ASSOCIATION OF CLINICAL PATHOLOGISTS:
57th GENERAL MEETING

The 57th general meeting was held at the Royal College of Surgeons, Lincoln's Inn Fields, London, from October 4 to October 6. Abstracts of the scientific papers and of the symposium arranged jointly by the Association of Clinical Pathologists and the Association of Clinical Biochemists follow.

Cross-matching Technique in Acquired Haemolytic Anaemia

W. Weiner (Birmingham) said that, until a short time ago, it was thought that one of the differences between acquired and inherited haemolytic anaemias was the fact that in the former the life span of transfused blood was shortened as against the normal survival time in the latter. It could, however, be shown that the short survival time was due to the fact that incompatible blood had been transfused into these patients.

Cross-matching for these patients using the serum as a reagent will not discover the incompatibility, as the antibody responsible for the rapid destruction cannot on many occasions be found in the serum but is present in the globulin coating the patient's cells. Cross-matching should therefore be performed with an eluate obtained from the cells, and an elution method was worked out to make this procedure practicable in a routine laboratory and at comparatively short notice. Details of this method were given.

Various examples were given of the distribution of the antibody in serum and eluate. It was pointed out that compatible blood must of necessity be non-homologous, and patients with acquired haemolytic anaemia, being good antibody producers, were thus liable to develop immune antibodies due to the stimulation by the transfused cells.

In addition to the autochthonous antibodies, immune antibodies of antithetical character could then develop, thus making transfusion treatment in an emergency impossible. The availability of steroid treatment would seem to call for transfusion treatment only in most desperate circumstances.

Blood Group A_m in Two Families

W. Weiner, R. R. Race, Ruth Sanger, and B. Lewis (Birmingham) reported the results of investigations of two families. One member of each of these families had been found to have the blood group A_m described by Wiener and Gordon a short time ago. This blood group can only be determined if both cell and serum groupings are performed.

One person was grouped in his cells as group O; his serum, however, lacked the a, but his saliva contained group A substance. The other person grouped by her cells as group B, by her serum as AB, and her saliva also contained group A substance. These cases are apparent exceptions to Landsteiner's law.

The genetics of this blood group were discussed in the light of the family investigations performed, and it was considered that the most likely explanation was the presence of a recessive gene in a homozygous state in persons showing this particular blood group.

A table was shown giving the relationships of other "abnormalities" found lately which tried to correlate the latter with the present findings. The importance of these findings was stressed, particularly with regard to transfusion treatment and a question of heredity.

Erythropoietic Porphyria

S. Varadi reported the case of a 4½-month-old female infant who presented with haemolytic anaemia (Hb, 6.1 g., R.B.C.s, 2.23 × 10⁶, reticulocytes, 8.6%, normoblasts + + +), thrombocytopenia, splenomegaly, and history of red urine since birth. The porphyrins found in the excreta and blood were mainly of type I. Half of the normoblasts in the bone-marrow were abnormal and normoblastic nuclei exhibited red fluorescence. The cytoplasm of 10% of the normoblasts contained fine basophilic needles, presumably porphyrin crystals. Central clear areas apparently corresponding to intranuclear haemoglobin were found in some of the patient's abnormal normoblasts but also in a case of abnormal erythropoiesis of a different type. As a whole the abnormal normoblasts are considered to be specific of the disease.

