ASSOCIATION OF CLINICAL PATHOLOGISTS:
49th GENERAL MEETING

The forty-ninth general meeting of the Association of Clinical Pathologists was held in London from October 2 to 4, 1952.

At the first session the Chairman was Dr. S. G. Platts, and three papers were read.

**Benign Polypoid Gastric Tumours**

Dr. H. G. H. Richards (Winchester) introduced his subject by describing briefly the clinical features of these tumours, and classified them into adenomas, pancreatic rests, fibromas, leiomyomas, neurogenic tumours, lipomas, angiomas, and miscellaneous rarities.

The incidence of these tumours was compared with that of peptic ulcers and gastric carcinomas in the Royal Hants County Hospital. The benign group formed about 10% of all gastric tumours (six benign and 53 malignant). The figures were compared with those of other writers.

The morbid anatomy and histology of the individual cases in the various types of tumour were discussed. It was pointed out that epithelial polyps varied from what amounted to simple hypertrophy of the gastric mucosa to frankly adenomatous tumours with early malignant change, all degrees between these extremes being encountered on occasion. A case of polyoid gastric tumour due to a pancreatic rest was described and its typical parenchyma demonstrated; the absence of islet tissue was commented upon.

Next three cases of leiomyoma were described and the similarity of one of them to what in any other situation would be called a neurilemoma was discussed. The central crater in these tumours and its importance to the radiologist was pointed out.

The typical lobulated appearance and ease of diagnosis in a lipoma of the stomach was demonstrated.

**The Pathology of the Gastric Arteries, with Special Reference to Fatal Haemorrhage from Peptic Ulcer**

Dr. G. R. Osborn said that although it had been taught for over 100 years that peptic ulcer was related to arteriosclerosis the gastric arteries were practically free from the usual forms of sclerosis. In 1951, 327 partial gastrectomies were performed at the Derbyshire Royal Infirmary. Arteriosclerosis was not seen in any of these.

The gastric vessels were examined in 32 sudden deaths from coronary thrombosis. There were 25 males aged 41-85 (average 61 years), and seven females aged 55-82 (average 74 years). The gastric vessels were all normal with four minor exceptions: three had localized lesions of inflammatory origin, only one (a woman of 74) had an atheromatous plaque, and this was too small to affect the size of the lumen. The splenic and superior mesenteric arteries did not share this immunity from sclerosis. A suggested explanation was that the five series of anastomoses protected the gastric arteries from stresses which produce sclerosis elsewhere.

Arterial lesions related to peptic ulcers should be termed chronic, subacute, or acute erosion. In chronic erosion mucinous infiltration of the intima greatly reduced the size of the lumen; this was the main reason why every patient with peptic ulcer did not bleed to death. In subacute erosion there was an organizing thrombus at the distal (eroded) end; elsewhere the artery was normal. The typical lesion of fatal haemorrhage was acute erosion, which would prove fatal if gastrectomy were not performed in the pre-uraemic stage.

**Difficulties in the Use of Antibiotics**

Dr. J. D. A. Gray said that antibiotics had conferred such benefits on mankind that the dangers from their use had tended to be overlooked.

The toxic effects, such as damage to the eighth nerve by streptomycin and the blood dyscrasias after chloramphenicol, were now well known and could usually be avoided. Modern penicillin was non-toxic, but large doses intrathecally were dangerous.

Sensitization of patients to antibiotics had occasionally produced alarming effects. To prevent sensitization a plea was made against the use of penicillin for minor skin ailments.

It was difficult to determine whether the mucous membrane lesions were due to avitaminosis or to a change in the flora.

When penicillin was used as a prophylactic in surgery of the urinary tract or meningitis infections with *Pseudomonas pyocyanea* were common and might be prevented by giving a sulphonamide at the same time. Moniliasis might occur after antibiotic therapy in the alimentary, respiratory, and urinary systems, and might be fatal in a case of lung abscess or bronchietatic cavity.

When an organism became resistant further treatment with the antibiotic might harm the patient and expose his contacts to infection with resistant organisms. The incidence of resistance was increasing; its mechanism was still a subject for speculation; its development could sometimes be avoided and its ill effects overcome by the use of other drugs.

