ASSOCIATION OF CLINICAL PATHOLOGISTS:
60th GENERAL MEETING

The 60th general meeting of the Association of Clinical Pathologists was held in Dublin. Summaries of the scientific papers follow.

A. B. Raper described 30 cases in which there were discrete, fibrotic nodules of the splenic pulp. Such lesions had been considered very rare, and had generally been regarded as hamartomata. The present examples had been collected in routine necropsies on Africans in Uganda, where amongst male subjects they affected over 5%, mostly young adults. Such a frequency demanded a revision of the accepted diagnosis, and a review of as many as 30 cases allowed a picture of the pathogenesis to be constructed. The essential lesion was proliferation of the reticular cells of the splenic cords. This gave way to hyalinization which, however, was not uniformly distributed, being minimal on the venous side of the (anatomical) splenic lobules, where some intact pulp might remain. The amount of surviving pulp varied, so that some lesions became wholly sclerotic and others showed sclerotic bands enclosing islands of pulp. To the naked eye, the nodules appeared as slightly raised bosses, usually on the anterior border, or as firmish spheres within the spleen; on section they were clearly demarcated, but without a true capsule, and they showed interlacing bands of greyish fibrous tissue enclosing islands of dark firm tissue. In discussing aetiology, the speaker gave reasons against regarding these lesions as hamartomata, or the result of parasitic (and in particular of filarial) infestation. Nor were they intraliteral splenunculi or implants. Trauma, however, if it left the reticular framework intact, could initiate these, or very similar lesions, and the high frequency of splenic trauma in young African males was noted. But not all cases could be so explained, and it remained a possibility that the lesions resulted from the action of an unknown but specific agent capable of exciting fibroblastic proliferation in much the same manner as in Kaposi's sarcoma, which was not uncommon in the same population, though not directly associated with splenic nodules.

Megaloblastic Anaemia of Pregnancy and the Puerperium

C. Giles and Eileen M. Shuttleworth reported an attempt to assess the true incidence of megaloblastic anaemia of pregnancy and the puerperium in north Staffordshire during the 12 months ending February 28, 1958. A haemoglobin level of 9.5 g. % was chosen as the critical level below which patients were included in the study, which comprised sternal puncture, serum iron determination, and an inquiry into the medical, occupational, and dietetic history of each patient. Altogether 369 cases came under review. In 67 of these sternal puncture revealed a megaloblastic marrow. Peripheral blood counts were seldom diagnostic. All patients were treated with folic acid, which produced reticulocyte counts varying from 8 to 33 % and, as a rule, a pronounced fall in the serum iron. Histamine test meals in 53 patients showed 18 cases of achlorhydria. Fat absorption was definitely impaired in three out of 11 cases and the faecal fat content exceeded 20% in seven out of 15 cases. No seasonal incidence was noted and the age distribution was much the same as in normoblastic anaemias. The disease was commoner in multigravida and there was a 9% incidence of twin pregnancy. The overall diet during pregnancy was judged adequate in only 54% of megaloblastic anaemias as compared with 82% of normoblastic anaemias and 96% of pregnant women without anaemia. Of women with megaloblastic anaemia of pregnancy, 83% had fewer than three meat meals per week. There was less variation regarding other items of diet.

Haemolytic Episodes in Pregnancy

H. B. Goodall (Dundee) said that haemolytic anaemia is uncommon in pregnancy, but that many different types are known. He described three types.

Haemoglobinuria in severe hypertensive toxaemia was discussed in the light of a case in which the usual haematological investigations failed to reveal the cause of the haemoglobinuria. It was considered after necropsy that the free haemoglobin had probably come from the lysis of widespread hepatic haemorrhages. Intra-caval catheterization might give proof of this hypothesis.

The assessment of haemolysis in megaloblastic anaemia was on the amount of erythropagocytosis in the peripheral blood. In three cases in which this phenomenon was particularly marked there was severe toxaemia and shock with intra-uterine death in two cases and accidental haemorrhage in the other. There was a possibility that masses of degenerating erythrocytes might cause micro-embolism and, thus, shock.