Splenectomy at the age of 6 months did not prevent the development of the full clinical picture, i.e., erythrodermatia, hydroa, hypertrichosis, shedding of finger nails. Because of the disappearance of the anaemia and the marked decrease of porphyrin and stercobilinogen excretion, it was thought that the photosensitivity was probably also favourably influenced. The platelet count returned to normal and, temporarily, porphyrin needles were present in circulating red cells. The child is now 13 months old and there is a lymphocytic hyperplasia (>60%) in the bone-marrow with peripheral lymphocytosis, the nature of which is not quite clear: it might be due to a stimulation of urine-synthesis following a depression of the porphyrin production. Of the highest importance is Professor Rimington's experiment, which shows that haemolysate prepared from the child's red cells, when incubated with δ-aminolaevulic acid, synthesizes uro-, copro-, and protoporphyrins in that descending order of magnitude (uroporphyrins >50% isomer I, coproporphyrins mainly III).
In the ensuing discussion Professor C. RIMINGTON (University College Hospital Medical School) said that members would recollect that nucleated avian erythrocytes, or haemolysates prepared therefrom, are capable, when incubated at 37° C. in the presence of radioactive glycine, of synthesizing porphyrins and haem bearing the radioactive label. Since preparations of the non-nucleated human erythrocytes are incapable of synthesizing haem under these conditions, it has, up to the present, been assumed that they have lost all biosynthetic capacity in this direction. This is not so. If human red cells or their haemolysates are incubated with \( \delta \)-aminolaevulic acid or with porphobilinogen as substrate, both these substances, being proven intermediates in the biosynthetic pathway to haem, produce copious amounts of uroporphyrin and coproporphyrin and also a small quantity of protoporphyrin. Paper chromatographic analysis shows that these porphyrins belong to the isomeric series III, as do those formed by nucleated avian red cells, and also, of course, the physiologically occurring haemoproteins such as haemoglobin, cytochromes, catalase, etc.

In congenital porphyria there is some gene-controlled abnormality of the haemopoietic system which leads to the production in the bone marrow of much uroporphyrin I and coproporphyrin I. In addition to the normal isomers, these series I porphyrins being excreted or, in the case of uroporphyrin, also laid down in the bones and teeth. Dr. Varadi kindly provided a specimen of blood from his congenital porphyria patient so that its behaviour could be examined when incubated in vitro with \( \delta \)-aminolaevulic acid. It was found in Professor Rimington's laboratory that the haemolysate of this blood produced roughly equal amounts of the I and III series isomers of both uroporphyrin and coproporphyrin. Since the proportion of nucleated red cells in the peripheral blood at the time this sample was taken was not more than 1 in 10,000, this finding must mean that the defect in the biochemical synthetic mechanism persists in the enzyme systems of the mature cell and cannot be confined merely to some early stage in the maturation of the erythrocyte in the bone marrow.

Indications for and a Method of Bone Marrow Aspiration in Children

JOHN L. EMERY (Sheffield) said that in children the most common indications for marrow aspiration were (a) in the diagnosis of leukaemia; any child with unexplained anaemia must be suspect until proved otherwise; (b) in the diagnosis and treatment of neuroblastoma; (c) in other general causes including the diagnosis of miliary tuberculosis and obtaining blood for general laboratory procedures.

The anaesthetic of choice is rectal thiopentone (Lorber, 1950).

The easiest site for puncture in children under 18 months is the upper end of the tibia, where the insertion must be made close to the end of the bone. In children over the age of 18 months the most convenient site is the posterior iliac crest, where no secondary epiphysis occurs, and where the needle insertion can be carried out while the child is being maintained in a sleeping posture.

Reference


**Mylelan Therapy in Chronic Myeloid Leukaemia**

E. K. BLACKBURN (Sheffield) described experiences in the treatment of 20 cases of chronic leukaemia (19 myeloid, one neutrophilic) with myleran. The optimum dosage averaged 0.06 mg. per kg. body weight orally a day. With larger doses, thrombocytopenia might be precipitated.

Eight of the nine cases not previously treated had satisfactory remissions, and myleran was life-saving in one of these who was quite unfit to travel for radiotherapy following gastrectomy. The ninth case was the one of neutrophilic leukaemia. Satisfactory remissions also occurred in four out of six radio-resistant patients, in three out of four who had had previous irradiation but were not radio-resistant, and in one patient who had had demecolcine therapy for 11 months.

The terminal event in seven of the 14 patients who died was acute relapse.

Mylelan can be used justifiably if radiotherapy is not available or contraindicated, or if the patient is radio-resistant. It is preferable to other chemotherapeutic agents, excepting possibly demecolcine. The best palliative therapy for chronic myeloid leukaemia which has stood the test of time is, however, radiotherapy. We cannot yet answer the question, "Is myleran an effective substitute?"