Antagonism between bactericidal and bacteriostatic antibiotics seemed to be limited to cases where the organism was sensitive to the bactericidal drug.
At the second session Professor W. D. Newcomb took the chair.

On the Origin of Visceral Melanomata

Dr. Oliver C. Lloyd said that primary melanomata occasionally arose in the adrenal or small intestine. He described a case of the former with multiple metastases, in which there was no evidence of a primary tumour in skin, eyes, or meninges; it was the largest in the body. Sixteen other instances had been described, in four of which there were no metastases, so that there could be no doubt that primary melanomata do occur in the adrenal.

Dr. Lloyd then attempted to demonstrate the cell of origin. Specific silver stains for melanin showed granules to be present in 57% of 44 human adrenals examined, irrespective of age or sex. They were found in the cytoplasm of the adrenal medullary cells (phaeochromocytes). The cells affected were often scanty. Fontana's silver solution applied for eight hours or less was not quite specific for melanin, as claimed by Masson, as it also blackens the pigment of ganglion cells. Becker's method, as described by Baker (1938), used 5% silver nitrate at 37° C. for one to two hours. If this were used for only one hour it would blacken nearly all true melanin, including that found in the phaeochromocytes. All adrenal pigments, including melanin, were bleached by nascent chlorine.

He concluded that adrenal melanomata were derived from medullary phaeochromocytes, and that they could be melanogenic was scarcely surprising in view of their common origin from the neural crest with the melanoblasts of the skin and meninges.

Reference

Lingual Biopsy in Mitral Stenosis

Dr. J. B. Enticknap said that specimens had been taken from the lingula segment of the left upper lobe during operations for mitral stenosis in the hope that their examination would be useful in assessing prognosis. They had not been found of much value in individual cases, but some useful information had been derived from the series as a whole.

While the parenchymal changes in the lingula were not typical of the entire lung, the vascular changes did appear to be. Arteriolar sclerosis and hypertrophy were common and both reduced the vascular lumina; as they occurred in similar morbid physiological conditions they had been assessed together by measurement of lumen to wall ratios. Considerable reduction of the lumina was seen, the mean ratio being about 4:1. All the patients had pulmonary hypertension and the lower ratios occurred with the higher pressures; a significant correlation was discovered which could be expressed by the formula:

\[
\text{arteriolar lumen/wall ratio} = 21.3 - 3.03 \times \frac{\text{mean pulmonary artery pressure}}{3.03}
\]

Arteriolar hyalinization was not seen in these sections; a possible explanation was to be found in the fact that the highest pulmonary systolic pressure in these patients (120 mm Hg) was much less than that found in the brachial artery in cases showing renal arteriolar hyalinization. In two cases with necrotizing arteriolitis the pressures were about 20 mm. lower than the highest in this series and both showed either histological or clinical signs of active rheumatism; it seemed probable, therefore, that arteriolar necrosis in mitral stenosis was related to circumstances other than simple excessive strain. Capillary dilatation was seen in about a third of the cases; their pressures did not differ from those of the remainder and they showed no arteriolar changes.

On October 3 Dr. E. N. Davey took the chair and five papers were read.

The Differentiation of Primitive Leukaemic Cells by In Vitro Culture

Dr. D. Robertson Smith described a method of differentiating myeloblasts, lymphoblasts, and monoblasts by a simple *in vitro* culture.

The bone marrow or peripheral blood is placed in heparinized Tyrode solution and the nucleated cells are separated by centrifugation or sedimentation. Cultures are set up in a medium consisting of equal parts of serum from the patient and Tyrode solution, the final cell concentration being about 2,000 cells per c.mm., and 3-ml amounts are placed in each of three McCartney bottles. A total and differential count is made and bacteria (either *Staphylococcus* or *Bact. coli*) are added to one tube. The bottles are incubated at 37° C. and examined after four hours and 24 hours.

Myeloblasts mature into promyelocytes and myelocytes after 24 hours, but show no phagocytic activity. Lymphoblasts do not mature or show phagocytic activity *in vitro*, but degenerate rapidly into smear cells. Monoblasts are highly phagocytic in culture and develop into more mature forms. In the Naegeli type of mononcytic leukaemia some of the blast cells are phagocytic (monoblasts) and others are non-phagocytic (myeloblasts), whereas in the Schilling type all are phagocytic.