Lytic episodes were described in women responding to treatment for hypochromic anaemia characterized haematologically by erythropagocytosis and
in four of five cases by abdominal upsets of the false labour type. In one of these cases the upset was probably due to the passage of incompatible foetal red cells through a defect in the placental barrier; a large central placental lesion was found at delivery. In the others there was no gross placental damage and the infants were compatible. Though the cause of the phenomenon was not yet known, it was considered possible that a rapid increase of iron in the blood, particularly with intensive parenteral therapy, might have damaged the maternal erythrocytes and made them more susceptible to phagocytosis. Was excessive destruction of transfused blood in transfusional siderosis possibly due to a similar mechanism? Another cause of the excessive tendency to erythrophagocytosis might be the depletion by response to iron of some factor essential for the integrity of the red cell. Reasons were given for pointing to vitamin E depletion as a possibility.

The Problem of Myasthenia Gravis

A. L. Woolf said that many curious facts relating to myasthenia gravis had been discovered in the last 50 years and the discoverer of each had been tempted to postulate his own discovery as of primary pathogenic significance. The problem lay in the need to integrate all data into a single theory of pathogenesis. He had himself reported (Woolf, Bagnall, Bauwens, and Bickerstaff, 1956) for the first time striking changes in the intramuscular nerve endings in a case of myasthenia gravis and had now found similar changes in two out of a further three cases.* The changes consisted of sprouting of the distal extremity of the subterminal nerve fibres (which were sometimes irregularly swollen) and formation of multiple, bizarre, or unduly complex end-plates. Cholinesterase preparations showed minute deposits of cholinesterase over a wide zone around the end-plates, indicating that there were far more sprouts than were detectable with the light microscope alone. The changes resembled those seen in myositis, dystrophia myotonica, and thyrotoxic myopathy. These diseases had little in common as regards contractility of muscle, but all showed evidence of endocrine imbalance. He thought the changes in the nerve endings were the result of muscle fibre degeneration and lymphorrhages caused by this imbalance, which was also responsible for the disordered contractility.

Reference

Histology of Chronic Bronchitis

W. K. Taylor described the microscopical appearances of biopsy material taken from the carina in 54 cases of chronic bronchitis. All cases showed an increase of goblet cells up to 10%, of the epithelial cells. Transitional cell metaplasia was a very common finding (60%, of cases) and about 12% showed squamous metaplasia. Carcinoma *in situ* was seen in a few cases. In more than half the sections there was some degree of thickening of the basement membrane. In the lamina propria there was often some oedema and occasionally dilated lymphatics. An increase in vascularity was frequently noted. Cellular infiltration was notably slight and in a high proportion consisted only of a few lymphocytes. Plasma cells were not infrequent, but polymorphs were rare and eosinophils were not seen. Elastic fibres were reduced in number and often absent. Fibrosis was uncommon and where present was fine and diffuse.

Cytological Observations on Carcinoma

R. A. Q. O’Meara and R. D. Jackson had applied standard cytological methods to fresh biopsy material containing carcinomas. The first aim had been to determine the way in which a primary carcinoma invades the tissues, using the cytological techniques as a means of orientating the growing cells. In particular, staining of the Golgi apparatus had been useful and had shown different results in man from those obtained by Ludford (1925) in animals. Squamous cell carcinomas were first examined and it was found that the arrangement of the Golgi apparatus in the normal epidermis corresponds to that found in carcinoma invading the tissues, in so far as the basal cells of the epidermis correspond to the peripheral cells of invading carcinoma, and the mature cells of the epidermis undergoing keratinization correspond to those of the central parts of the carcinoma. Thus in squamous carcinoma the tumour is maturing by mitosis and keratinization towards the centre of the growth, while the peripheral cells of the carcinoma are growing outwards into the tissues in the opposite direction.

Concentrating on the cytological features of these cells, as representing the more important part of the growth, it was found incidentally, when stained to show mitochondria, that the cells appeared to be forming fibrils. Further investigation showed that these fibrils were not artefacts, but are deposited on the outlying invading cells. All tests so far applied indicate that they are fibrin or of a fibrinous character. This point continues to be investigated. Sometimes the invading cells are overwhelmed by the deposit and they are then removed by phagocytes: for the most part they thrive and grow outwards into the deposit. The fibrils can be found in advance of the main body of the tumour, forming a coagulum in the tissue spaces, thus interfering with the nutrition of the cells of the part and providing the carcinoma cells with an advantage. It is also considered that successive fibrinous deposits on the invading carcinoma are of importance in leading to the formation of the stroma of the tumour as a result of the organization thus induced.

