ASSOCIATION OF CLINICAL PATHOLOGISTS:
58th GENERAL MEETING

The 58th General Meeting was held at Torquay on April 11, 12, and 13, 1957. Abstracts of the scientific papers follow.

 Symposium on Radioactive Materials and the Pathologist

D. H. Collins (Sheffield) introduced the symposium. Those hospital pathology departments already handling radioactive tissues or substances in their routine work have had to devise techniques that not only guarantee safety but ensure the best use of scientific material. Where possible the pathologist should collaborate with a team of medical scientists and physicists, but the use of isotopes will undoubtedly become wider and all pathologists should know about them. Moreover, everything that relates to pathogenic agents and pathological mechanisms is a proper field of study for the pathologist. He is in the position to detect the effect of noxious elements in man's environment.

While most of the radioactive materials used clinically pass the laboratories, a considerable amount converges there through various channels. The risks involved are sometimes serious, but they are also often negligible. The pathologist himself must know how to assess these risks. He must therefore be informed of the radiation history of every piece of potentially radioactive tissue, cadaver, or other biological material coming into his department.

Pathologists must adopt techniques for handling radioactive tissues safely, just as they adopt safety methods in bacteriology and in other branches of their craft.

In considering the dangers, it must not be forgotten that radioactive substances are used in medicine for good purposes, among which are their uses in pathological studies. Radio-assay and perhaps autoradiography may soon become standard laboratory methods.

The purpose of this symposium is to arouse the interest of clinical pathologists so that they may play their full and proper part in the coming era of radiation medicine.

The Injurious Biological Effects of Radiation

R. H. Mole (Harwell) said that what is known of the cellular effects of irradiation was of little help in understanding the delayed effects in tissues and in the whole animal. Interference with the processes of repair is a characteristic feature, and one basic problem is whether malignancy is a sequel to prolonged and inadequate repair or is due to some sub-microscopic cellular change such as "mutation." Another characteristic feature is the time required for biological damage to show itself. The time scale is even more drawn out in man than in animals, so that the sperm count may be at a minimum six to 10 months after a sublethal exposure. A limited exposure to radiation in early adult life may shorten the eventual life span. In fact the sequel of low-level irradiation may be conveniently, if not very illuminatingly, described as premature ageing.

Clinical experience has shown that 1 μg. of radium in the body may cause bone tumours. Thus 0.1 μg. is taken as the body burden which will not do harm, i.e., the maximum permissible level (m.p.l.) for occupational exposure. The m.p.l. for other radioactive materials, e.g., radioactive strontium, is based on this and takes into account the amount of radiation energy absorbed in tissue, differences from radium in degree of uniformity of deposition, and metabolic differences in excretion and in uptake from food and water. Each radioactive species has to be considered on its own merits. Maximum permissible levels for the general population are set at one-tenth of those for occupational exposure.

Handling of Radioactive Tissues in Laboratory and Post-mortem Room

R. C. Curran (Sheffield) said that already a considerable number of radioactive isotopes were in use in hospitals. Many of these are given in trace doses; and, though it is always wise to avoid all unnecessary radiation in view of the lack of knowledge of the effects on man of even a small increase over his "normal" radiation background, the hazard from handling these is comparatively slight. However, some isotopes, including iodine 131, gold 198, and yttrium 90, are administered in far greater doses for therapeutic purposes. When the pathologist is asked to examine a surgical specimen or perform a post-mortem examination of a body containing such large quantities of radioactive material, it is essential for him to have all the relevant information. The hospital physicist should determine the residual radioactivity, and this information allows the pathologist to decide how to conduct his examination.

Several tables illustrated the procedure to be followed in handling such specimens or bodies.
The importance of scrupulous cleanliness in the laboratory and post-mortem room cannot be over-emphasized after dissection of a highly radioactive specimen, and all appropriate areas of these departments should be monitored by the physicist, who will be able to detect any persistent foci of radioactivity that must be eliminated. This not only avoids any danger to health, but also rules out the possibility of vitiating other investigations which employ trace doses of isotopes. To illustrate this type of study, the application of autoradiographic location of radioactive isotopes in pathological lesions in man was briefly demonstrated.