This simple *in vitro* culture method was found to be valuable where routine methods of distinguishing primitive leukaemic cells failed.

The Testing of Plasma and Its Fractions for Anti-haemophilic Potency

Dr. W. R. Pitney and Dr. J. V. Dacie briefly described a method of studying the generation of thrombin in recalcified citrated plasma by means of subsampling into fibrinogen solution. Normal thrombin generation curves were shown, and contrasted with the far slower evolution of thrombin in cases of haemophilia.

Illustrations were given of the value of thrombin generation curves in assessing the effects of normal plasma or fibrinogen fraction on haemophilic plasma both *in vivo* and *in vitro*, and on the effects of storage at 4° C. on anti-haemophilic globulin.
The Value of Cytochemical Methods in Diagnostic Haematology

Dr. F. G. J. Hayhoe discussed five cytochemical staining methods from the viewpoint of their applicability to routine diagnostic work. The Feulgen reaction specifically demonstrates desoxyribonucleic acid, which is confined to the cell nucleus. The pattern of nuclear staining resembles that seen in a good Leishman preparation. The intensity of the reaction increases with maturity of the cell. Nucleoli are invariably negative and surrounded by a strongly positive ring of nucleolus-associated chromatin, which makes them readily recognizable. The reaction has a practical application in assessing cell maturity, enumerating nucleoli, and defining nuclear chromatin patterns.

The demonstration of glycogen by the periodic-acid-Schiff reaction gives consistent results and the method is satisfactory and valuable in academic studies. Glycogen is confined to the cell cytoplasm; the erythropoietic series is negative at all stages; the myeloid series shows increasing numbers of positive granules with increasing maturity; eosinophil granules are negative against a positive cytoplasmic background; and lymphocytes and monocytes contain small amounts of glycogen. No significant variations in cell glycogen content seem to occur in blood dyscrasias, however, and the method has no great practical application in diagnostic work.

Sudan black B stains lipids excellently. The myeloid series show increasing numbers of positive granules with increasing cell maturity. Eosinophil granules show a positive periphery with unstained centre. Basophil granules are negative. Lymphocytes and monocytes contain scattered granules. Although cells from blood disorders do not greatly differ in reaction from comparable normal cells, the reaction is of diagnostic value in demonstrating positive granules in early cells of the myeloid and monocytic series. For this purpose Sudan black seems superior to the commonly used peroxidase reaction.

The techniques of Gomori for acid and alkaline phosphatase demonstration are too unreliable to be of value in routine work.

The Coagulation Defect in Tromexan Therapy

Dr. A. S. Douglas described the investigation designed to elucidate the prolonged one-stage clotting time following the administration of tromexan. Factor V was found to be normal. The addition to tromexan plasma of small proportions of normal serum, which contained no prothrombin, was found to shorten the one-stage clotting time often to normal, while tromexan serum was deficient in this property. This action of serum was described as its factor VII property. Therefore the one-stage test in tromexan therapy reflected a deficiency other than prothrombin. A comparison was made between tromexan plasma and that of a patient with an idio-pathic prothrombin deficiency, an excessively rare condition. From a study of this patient's plasma a new two-stage method for the measurement of prothrombin had been devised (Biggs and Douglas, 1952, unpublished data). This patient's plasma, with 10% prothrombin determined by this method, had a one-stage clotting time only slightly prolonged, and his plasma was able to correct the defect in tromexan plasma as well as did normal plasma. By the two-stage technique the prothrombin content of tromexan plasma was estimated and was only occasionally found to be lower than 40% of normal, and usually lay between 40 and 100%. It was concluded that the one-stage clotting time in tromexan therapy was measuring factor VII rather than prothrombin. Using these methods the effects of therapy on prothrombin and factor VII were followed. The factor VII dropped at the onset of therapy to a very low level, the prothrombin some days later, and to a much less extent.