Reference
The Thyroid Gland After Treatment of Thyrotoxicosis by Partial Thyroidectomy or $^{131}$Iodine

R. C. Curran, H. Eckert, and G. M. Wilson said that methods of treating hyperthyroidism included partial thyroidectomy and incomplete destruction of the gland by ionizing radiation, using $^{131}$iodine. By searching the hospital's pathological records of many years, seven cases were found in which the portion of gland left after partial thyroidectomy was available for histological examination: the interval between the surgical operation and the necropsy or biopsy ranged from one to $14\frac{1}{2}$ years. For comparison, 13 glands treated by therapeutic doses of radioactive iodine were obtained: these 13 patients died in hospitals at intervals of a few days to five and a half years after treatment. Full necropsies were performed.

After partial thyroidectomy, the portion of gland left shows, with the passage of time, progressively decreasing cellular hyperplasia and increasing colloid storage. In the first weeks following treatment with $^{131}$iodine, the irradiated glands showed disruption of follicles, necrosis and damage to epithelial cells, oedema and early fibrosis of the stroma, and widespread vascular lesions, including thrombosis. The more chronic effects of radiation treatment include stromal fibrosis and increasing numbers of altered epithelial cells, but more striking are the microfollicular structure and the comparative lack of colloid.

The two series of glands therefore differ histologically. They also appear to differ metabolically, as judged by the results obtained after giving a tracer dose of $^{131}$iodine to patients rendered euthyroid by one or other of these two methods of treatment. In the post-thyroidectomy cases, the level of protein-bound radioiodine 48 hours after the test dose is almost invariably normal, that is, less than 0.4%. This suggests a normal iodine pool in the thyroid gland. In contrast, in the irradiated series, the protein-bound radioiodine remains high on average. This suggests a reduced iodine pool in the thyroid gland, a result apparently in harmony with the histological finding of colloid lack.

The distribution of female lung cancer histological types is very similar to the distribution of all lung cancers in western Europe, even after taking into account the controversial classification of adenocarcinomas which Kreyberg includes in his group II. In group I he places epidermoid, large and small round-cell carcinomas; in group II, adenocarcinomas, squamous-cell, and bronchial glandular tumours.

Of 33 women, eight were living in country areas; only 3 among them were to be classified in group II. The data are not sufficient to draw any significant conclusions.

The main interest of Kreyberg's work is that it yields information on areas where atmospheric pollution is relatively low and lung cancer incidence has been growing more recently than in our own areas of observation.

Control of Staphylococcal Infection in Surgical Wards

W. A. Gillespie, G. Ayliffe, and V. G. Alder said that cross-infection by Staph. aureus in three surgical wards had been studied for two years. About 3% of all wounds were infected by Staph. aureus in the theatre, sometimes with serious results. Thirty-eight per cent. of all open and drained wounds were subsequently colonized by Staph. aureus in the wards, but often with little effect on the wounds.

Staph. aureus caused about 10% of the post-prostatectomy urinary infections, and in about 1% of all patients caused other staphylococcal complications (pneumonia, enterocolitis, parotitis, boils).

Staphylococci with multiple antibiotic resistance caused nearly all the wound and other infections in the wards and often spread from patient to patient, causing "epidemics" of ward cross-infection. They also caused almost one-third of the theatre staphylococcal infections. The chief sources of these strains were the patients' infected open lesions and noses. They rarely spread from nurses' noses. Strains which were sensitive to all antibiotics or resistant to penicillin only were common in the noses of the staff and caused most of the theatre infections, but rarely spread in the wards.

