error of metabolism" of an amino-acid, than it would be to describe the well-known condition of renal glycosuria as an error of glucose metabolism. The question now was why this usually inherited condition of renal amino-aciduria could be associated in some cases with no ascertainable disease at all, and in other cases with such serious organic diseases so utterly unrelated to each other clinically as the Fanconi syndrome and hepatolenticular degeneration. Dr. Dent said that he could not account for this curious anomaly, but could only take advantage of the excellent diagnostic facilities offered by the amino-aciduria.

After tea a paper was read by F. T. G. Prunty (London). Dr. Prunty's paper, "Laboratory Aids in the Assessment of Adrenal Cortical Function," is published in full on page 87 of this issue. Professor G. W. Pickering (London) and Professor Dorothy Russell (London) opened a discussion on aspects of benign and malignant hypertension.

The papers on January 28 were read by C. W. Taylor (Birmingham), N. H. Ashton (London), K. S. Thompson (Birmingham), and G. R. Osborn (Derby), and summaries follow.

Fatal Maternal Embolism by Amniotic Fluid Contents

Dr. C. W. TAYLOR demonstrated the findings from a case of fatal maternal embolism due to amniotic fluid contents, and stressed the importance of microscopic examination of the lungs in patients dying either during or soon after delivery of apparent obstetric shock.

The main clinical features are usually those of a healthy multigravida with a large foetus, very strong uterine contractions, and stillborn baby. During labour or shortly after delivery there is a sudden onset of profound shock with cyanosis and dyspnoea terminating fatally in a few minutes or, at the most, a few hours.

At necropsy there is usually no obvious cause of death, and the emboli are only seen on microscopy.

In the case described, emboli were present in the brain and heart muscle as well as in the lungs. The emboli consisted of plugs of epithelial squames similar to those seen in the alveoli of infants who have attempted to breathe *in utero*.

New Techniques in the Laboratory Examination of the Eye

Dr. NORMAN ASHTON described new methods which had been devised and familiar techniques which had only recently been applied in the research and routine examination of the eye. He showed how the retina might be examined macroscopically and microscopically by mounting it in a glass sphere the same size as the sclera, and retinal micro-aneurysms demonstrated by benzidene staining; by the periodic method of McManus; by indian ink; and by neoprene injection of the central retinal vessels. Dr. Ashton showed neoprene casts of

Schlemm's canal and its branches, and pointed out the value of this technique in the study of aqueous veins. He described his museum mounting techniques, particularly those using solid "perspex" and "marco" resin. He concluded with an account of the examination of the aqueous humour by puncture of the anterior chamber, and spoke of his experiences with the "hot" celloidin embedding technique for rapid section of the whole eye.

A Correlation of the Histology and Cytology of the Female Genital Tract

Dr. G. R. OSBORN gave an account of his experiences with the Papanicolaou method of cytodiagnosis applied to the female genital tract.

The Papanicolaou smear was at its best in cases of carcinoma *in situ* and typical squamous-cell carcinoma of the cervix. Some tumours, especially adenoacanthomata of the endocervix and recurrences after radium treatment, had been found to desquamate few or no cells, although studied repeatedly. It was because of the existence of this type of tumour that smears of cervical scrapings should be examined. The surgeon had to be skilled technically or the smears were hopeless for diagnosis.

Adenocarcinoma of the body of the uterus often shed actual tumour fragments containing mitotic figures, as a rule fully diagnostic, as they drew attention to the importance of seedling implants in the Fallopian tubes, endocervix, vagina, and operation wounds.

The most important pitfalls in cytodiagnosis were badly taken and badly stained material; trichomonas vaginalis, which could be recognized with experience to give pathological smears unlikely to be confused with cancer; degenerating epithelial cells (especially in atrophic vaginitis) which should not be confused with mitotic figures; atrophic vaginitis, responding to oestrogens; histiocytes, which could cause endless mistakes, as with clusters of endometrial cells they gave false positive diagnoses; inflammatory reactions giving very bizarre appearances; the carcinoma without desquamation would be missed by the ordinary methods, a negative report not necessarily indicating the absence of a tumour; and the carcinoma which desquamated so few cells that a confident diagnosis was not made.

Dr. Osborn did not think that the value of the Papanicolaou smear in the diagnosis of radiation response was proved.

The meeting ended with a discussion on "The Reconstruction of Human Remains" which was opened by Professor J. D. Boyd (London), and those taking part included C. Keither Simpson (London), R. D. Teare (London), F. D. M. Hocking (Truro), and D. E. Price (Barnsley).

About 200 members and guests attended the meeting. The guests included Dr. D. Jaumain (Belgium) and Professor Raoul Kourilsky (Paris), president of the Societé Française du Biologie Clinique.

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Notes