**A Quick Method for Detection of Thallium in Urine**

F. RAPPAPORT and F. EICHHORN (Beilinson Hospital) said that with the increasing use of thallium compounds as rodicides there is a risk of thallium poisoning of men and animals due to careless or inexpert handling. Several cases have been reported among agricultural workers in Israel. In cases of thallium poisoning 1–2 mg. thallium per litre is excreted in the urine (100 \( \mu \)g. per 100 ml.). The excretion is slow, and excretion has been observed for many months, and in one case for two years, after thallium poisoning. A qualitative test for thallium in urine was demonstrated. Thallious ions are precipitated by sulphide in the presence of excess cadmium, which acts as a "trace catcher" in the precipitation process. Cadmium was chosen as the carrier because it is soluble in HCl and does not react with rhodamine B. Thallious ions are oxidized to thallic by bromine, and excess Br\(_2\) is removed by sulphasalicylic acid or ethylene. The thallic ions are detected by a colour reaction with rhodamine B. Iron, mercury, and antimony do not interfere when the urine is treated as described. The test may be made quantitative.
Symposium on Diseases of Lymph Glands

Professor C. V. Harris was in the chair.

Reactive Overgrowths of Lymph Nodes

A. H. E. Marshall (London Hospital) said that reactive overgrowths of lymphatic tissue might be considered as essentially physiological hyperplasias, produced by regeneration following loss of normal tissue, by excessive stimulation of one or more of its normal functions, or by altered endocrine control of the tissue; the prognosis of such conditions therefore depended on the nature of the exciting stimulus. Such proliferations show similar biological features to hyperplasias in other tissues, and their separation from the neoplastic varieties of lymphatic enlargement may be made on the basis of such behaviour. The term "reticulo-" essentially avoids any attempt to draw the boundaries between hyperplasia and neoplasia in lymphatic tissue and should be employed only when the term has become attached to individual conditions.

The majority of reactive overgrowths of lymphatic tissue of diagnostic importance fall within the group of functional hyperplasias and represent stimulation of the phagocytic function of the node and of its capacity to form antibodies. In chronic inflammation of a lymphatic node these functions appear as the formation of "sinus catarrh" and the proliferation of plasma cells. In rheumatoid arthritis the same basic proliferation is present with the addition of marked hyperplasia of germinal follicles. In measles the formation of the characteristic "mulberry" giant cells is a diagnostic feature. In dermatopathic lymphadenitis lipid and melanin are present in the sinuses of the inflamed node. In lymphogranuloma venereum and Boeck's sarcoïdosis, focal areas of necrosis surrounded by epithelioid cells and nodules of epithelioid cells and giant cells respectively form the diagnostic features.

Reticular Overgrowths of Limited Invasiveness

G. D. Lumb (Westminster Hospital) said that when discussing cellular proliferations anywhere in the body it was important to distinguish between hyperplastic conditions and true tumours. The distinction in the reticular tissues was even more difficult than in other tissues, because then those very cells which are normally associated with the inflammatory process are being studied. Once a decision has been made that this is a true neoplasm then it is of little value to attempt a division into benign and malignant variants. This is a group of tumours, some of which have a better prognosis than others, some of which develop invasive and even metastatic potentialities more rapidly than others, but which cannot be separated into two groups, benign and malignant, because even those which might be regarded as most benign, namely, follicular lymphoma and reticular lymphoma (a benign form of Hodgkin's disease), finally undergo malignant change and cause death with diffuse invasion; or, on the other hand, the most malignant tumours, when viewed histologically, may occasionally remain completely localized with no evidence of invasiveness. For this reason it is preferable first to separate the tumours into their histological variants and then to examine the prognostic significance of such a separation with the full realization that the histological appearances may well change in any particular case during the natural history of the process. For this reason it is essential to study these diseases by means of multiple biopsies during their course and to make careful necropsy examinations after death. In collaboration with K. A. Newton, radiotherapist to Westminster Hospital, Dr. Lumb had made a survey of the Westminster series of lymphoid tissue, paying particular attention to those with a good prognosis. He divided the tumours into the following types: lymphosarcoma and follicular lymphoma; Hodgkin's disease and reticular lymphoma (a benign form of Hodgkin's disease); reticulum cell sarcoma and anaplastic sarcomas of lymphoid tissue, among which are included lymphoblastic reticulosarcoma; stem cell carcinomas and Hodgkin's sarcoma.