After a seven-month control period, extra precautions were introduced into one ward during the next six months. Nasal carriers were treated with neomycin-bacitracin ointment, the blankets and pillows disinfected with formalin after use; the barber's brushes, the crockery, and baths were disinfected; open wounds were sprayed with "poly-bactrin"; paper towels and "hibitane" hand cream were issued to nurses. There was a moderate reduction in cross-infection, of doubtful significance. Finally, in February, 1957, the disinfection of baths was improved by adding hexachlorophene to the bathwater, and nasal prophylaxis was started by supplying twice daily neomycin-bacitracin ointment to the noses of all patients from the day of admission. Following these steps the rate of cross-infection fell immediately. During the subsequent year (to February, 1958) 13% of 106 open wounds became infected by Staph. aureus.
compared with 47% of 60 similar wounds in the control period. There was a corresponding fall in incidence of other staphylococcal complications in the experimental period, and a reduction in theatre infection which was probably partly caused by the changes in the ward.

Subsequent study showed that nasal prophylaxis by itself gave only a slight reduction in wound cross-infection, much less than when combined with other precautions.

**Haemoglobinopathies in General Hospital Practice**

H. M. RICE (Nottingham) described two cases of haemoglobin C disease (CC), which were discovered within one year, from a West Indian population of about 2,000. Both cases were pregnant women and both had been quite well between pregnancies. Their haemoglobin dropped in pregnancy and haemoglobin C was not detectable in the cord blood of the infants.

Within about one month after, the haemoglobin F level was noted to drop and that of haemoglobin C to rise, and at about one year haemoglobin F was not detected and haemoglobin C was about 40%.

He also described a sickle cell anaemia in a male Jamaican aged 40 years with characteristic findings in the red cells and Hb and in the electrophoretic pattern.

**Phenol Reaction for Blood Sugar Estimation**

T. M. HEALY (Dublin) reported on the use of this test. Simple sugars, when treated with phenol and concentrated sulphuric acid, gave an orange-yellow colour, stable for some hours (Dubois et al., 1956). Experience of the test over two years had shown that the method gave accurate and consistent results. With standard sugar solutions of 121 mg. % concentration “labtrol” the mean of 30 readings was 116.5 mg. % (S.D. ± 3.3) and with reconstituted plasma containing 400 mg. % the mean was 398.5 mg. % ± 7.0. Compared with Folin and Wu’s method the results were of comparable accuracy, but the phenol method took one-third of the time and needed less apparatus and cheaper reagents. There had been no accidents with the concentrated sulphuric acid.

The method was found suitable for routine use.

**The Prophylaxis of Pulmonary Embolism**

N. B. GIBBS (Royal Surrey County Hospital, Guildford, Surrey) said that he had investigated fully the relationship between venous thrombosis of the lower limbs and bed rest. Thrombosis begins in two separate and quite clearly defined sites, namely the leg and the thigh, where venous stasis is particularly liable to occur. Venous stasis is prevented in hospital patients by early ambulation and other efforts.

A simple apparatus has been designed which completes control of exercise of leg and thigh muscles to be effected in patients confined to bed. The “soleus ergometer” consists of a pivoted platform attached to a spring of variable tension. The platform is depressed by plantar flexion of the feet, and each full movement of the platform is recorded on a meter. The apparatus is mounted on an adjustable carriage which is clamped to the foot of the bed. The spring tension and position of the apparatus may be adjusted with regard to the height, age, and strength of the patient. An average of 30 recorded acts of plantar flexion per hour during the waking hours is thought to be adequate.

**Ornithine Carbamyl Transferase in Serum**

PATRICK T. MOORE (St. Kevin’s Hospital, Dublin) reported that following the method of Reichert estimates were made of the enzyme ornithine carbamyl transferase (O.C.T.) in serum. Normal values range from 1 to 5 units and elevated values are observed in cases of acute liver damage.

The simple method of estimation is based on the arselenolysis of citrulline described by Krebs, Eggleston, and Knivett as follows:

1. Incubate 1 ml. of serum for 24 hours at 37° C. with 1 ml. 0.2M dl-citrullin in 0.5M arsenate buffer at pH 7.1 and a further 1 ml. of serum under similar conditions but without the substrate citrulline.