Role of the Laboratory in the Control of the Hypothemic State

P. I. Reed (Maida Vale Hospital for Nervous Diseases, London) reported investigations carried out in the Department of Neurosurgery, Maida Vale Hospital, on 31 patients undergoing craniotomy under hypothermia for vascular abnormalities to demonstrate possible differences in biochemical findings compared with cardiac surgery, and what screening tests, if any, were of value in anticipating any of the recognized or theoretical complications of hypothermia.

Significant changes were found in the serum bicarbonate, blood sugar, and urea levels, the bicarbonate decreasing from 28.0 mEq./l. pre-operatively to 24.2 mEq./l. at 27.5°C and 24.4 mEq./l. post-operatively; the sugar rising from 107 mg./100 ml. to 207 mg.% at 27.5°C and 148 mg./100 ml. post-operatively, and urea from 25 mg./100 ml. to 30 mg./100 ml. and 40 mg./100 ml. respectively. The changes in the serum sodium, potassium, calcium, chloride, and prothrombin and serum fibrinogen concentrations, coagulation time and platelet counts, were not significant.

These findings were compared with those described in cardiac surgery, and it was concluded that there are significant differences in the electrolyte picture. Neurosurgical cases are much less prone to acid-base imbalance, stimulating ventricular fibrillation, are potentially less likely to develop liver damage and in consequence less prone to abnormal bleeding tendencies.

The following tests are therefore worth carrying out. The serum potassium and bicarbonate before and during hypothermia, as a guide to correcting an acidosis, and blood sugar and urea to exclude diabetes and early uraemia respectively. Other tests are only indicated when complications unassociated with hypothermia are suspected, and it is therefore felt that there is no way at present in which the laboratory can anticipate any danger due to hypothermia itself, apart from helping to exclude from it those patients in whom this procedure is contraindicated.

5-Hydroxytryptamine Deficiency in Phenylketonuria

C. M. B. Pare, M. Sandler, and R. S. Stacey (London) presented evidence of a defect in the 5-hydroxyindole pathway of tryptophan metabolism in phenylketonuric patients. They showed that there was a significant decrease in serum 5-hydroxytryptamine (5HT) levels and urine 5-hydroxyindoleacetic acid excretion compared with control groups. Surprisingly high levels of serum 5HT were found in some control mental defective children.

They thought that the low levels of circulating 5HT might be of importance in providing an explanation of the cause of the mental deficiency in phenylketonurics, for there was a body of evidence which suggested that 5HT was functionally important in the central nervous system.

The improvement in mental function which occurs in some phenylketonurics on a low phenylalanine diet may be related to the rise in circulating 5HT which they had observed in a small number of patients undergoing this form of treatment.

A possible form of treatment upon which they had embarked was the administration of 5-hydroxytryptophan which passes the blood-brain barrier to be metabolized to 5HT in the central nervous system.

As a working hypothesis to explain the 5HT deficiency, it was suggested that the defect in hydroxylation of phenylalanine may affect hydroxylation of tryptophan, either by providing competition for a limited hydroxylation mechanism or by the inhibition of tryptophan hydroxylase by phenylalanine one of its metabolites.

The Proteins of Normal Urine

G. Grant (Shrewsbury) said that the proteins of normal urine were concentrated by negative pressure ultra-filtration and then examined by the technique of Grabar and Williams, a technique by which the proteins are first separated electrophoretically and then identified immunochemically by means of the precipitin reaction.

The urine proteins of a small number of normal men, women, and children have been analysed in this way, using three rabbit antisera prepared against serum proteins, urinary tract proteins, and the Tamm-Horsfall mucoprotein respectively.

It was concluded that: (1) Normal urine contains small quantities of a large variety of different proteins. (2) Many of these are electrophoretically and antigenically identical to the serum proteins. (3) The glomerular pores must be much larger than used to be supposed, but they are apparently not wide enough to let through the large β-lipoprotein molecule. (4) There are probably at least three proteins in the urine unrelated to the serum proteins and so presumably arising from the urinary tract. The principal one is the mucoprotein described by Tamm and Horsfall, which is precipitated from normal urine on increasing the concentration of NaCl. (5) The high proportion of α-globulins found in normal urinary protein by moving boundary or paper electrophoresis is not due to differential tubular reabsorption of plasma proteins but to urinary tract protein.
Biochemical Treatment in Renal Failure