Discussion of the sarcomas did not fall within the province of the symposium.

Reticulosarcoma

A. H. T. Robb-Smith (Oxford) said that the reticulosarcoma had all the characteristics of a typical malignant neoplasm, cells of atypical appearance, local infiltrations, and metastases to distant sites: the tumour might arise in a single site or be multicentric.

There is a considerable range in the morphological characters of the tumour cells, but a review of the natural history of 450 cases of reticulosarcoma has shown that the degree of cellular differentiation has no significant influence on prognosis or response to therapy; it is the site of origin of the tumour and the age of the patient that are the most significant factors provided it has been shown histologically to be a reticulosarcoma.

The incidence of reticulosarcoma, like other malignant neoplasms, increases with age, the maximum lying in the seventh decade, and the total incidence is about 12 per million population (about half the frequency of Hodgkin's disease). About 4% of all reticulosarcomata arise as a result of malignant transformation of the progressive hyperplastic lymphadenopathies and 15% are multicentric when the patient first comes under observation. The multicentric group have a very poor prognosis with a 9% three-year survival and 5% five-year survival, whereas the whole group of reticulosarcomata have a 26% three-year survival and 15% five-year survival. Fifty per cent. of cases have their primary origin in lymph nodes, and those arising in the superficial groups have a much better prognosis than those of mediastinal or abdominal origin. Primary reticulosarcoma of the skin and nasopharynx have a relatively better prognosis, whereas tumours arising in bone marrow, liver, or spleen have a very grave prognosis.
Comparison of the results of treatment for the group as a whole reveals that radiation alone gives far inferior results in cases treated by surgery whether or not it is combined with radiotherapy, and a study of a group of 60 cases in which the involvements at the outset of treatment were limited to a single group of superficial lymph nodes shows that cases treated by radiotherapy alone had an average survival of 1.7 years with only 3% of cases surviving 10 years, whereas similar cases treated surgically had an average survival of 6.0 years with 14% surviving 10 years; a group in which the treatment was surgery with radiotherapy had an average survival of 6.3 years with 29% surviving 10 years. These results are much better than those observed in a series of 475 cases of Hodgkin’s disease, and this is even more striking when it is considered that Hodgkin’s disease affects patients at a lower age group with a better natural expectation of life. However, cases of Hodgkin’s disease treated surgically have a much better prognosis than those treated by radiation.

The Radiotherapy of Diseases of Lymph Glands

W. M. Levitt (St. Bartholomew’s Hospital, London) considered the subject under three headings.

1. Reactive Hyperplasia—Since by definition reactive hyperplasia reverses with removal of the cause, radiotherapy plays only a subsidiary part in its treatment.

2. Lymphadenopathies of Limited Invasiveness—A rational approach to radiotherapy is only possible if the aim of treatment is clearly defined. This in turn depends upon the radiotherapist’s view of the nature of the disease. Are the local swellings merely manifestations of some deep underlying process which itself is little influenced by radiations, or are they to be regarded as part of the essential disease demanding treatment wherever they appear? On the former view, however satisfactorily the local manifestations are controlled, duration of life is not greatly influenced although comfort may be. On this view treatment is therefore symptomatic only and should be conservative, demanding the minimum dosage necessary to control the swellings and the minimal constitutional disturbance. This is the view taken by the author. Dosage and scope of treatment were discussed.