2. Stop the reaction by adding 0.2 ml. 4N perchloric acid and centrifuge down precipitate.

3. Estimate ammonia in 1 ml. of supernatant by the Conway microdiffusion technique. Final Nesslerization is to be preferred to titration because of the range of values observed.

4. After subtraction of the blank, results are expressed in O.C.T. units (1 unit = 1 μg NH₃—N liberated in 24 hours per ml. serum).

The importance of elevated levels of O.C.T. lies in the fact that the enzyme can only have been derived from liver where it is specifically located as one of the enzymes of the urea cycle.

**The Further Investigation of Hypercalcemia**

H. G. MORGAN (Dundee) reported that few patients with hyperparathyroidism were now seen with the classical biochemical and skeletal changes. Far more show renal tract signs, often with no specific bone changes, with but slight or even intermittent hypercalcemia, and sometimes equivocal levels of plasma phosphorus, even in the absence of renal failure.

In further support of the diagnosis, and in the differentiation of the many other possibilities, newer tests have been adopted, sometimes over-enthusiastically, and are here assessed. Strontium infusion to assess skeletal uptake rates, the ionized fraction of plasma calcium, and phosphate deprivation are all potentially helpful, but little experience is yet avail.
ASSOCIATION OF CLINICAL PATHOLOGISTS MEETING

5-Hydroxytryptophan-secreting Carcinoid Tumours

M. SANDLER (Royal Free Hospital, London) described two patients with metastasizing carcinoid tumours who were excreting 5-hydroxytryptophan and greatly increased amounts of 5-hydroxytryptamine in their urine as well as 5-hydroxyindoleacetic acid. The tumour tissue was of an unusual histological pattern. It was suggested that these patients, together with three others in the literature, form a distinct clinical subgroup of 5-hydroxytryptophan-secreting carcinoid tumours. Other unusual features included atypical flushing attacks—bright red, prolonged, and of “geographical” pattern—osseous metastases, low tumour 5-hydroxytryptamine concentration, and an enormous urinary histamine output.

Phenylacetic acid, which has recently been shown to have an inhibitory action on 5-hydroxytryptophan decarboxylase \textit{in vitro}, was administered orally to one of these patients. Serial urinary indole analyses afterwards showed a response compatible with an inhibition \textit{in vivo} also.

The Gliadin Loading Test in Coeliac Disease

W. J. E. JESSOP and W. F. HEMMENS (Dublin) said that gliadin (350 mg./kg. body weight) is given by mouth to a fasting patient. Blood specimens are taken fasting at one, two, three, and four hours after gliadin. The glutamine concentration in each specimen is measured.

The method of Harris (1943) was adapted to the measurement of serum glutamine concentration; 1 ml. serum was added to 3 ml. 14% trichloroacetic acid and a sample of the filtrate was heated to 70°C. for 60 minutes; the glutamine was determined as ammonia with Nessler’s reagent; urea does not interfere.

According to Weijers and van de Kamer (1955), a normal child shows no change in serum glutamine during the gliadin loading test; an increase of at least 50% of the fasting level is observed in wheat-sensitive patients with coeliac disease.

Two normal children were included in this series. One showed no change in serum glutamine till the fourth hour, when a 25% rise was observed, and the other showed a decreased level after two hours. Of 10 children with coeliac disease who were known to be sensitive to wheat from previous dietary experiments, five showed the 50% increase over fasting serum glutamine indicated by Weijers and van de Kamer; three showed a rather smaller increase, and the blood glutamine level was unchanged in two.

It was concluded that the gliadin loading test did confirm the presence of wheat sensitivity in the majority of cases, but that in its present state of development it is not suitable as a routine test for coeliac disease.