J. W. CHAMBERS (Glasgow) reported that in the Glasgow Northern Hospital Group in the past seven years 12 obstetric cases out of 13 with acute renal failure had been successfully managed by conservative treatment. Cases had been sought in other units (medical, surgical, paediatric, tuberculosis) to which the same principles of treatment apply. In the past three and a half years some 20 cases of uraemia had been treated and recovered; all had normal serum urea levels before discharge (except two with ureter transplanted to colon). Half of these patients had acute renal failure; the others were mainly patients with renal insufficiency associated with severe electrolyte depletion or toxaemia. Maximum serum urea levels ranged from 100 to 500 mg./100 ml. Half of the patients were semicomatose or confused; one-third had an episode of circulatory failure.

Treatment was according to the principles and practice of Professor Bull, with special attention to rectification of electrolyte depletion as indicated by the history and clinical status, and the biochemical findings (urine and serum). In 75% of cases the prognosis at the start of the therapy was very bad. Three of the most severely ill patients are known to be well two and a half years later.

No case described had simple extrarenal uraemia of the type frequently produced by persistent vomiting, diarrhoea, etc. It is rewarding to look for, assess, and treat actively many severely ill patients who present in the uraemic state.

The Bacteriology of Human Pasteurellosis

J. M. TALBOT (London) said that Pasteurella septica was well known to occur as a human pathogen and was now being isolated with increasing frequency from a number of situations. In this series of 52 strains of the organism from cases of human infection, approximately half were obtained from cat and dog bites; while of the remainder, 17 were found in the sputum, mostly in patients with chronic bronchitis or bronchiectasis, two in the C.S.F. in cases of meningitis, one of which was fatal, and the remainder from a number of other situations, such as an empyema, renal abscess, or submandibular abscess.

These strains were compared in their cultural, morphological, and biochemical characteristics with six strains from animals, three of which were the type strains A, B, and C of Carter and Byrne (1953). No differences were found either between the human and animal strains or between the human strains from various situations, except for certain carbohydrate fermentations of doubtful significance, but a number of points did emerge which might be of assistance in the identification of a suspected organism.

All the strains produced acid in glucose, sucrose, mannose, and laevulose within 48 hours; they all produced indole, reduced methylene blue, and formed catalase, nitratase (with one exception), and phosphatase. None of them grew on potato, on McConkey's agar medium, or in citrate, or fermented adonitol, inositol, or dextrin. All gave negative methyl-red and Voges-Proskauer tests. None produced any haemolysin.

A number of unusual biochemical tests are noted, particularly the formation by one strain of gas from certain carbohydrates, which has not before been observed.

Although the possession of a capsule was generally associated with virulence for mice, it was not invariably so.

REFERENCE

Post-valvotomy Complications

A. DICK and R. MATHIESON (Glasgow) said that during the years 1952-6 41 patients were subjected to the operation of mitral valvotomy; five deaths occurred within six months of operation. Histological examination of the auricular appendages of these patients revealed evidence of rheumatic disease in all, but of activity in only one. The necropsy findings indicated that two patients died of thrombotic lesions, one of cerebral emboli, and two of infection within three weeks after operation.

In one of the latter cases detailed bacteriological studies were performed and two varieties of penicillin-resistant, coagulase-positive staphylococci were isolated, one from the lung, pleural, and pericardial cavities, and one from the blood stream. This patient had profound toxaemia which failed to respond to antibiotics, and at necropsy there were fibrinous pericarditis and pleurisy, but the classical features of acute infection were absent. These features were discussed, as was the source of infection and its prevention during cardiac surgery, the scope of which still widened.

Assessment of Blood Loss After Trauma, Including Burns

ELIZABETH TOPLEY (Birmingham) said that comparison of different red cell volume techniques in parallel showed that equally accurate results were obtained using Cr⁵¹ labelled patient's blood, Cr⁴¹ labelled bottled blood, and P⁴¹ labelled patient's blood. T.1824 and haematocrit was less accurate but clinically useful. A variety of assessments (128 patients) suggested that all these red cell volume methods give results accurate enough to be clinically useful in measuring the blood lost in the hours and days following trauma. The greatest inaccuracy is in the uncertainty of the patient's "normal" red cell volume.