The Significance of Histologically Demonstrable Iron in Sternal Marrow Biopsies

Dr. H. E. Hutchison said that the amount of stainable iron in sternai aspirates had been estimated by the Prussian blue reaction. The superiority of histological sections over smears for the purpose of iron staining had been stressed. The results of applying this simple histochemical test routinely to sections prepared from 141 marrow samples were described, and it was shown that, in disorders of the blood, the procedure provided a simple means for the control of iron therapy. It was held as established that in an anaemic subject the absence of stainable iron meant that the patient was deficient in iron, and it was believed that this technical method gave the most decisive haematological indication yet devised for the recognition of iron deficiency. So far as his experience carried him, the converse had also proved true—notably, that if stainable iron were present in the marrow the anaemia would not be improved by the administration of iron whether by mouth or intravenously. Over the past two years, used in this way for the control of iron therapy, the method had not failed, and he had been able to predict when an anaemia would be likely to respond to iron. The method also indicated when enough had been given, and so could be used to obviate the danger of overdose when iron was being administered by the intravenous route. The method was put forward as a possible means of disintinguishing the hypochromic anaemias of chronic intoxication from those due to iron deficiency.

Dr. A. F. Sladden then took the chair, and four papers were read, ending the morning session.

The Early Detection of Potassium Deficiency

Dr. L. Naftalin said that in the post-operative state a correlation existed between the amount of potassium excreted in the urine and the clinical condition. The relation was not a directly causative one in every case, but it had been found that patients in good nutritional status excreted a larger amount of potassium and had an easier clinical course than the
more poorly nourished patients who were unable to excrete potassium. He pointed out that at present this was known only after the event, and he proposed an approach to the earlier detection of potassium deficiency based on the finding of a high serum bicarbonate level, low serum chloride level, normal or near normal serum sodium level, and a history or clinical evidence of reduced food intake. Relevant data from nine case histories were given.

The Interpretation and Principles of Strip Paper Electrophoresis

Professor N. H. Martin said that analysis of serum proteins by paper strip electrophoresis had become a routine technique in many laboratories. Because the procedure was still very largely empirical it was vital that its limitations and pitfalls should be appreciated. In the laboratories at St. George’s Hospital, London, because of atypical separations liable to be seen when the serum proteins are dried on the filter paper, "wet boundary" techniques have been elaborated. This has involved some loss of clarity of outline. A rigid comparison with the classical electrophoresis has been carried out and it is certain that differentiations achieved by the old technique are not constantly mirrored in the new. The variations in mobilities obtained with purified factions, whether using dextran or albumin markers, indicate that these can only form the crudest means of identification. Quantitative studies show that the binding capacities of the various proteins differ so markedly that correlation between the classical and the paper method by dye elution techniques is impossible, unless some form of correction factor is used. It is impossible, with our present knowledge, to forecast how far empirical correction factors for normal sera are valid for pathological sera. It is difficult to understand the good agreement obtained by some authors between the classical and the paper technique using the Folin reagent if the known difference in tyrosine content of the purest isolated protein fractions is borne in mind. While the Kjeldahl analysis on sectioned strips would be satisfactory, the labour involved seems to negative this as a routine procedure.

With widening experience the hope that the classical and paper strip methods might be equated, a hope which has little justification in theory, is diminishing. It is clear that the technique of paper electrophoresis should, in future, stand alone and, when subject to criticism, must not necessarily invoke apparent qualitative or quantitative similarity with the classical technique as a defence.

The Tubeless Test Meal

Drs. John Harkness and John A. Durant reported their results with Segal’s tubeless gastric analysis, which depends on the power of the hydrogen ion of free hydrochloric acid in the gastric juice at a pH below 3 to displace the quininium ion of a quininium-resin compound; the liberated quinine is absorbed from the intestine and excreted in the urine, where it can be estimated readily. Segal’s quininium-resin indicator is not available commercially in Britain, but an equivalent product has been made from material available here.

(The details of the techniques for the preparation of the quininium-resin, for the estimation of quinine in urine, and for the ward procedure were duplicated for issue to interested persons, and presented separately as a demonstration.)

One hundred and twenty-four duplicate gastric analyses were made in 104 subjects by the ordinary alcohol method and the quinine-resin method. Histamine had to be given to some cases to ensure the secretion of hydrochloric acid for both methods. The patients with free hydrochloric acid excreted considerably more quinine than did the achlorhydric patients; the results of the two groups did not overlap. Four patients showed discrepancies; two patients with free HCl could not excrete quinine because of poor renal function, one achlorhydric patient excreted quinine because of interference by aluminium silicate therapy, and one apparent discrepancy is still being investigated to decide which method is actually yielding the correct results. Patients with achlorhydria due to pernicious anaemia excreted less quinine than those patients with achlorhydria following partial gastrectomy.