REFERENCES


Chorion Epithelioma of the Pineal with Precocious Puberty

J. H. STEPHENS, D. F. CAPPELL, and J. L. EMERY (Glasgow) reported two cases of pineal tumour associated with precocious development in boys. (1) A precociously developed boy of 4 years was admitted with headache, vomiting, and a tumour of the pineal region. This was treated by x rays and about four weeks later he died suddenly of intraventricular haemorrhage. At necropsy the cerebral tumour was wholly necrotic, but the lungs contained multiple haemorrhagic nodules with the structure of chorion epithelioma, from which it is inferred that the pineal growth was of this nature. (2) A boy of 10 years presented about a year before death with signs of somatic and sexual precocity with mild polyuria. After about nine months nervous symptoms developed, with diplopia, mild fits, athetoid movements, and drowsiness. Examination revealed a pineal tumour with hydrocephalus, for which a short-circuit operation was performed. Death occurred suddenly from intraventricular haemorrhage and necropsy revealed a large haemorrhagic growth replacing the pineal gland and invading the adjacent brain substance. There were no secondary growths.
Despite the known activity of such growths in secreting chorionic gonadotrophin, the development of somatic and sexual precocity is not likely to be attributable to these effects but is thought to be similar to that responsible for precocity in other pineal growths, e.g., teratomata, and in certain lesions, both tumours, and inflammatory or destructive, in the posterior part of the hypothalamus, viz., by the interruption of the inhibitory nervous pathways which control the release of hormones from the anterior pituitary.

**Familial Hypoplastic Anaemia: the Fanconi Syndrome**

M. G. Nelson and J. T. Lewis (Belfast) said that the syndrome of marrow failure with congenital anomalies first described by Fanconi had been seen in many parts of the world both in familial and sporadic form. They described a family in northern Ireland in which, of eight children, three are known to be affected. One with pancytopenia and pigmentation had died of a haemorrhagic diathesis, the parents and two children are unaffected, and two children, as yet normal, are too young for any syndrome to have developed.

The onset of the disease usually occurs at about the age of 6; the clinical course is progressive and normally death takes place after a period of some four years.

The clinical, haematological, and pathological findings were presented. Its aetiology was discussed both from the hereditary and environmental points of view. No evidence to support an endocrine dysfunction or metabolic error could be found at all. Laboratory tests for these functions are normal.

No therapeutic benefit was obtained from haematinics, B group substances, steroid hormones, or erythropoietin containing plasma.

Splenectomy has been performed on two of the cases. One severe case has been much improved. He is free from symptoms and fully employed 11 years after operation. The other case submitted to splenectomy has been less benefited.

**Bronchiolitis from Nitrous Fumes**

A. J. N. Warrack (Sheffield) said that nitrous fumes were produced in industry in such processes as the dipping of metals, the manufacture of toluene and cellulose, and the descaling and pickling of steel sheets after firing and rolling.

Symptoms of poisoning varied with the concentration of the gas and the duration of exposure. Low concentrations merely provoked a mild irritation of the eyes and upper respiratory tract. Strong concentrations were liable to cause severe pulmonary oedema. Some cases, however, showed a latent interval of many hours, or even days, before symptoms appeared. These took the form of dyspnoea, cyanosis, and paroxysmal cough. Secondary infection is a danger and appropriate treatment might save the patient's life.

One patient had recovered after exposure to nitrous fumes whilst dipping cadmium-lined bolts in an acid mixture. He was admitted to hospital 17 days afterwards. His symptoms had not started until seven days after exposure. He recovered with massive doses of antibiotics. Chest radiographs taken at the height of his illness showed soft irregular opacities through both lung fields which disappeared eight weeks later.

Another case ended fatally. A man carrying nitric acid in a rubber bucket noticed a leak and held the bucket over another of galvanized iron. He was enveloped in thick brown fumes for 30 seconds. He complained of no ill effects and finished his work. It was not until seven days after exposure that he sought medical attention. He was admitted semi-comatose and cyanosed to hospital two weeks after the original accident. A radiograph of the chest had an appearance similar to that of the first case. Post-mortem examination showed both bronchial trees filled with thick mucopus. The lungs were solid and on section showed generalized tiny yellow areas having the appearance of purulent bronchiolitis.

Microscopical examination showed varying degrees of obliterative bronchiolitis from simple epithelial desquamation to complete bronchiolar destruction with surrounding alveolar organization. There was presumably secondary infection of these areas and it was thus important that antibiotic therapy should be given.

Both the cases described had the typical features of the condition—a latent interval between exposure and symptoms, the typical radiological appearances of obliterative bronchiolitis, in one instance recovery with adequate treatment, and in the other classical post-mortem histological findings.