Comparison of clinical assessments of blood loss with red cell volume studies has served to define the common degree and timing of blood loss in a variety of injuries, and has demonstrated the limitations of clinical assessment of the degree of blood loss.

The question is raised whether the availability of efficient red cell volume studies during the first 24
hours combined with later haemoglobin tests could considerably improve the standard of blood transfusion management of acute trauma.

Red cell volume estimates in burns (115 patients) may be inaccurate if more than 20% of the body surface is involved. In spite of this, estimates six and 24 hours after injury have increased the usefulness of hourly haematocrits as a guide to plasma therapy during the shock stage in extensive burns.

Inspection of a blood film taken on admission helped to decide whether red cell volume studies were necessary. A loss of more than 25% of the normal red cell volume has so far not occurred in patients showing less than 1% of microcytes (less than 3 μ) in the head of the blood film.

**Ulceration of the Oesophagus in the Newborn**

G. R. Osborn (Derby) reported that ulceration of the oesophagus had not been observed in the embryo, the foetus, or in stillbirths. It is not a consequence of shock. Pus may be found in the lumen of the oesophagus in the foetus and stillborn babies as a result of swallowing some of the inflammatory exudate in the amniotic fluid. There is no reaction to this in the epithelium.

In babies born alive ulceration is uncommon in the absence of intubation. It may be of the infectious acute inflammatory type or of peptic type. These have a characteristic histological appearance which is quite different from that following intubation.

If intubation is intermittent or of short duration, little damage may be done to the oesophagus, though some drugs, such as chloral and alcohol, may be harmful. The incidence of ulceration in a nursing-home where intubation is frequently necessary was found to be 12% when this was not for the purpose of administering intragastric oxygen; babies treated with intragastric oxygen showed ulceration in 71% of cases.

The lesions resulting from intubation also have characteristic appearances. There is first pressure atrophy of the epithelium progressing to its total destruction. Next, but very early, there is intense congestion frequently associated with haemorrhage into the muscularis mucosae and main muscle coat. At a later stage a leucocytic response develops and after this large bacterial masses may form on the eroded surface. These bacterial masses may invade the blood stream in great numbers, or, being swallowed, become obvious in the gastric mucus: staphylococci are most important.

It is not the rule to find severe ulceration if the intubation has been for less than two hours. If it lasts more than four hours or is frequently repeated, the ulceration and inflammation may be so gross that the lesion by itself is necessarily fatal.

The most prolonged treatment by intragastric oxygen was for bad cases of the pulmonary syndrome, mostly alveolar duct (hyaline) membrane and pneumonia. It does not appear to be a satisfactory treatment for these conditions.

**Venous Thrombosis of the Lower Limbs, with Particular Reference to Bed Rest**

N. M. Gibbs (Derby) said that venous thrombosis in hospital patients almost always occurs in the lower limbs and is rare under the age of 20 years, but common over 40 years of age. It is associated with alteration of the normal environment of the individual by confinement to bed during the waking hours, and is as common in medical as in surgical cases. These facts suggest the influence of potent local factors which operate in the lower limbs. The site and incidence of venous thrombosis of the lower limbs at necropsy was compared with the duration of bed rest in hospital in 239 cases in which the period of confinement to bed was accurately known. The cases were otherwise unselected.

**Results.**—The incidence of venous thrombosis rose progressively to a peak in 163 cases dying within two weeks of admission to hospital.

There were 76 cases dying after a period varying from two to 12 weeks' bed rest, and the periods of bed rest were subdivided into weekly periods. The high incidence of venous thrombosis is maintained throughout. The age of the thrombi corresponded to the duration of bed rest.

The incidence of thigh vein thrombosis rises progressively after the first week of confinement to bed.

The highest incidence of pulmonary embolism was found in cases dying after two to three weeks in hospital.

In cases of traumatic injury and of surgical operation, dying within a week of admission to hospital, there was an early onset of venous thrombosis. There was no difference between the incidence of venous thrombosis in medical and surgical patients who had been confined to bed for more than a week.

**Site of Thrombus.**—In the leg the maximum incidence of venous thrombosis was seen in the veins of the soleus muscle. In the thigh veins the thrombi were formed around the termination of the tributaries of the common femoral vein near the inguinal ligament. The primary site was usually in valve pockets.