Wider trials of the new technique were recommended.

Normal Values for Blood Constituents and Comparative Results from Different Laboratories on the Same Samples

Dr. I. D. P. Wootton and Professor E. J. King said that normal values for most of the commonly determined blood constituents had now been determined. Only some of the constituents are distributed among the population as "normal curves," almost all the remainder showing frequency distributions which are skew and fitted by lognormal curves. For ready reference tables have been calculated showing the limits within which 80% to 98% of the population of healthy people fall.

To determine the possibility of using these tables in other hospitals, agreement between hospitals has been examined. One blood sample and one synthetic solution have been analysed and reported by 25 and 36 laboratories respectively. For most constituents, there are very wide variations in the results, the highest figures reported being two to five times the lowest figures, although results for the solution were better than for the blood. This gives a very bad impression of the reliability of the work of clinical laboratories.

In the case of haemoglobin measurements, an attempt has been made to improve the situation by the Medical Research Council Haemoglobin Standard Scheme, operated from the Postgraduate Medical School of London. Each month a large sample of blood is analysed carefully for its haemoglobin content, using three standard methods of proved accuracy which are described. The blood sample, whose
haemoglobin content is now known with an accuracy better than 2%, is then distributed to interested laboratories in all parts of the world, where it is used to check and calibrate apparatus. At present about 75 laboratories receive the monthly samples.

For the first of the last two sessions on October 4 the chair was taken by Dr. A. Renshaw, when four papers were read.

The Mode of Action of Calciferol in Lupus Vulgaris

Dr. G. Wetherley-Mein briefly discussed the possible ways in which calciferol might act in lupus vulgaris, and the results of an experimental study of this problem were reported.

Standard virulent tubercle bacilli (H37Rv) were cultured and subcultured on a variety of media containing calciferol in concentrations ranging from 50 to 100,000 units per ml. of medium. Certain strains were subcultured at approximate intervals of 30 days for over two years. Comparison with controls showed that calciferol had no effect on the rate of growth, the colonial or bacillary morphology, or the virulence. Similar studies using lupus strains of attenuated virulence showed that they also were unaffected by culture and subculture in the presence of calciferol.

The sera of various tuberculous and non-tuberculous patients receiving high doses of calciferol (150,000 units daily) had no effect on the growth or morphology of tubercle bacilli.

The rate of healing and histological changes occurring in experimental wounds in guinea-pigs were in no way modified in animals receiving high doses of calciferol.

The findings, coupled with the clinical and experimental observations of Stringer (1948), Jensen (1948), and van der Lugt (1952), suggest that calciferol acts by enhancing the specific cellular reaction of the body to the chemical fractions of the tubercle bacillus following local accumulation of the vitamin in the skin.

References

Van der Lugt, —. (1952). Personal communication.

The Sensitivity and Resistance of Myco. Tuberculosis to Isoniazid

Dr. R. Knox reported that strains of tubercle bacilli freshly isolated from the sputum of 40 patients with pulmonary tuberculosis were found to be initially sensitive to isoniazid in a concentration of about 0.01 µg per ml. when a tube-titration method in Dubos liquid medium was used. Standard conditions must be used because the end-point changes with prolonged incubation, partly because of deterioration of the drug, partly because of the development of resistance.

In the course of treatment of a small series of patients with isoniazid resistant cultures developed very rapidly. The numbers of tubercle bacilli in the sputum showed a rapid decrease at first, followed by a reappearance associated with the development of resistance. The relation, however, between resistance in the test-tube and in the patient was not always clear.

Compared with resistance to streptomycin, resistance to isoniazid seems to be produced even more easily, at least in the laboratory, but it does not seem to reach the very high levels which can be obtained by streptomycin-resistant organisms. Isoniazid-resistant organisms retain their sensitivity to streptomycin and vice versa. On the other hand, streptomycin appears to delay the development of isoniazid resistance both in the laboratory and in the patient.

Risk in Routine Tuberculosis Investigations

Dr. W. Howard Hughes reported that, in an attempt to assess the risk to laboratory workers from the spilling of tubercle bacilli during the routine investigation of infected material, cultures were made regularly from the working site after the batch of work had been completed. The method used was to work in large enamel developing dishes lined with cellophane and then to wash this and culture the centrifuged deposit.