**Mechanism of Venous Stasis.**—Venous stasis occurs in the intramuscular veins of the leg, particularly the soleus muscle and in the common femoral vein and tributaries near the inguinal ligament. Muscular activity of the lower limb is essential to maintain venous flow.

**The Doubtful Lymph Node: Chronic Inflammation Versus Early Malignant Lymphoma**

K. R. Dempster (London) described how lymph node biopsy sections from 102 patients, previously reported either as "non-specific chronic inflammation" or as "doubtful," in the sense that a malignant lymphoma could not be excluded, were re-examined and the patients followed up. It is considered that 10 cases could be definitely diagnosed as malignant.
lymphoma (follicular lymphoma, 3; Hodgkin’s disease, 4; lymphosarcoma, 1; and reticulum cell sarcoma, 2), unequivocal evidence of which was subsequently obtained at further biopsy, splenectomy, or necropsy.

It was also found that certain histological manifestations of chronic inflammation were particularly prone to confusion with malignant lymphoma, and the usefulness of various criteria for differentiation was assessed.

A marked degree of reactive follicular hyperplasia was frequently mistaken for follicular lymphoma, but the uniform distribution of macrophages in the follicles and the presence of patent sinuses in the inflammatory condition were useful in differential diagnosis.

Many of the microscopical features of Hodgkin’s disease are mimicked by the multiple abscess formation characteristic of cat-scratch disease and lympho-granuloma inguinale, reactive hyperplasia with focal histiocytic hyperplasia (Robb-Smith’s lympho-histiocytic medullary reticulosis), and pleomorphic interfollicular hyperplasia. Sternberg-Reed cells were not found in inflammatory glands and their presence indicates Hodgkin’s disease.

Lymphosarcoma with persistent follicles is distinguished from lymphocytic interfollicular hyperplasia by the absence of patent sinuses and the presence only of a single cell type in the neoplastic condition. The occurrence of widespread necrosis or the persistence of follicular architecture may obscure an otherwise obvious diagnosis of reticulum cell sarcoma.

**The Significance of the L.E. Cell**

E. K. Blackburn (Sheffield) described the L.E. cell phenomenon. Its nature is not yet fully understood, but there is much evidence that it depends upon an antigen–antibody reaction.

Laboratory techniques for the detection of L.E. cells and their interpretation are discussed. L.E. cells have to be differentiated from tart cells and from erythropagocytic leucocytes.

L.E. cells are not absolutely pathognomonic of systemic lupus erythematosus. They have been found rarely and to a lesser degree in the chronic discoid form, in a group of miscellaneous diseases, and after penicillin, sulphonamide, and hydralazine hydrochloride therapy.

The L.E. cell test is valuable in the differential diagnosis of lupus erythematosus. In general the degree of positivity in cases of lupus erythematosus parallels the clinical severity of the disease, although there are exceptions to this rule.

The relationship between modern therapies of lupus erythematosus (including steroid hormones, meparicine, nitrogen mustard, and parenteral blood and leucocyte fractions) and the L.E. cell phenomenon was discussed.

**Theoretical and Practical Aspects of the Separation of Antibodies**

W. Weiner (Birmingham) said the definition of a blood group antigen depends on the specificity of the antibody. Mixtures of antibodies, frequent in transfused patients, must, to obtain suitable reagents, be absorbed. This operation always loses one or more antibodies which otherwise might be valuable. A method was therefore worked out to obviate this loss, and sera containing more than one antibody were absorbed with appropriate cells and those absorbed cells eluted. The eluates contained the absorbed antibody whereas the supernatants contained the rest. The latter, if necessary, could be processed in the same way if still more than one antibody was present.

The method has been found useful in the analysis of sera, and a short cell panel can produce clear-cut results to a statistically significant level. This was demonstrated on a serum which contained anti-Duffy, anti-E and anti-c, and a further serum containing anti-D and anti-Cellano. Both these sera appeared on screening to contain a pan-agglutinin, and the method made it possible to distinguish a "pan-agglutinin" from an antibody mixture. A serum containing anti-Kell and anti-E was mentioned, and from this serum the two antiserum were prepared by the method indicated. The method was used to investigate typical anti-rhesus antibodies. Two sera containing both anti-C and anti-D and one serum containing anti-D and anti-E were shown which could easily be separated by the elution method. The possibility of separation made it clear that the two antibodies were carried on different molecules in the serum. A further serum acting similarly could not be separated into its components, and the significance of this was discussed.

The antibodies in haemolytic anaemias often appear as pan-agglutinins. The elution method was used to show that at least in some cases the serology can be analysed satisfactorily. The eluate of cells from one patient appeared at first to be a pan-agglutinin, but could be, by absorption and elution, shown to consist of an anti-D and anti-e. Another case, however, which appeared identical serologically, gave an eluate which was not separable, and again the significance of this was discussed.

The method thus makes it possible first to prepare suitable reagents from sera containing a mixture of antibodies; second, to give an idea of the composition of sera using a short cell panel only; and third, to add something to our understanding of the conditions obtaining in complex antisera.

**Hereditary Factor VII Deficiency**

F. E. Dische (London) described the findings in a woman with a mild haemorrhagic diathesis due to hereditary deficiency of factor VII. The one-stage prothrombin time was greatly prolonged, and investigations revealed an isolated deficiency of factor VII with no abnormality of thromboplastin generation or deficiency of any other clotting factor. The patient’s brother was found to be similarly affected, while her two children and one of her parents had a partial deficiency, unaccompanied by symptoms. The parents were first cousins. A review of the inheritance of factor VII deficiency showed that the gene
usually acts as an autosomal dominant, with incomplete penetrance and partial expression in the heterozygous state. Individuals homozygous for the gene have a greatly prolonged prothrombin time and invariably bleed, while heterozygous persons either show no abnormality, or have a slightly prolonged prothrombin time with or without bleeding symptoms. In a few of the families the genes concerned may have been recessive. The varieties of the disorder are probably due to different genes.

**Carcinomatous Neuropathy**

A. L. Woolf (Smethwick) said that intramuscular nerve endings were studied in two cases by Coers' techniques. In one there was collateral reinnervation of denervated muscle fibres with diffusely swollen nerve fibres. "Strength-duration" curves indicated partial denervation of distal muscles of upper and lower limbs. Necropsy showed a bronchial carcinoma with carcinomatous infiltration of cervical, lumbar, and sacral spinal roots examined, between the arachnoidal cul-de-sac and the ganglion. The cord (apart from Wallerian degeneration from a metastasis in the pons), cauda equina, and dorsal root ganglia were practically normal. There were many necrotic cerebral metastases, one bordering on the ventricle.

The second case presented as a predominantly sensory "peripheral neuritis." Muscle biopsy showed collateral reinnervation, many slightly oval swellings, with dark lilac central and paler outer zone (methylene blue) on the nerve fibres and reduction of some end plates to single, approximately spherical, masses. Necropsy revealed an anaplastic carcinoma the size of a cherry stone in the bifurcation lymph node. Plasma cells mixed intimately with the tumour. There was posterior column degeneration and severe loss of nerve cells from the anterior horns. The medulla showed cellular nodules in the olives and other nuclei, suggesting virus encephalitis, but further examination showed the nodules to consist of plasma cells developing Russell bodies and what were probably carcinoma cells. The carcinoma cells appeared to have been surrounded by plasma cells from the tumour stroma, during their passage through the blood stream to the brain.

**Familial Non-spherocytic Haemolytic Disease**

J. R. H. Pinkerton (Salisbury) described a family in which nine people, all males, in three generations, suffered intermittent attacks of jaundice and anaemia, associated with splenomegaly. None had leg ulcers: one had gall stones.

Three of the patients have been investigated further: all three bloods behaved in substantially the same way. No abnormal haemoglobin was present in the red cells; alkali-resistant haemoglobin was present in normal quantities. The disease is distinguishable from hereditary spherocytosis by the normal cellular morphology, normal saline osmotic fragility, normal post-incubation saline osmotic fragility, normal rates of autohaemolysis, and normal changes in cellular volume on sterile incubation with and without added glucose. Addition of glucose and adenosine, separately or combined, however, did not reduce autohaemolysis to that of normal red cells similarly treated, although glucose utilization (after allowance for the increased reticulocyte content of the blood) was within the normal range. The spleen has not been removed in any of these patients, as splenectomy has not modified the disease in other reported cases, a further distinguishing feature between hereditary spherocytic and non-spherocytic diseases.

There has been no linkage of this family disease to blood group A, as has been observed in some other families.