Results showed that the spluttering seen when wires were sterilized was of little importance, whereas pouring of homogenized material from the original containers to 15 ml. centrifuge tubes was accompanied by heavy contamination. This spilling could be reduced or eliminated entirely by special care at this stage, and by substituting 25 ml. wide-mouth bottles or 25 ml. centrifuge tubes for the smaller sizes.

Change of staff or alteration of method always caused a temporarily increased risk.

The Renal Localization of Ps. Pyocyaneus in Mice

Dr. R. H. Gorrell said that the pathogenicity of Ps. pyocyaneus was tested by injecting varying numbers of organisms intravenously into groups of white mice. It was found that with large numbers of organisms the mice died of septicemia in one or two days. When the dose was lowered the mice survived the first two days and then died within the next fortnight of renal carbuncles. In all 12 strains were tested, derived in equal numbers from wound and urinary tract infections, and the same results were obtained with them all.

The earliest renal lesions were seen at 20 to 26 hours following injection of a suitable dose of Ps. pyocyaneus, and consisted of a focal embolic nephritis.

When the distribution of bacteria was followed in the blood, lungs, liver, spleen, and kidney following intravenous injection, it was found that the blood became sterile in 12 to 18 hours and in the lungs in 24 to 48 hours. The liver showed progressively fewer organisms throughout the experiment, but rarely became sterile. The spleen remained about constant or fell, but the kidneys showed a progressive rise in bacterial count.

It is suggested that owing to its special anatomical and physiological properties the kidney tends to filter
out all types of particulate matter passing through it. When pathogenic bacteria like *Ps. pyocyaneus* are filtered out they multiply and rapidly attack the kidney tissue producing renal abscesses.

At the last session Dr. A. G. Shera took the chair.

**Physiological Variations in the Blood Count**

Dr. John Marks said that the value of a laboratory test depended on the accuracy of the method balanced against the range of physiological variation.

The magnitude of this physiological variation and the factors involved were discussed. Venous blood gave lower results than capillary and was used throughout.

**Age.**—Variations in age have no effect on the blood count.

**Sex.**—The red-cell counts are well known to be lower and the sedimentation rate higher in the female. The leucocyte count is also significantly higher in females.

**Time of Day.**—The blood count in both red and white cells is lowest in the early morning and rises to a maximum in the afternoon.

**Exercise.**—Even moderate exercise produces a rise in the leucocyte count.

**Environmental Temperature.**—During acclimatization to heat there is a fall in the red-cell count. Exposure to cold causes a rise.

**Menstruation.**—No significant differences occur during the various stages of the menstrual cycle. It was suggested that serial counts should be performed under "basal" conditions—that is, in the early morning, with activity and temperature change restricted. A set of basal normals was given. This range was smaller than the "random" range for routine diagnostic counts on capillary or venous blood with the causes of variation not controlled.

The values quoted differ from the accepted range.

**Papain-treated Red Cells in the Detection of Rhesus Antibody**

Dr. K. Goldsmith said that papain was a proteolytic enzyme extracted from a plant called carica papaya, or pawpaw. Like trypsin, it might be used to render a saline suspension of Rhesus-positive cells agglutinable by incomplete Rhesus antibody.

The method used was modified from a technique described by Kuhns and Bailey (1950). Six hundred and fifty maternal sera were examined and only two false positive results were obtained, these both being due to cold agglutinins.

Twenty-one sera containing Rhesus antibody were examined, and in no case was agglutination absent when papain-treated cells were used. The antibody titre was noted, using saline, albumin, antiglobulin, trypsin, and papain techniques. In all cases the titre with the papain method was as high as, if not higher than, with the others.

During tests with papain-treated red cells in other blood-grouping systems enhancement of agglutination was noted with anti-A, anti-B, anti-P, anti-M, anti-N, anti-Le\(^a\), and anti-Le\(^b\). No agglutination occurred with anti-Fy\(^a\).

Papain is a readily obtainable laboratory reagent cheaper than commercial bovine albumin or crystalline trypsin. Rouleaux formation is largely obviated and the technique seems to be as sensitive for routine use as any other yet available.

**Reference**