3. The Invasive Lymphadenopathies—These probably comprise two groups of cases, those which are unifocal, at least at first, and those which are multiple at the outset. The treatment of the former is precisely on the same lines as the treatment of any other unifocal malignant growth with, however, some reduction of dosage because of the greater radiosensitivity. The treatment of the second subgroup is much on the lines of that of the previous group (lymphadenopathies of limited invasiveness), but generally speaking considerable extent of involvement is not a contraindication to massive regional irradiation provided that the greater part of the disease is contained within a single region, e.g., lesions in liver, lung bases, and epigastrium, or lesions limited to the chest. Surprisingly enough the outlook in lymphosarcoma of this distribution in the abdomen is actually better with radiotherapy than in the Hodgkin’s group, with the exception of follicular lymphoblastoma.

Clinical Appraisal of Diseases of the Lymph Glands

R. Bodley Scott (St. Bartholomew’s Hospital) said that the aetiology of the lymphomata was unknown and the diagnostic criteria, therefore, histological. The clinician required the pathologist to tell him whether the patient with lymphadenopathy had a lymphoma or not, and, if so, of what variety.

The first decision is usually easy, but a small satellite lymph node may mislead by failing to show characteristic changes, and some patients with visceral lymphoma present only a reactive picture in the superficial nodes.

Different pathologists employ different forms of words in answering the second question, but a basis of agreement can be discerned. Hodgkin’s disease, lympho-reticular medullary reticulositis, and lymphoid follicular reticulositis are generally recognized, and these diagnoses, under whatever labels they may hide, give some notion of prognosis and indications for treatment.

In the reticulosarcomata and lymphosarcomata prognosis appears to depend much more on the clinical type of the disease than on the histological picture.

The general principles of treatment are the same for the whole group, but the response to different chemotherapeutic agents varies with the histological type.

The Genesis of Bullae

E. Milford Ward (Leicester) described the development of bullae in subacute dermatitis, in bullous pemphigoid, pemphigus vulgaris, pemphigus vegetans, and in the Hailey-Hailey syndrome.

In subacute dermatitis the bulla develops intra-epidermally, beginning as an area of spongiosis with subsequent rupture of intercellular bridges, desmorrhexis, and the fully formed bulla has smooth sides and may contain a few inflammatory cells. In bullous pemphigoid and dermatitis herpetiformis the bulla is subepidermal. It arises by the development of papillary oedema and subsequent rupture of the denticulate fibres anchoring the epidermis to the corium.

In true pemphigus the bulla is intraepidermal. It begins by a specific form of degeneration of the prickle cells so that they become swollen and rounded. They lose their adherence one to another and a cleft bulla with acute angles at its edges is formed. The bulla has an irregular margin due to the presence of swollen prickle cells, and similar degenerate cells, acantholytic cells or Tzanck cells, are found lying free within the bulla. The presence of such acantholytic cells is necessary before a diagnosis of true pemphigus vulgaris is made. The disease carries a 100% mortality. Pemphigus vulgaris and pemphigus vegetans only vary in degree. In the latter there is a marked inflamma-
ASSOCIATION OF CLINICAL PATHOLOGISTS MEETING

Changes in the Nervous System in Vitamin B\textsubscript{12} Deficiency

A. L. Woolf (Smethwick) said that it had been recognized for many years that mental symptoms might occur in cases of subacute combined degeneration, and Dr. MacDonald Holmes had made a special study of these cases. He had not only noted the striking amelioration of symptoms after administration of vitamin B\textsubscript{12} but had also shown that the mental symptoms may be unaccompanied by signs of spinal cord involvement or other evidence of vitamin B\textsubscript{12} deficiency such as megaloblastic anaemia or sternal marrow. Some other indirect indication of the deficiency is therefore desirable, especially in view of the technical difficulties attendant upon the direct estimation of the serum B\textsubscript{12} level.

The effect of B\textsubscript{12} deficiency upon the nervous system was reviewed in the hope that some part of it might be more accessible to morphological examination during life than the spinal cord.

The brains from two cases of subacute combined degeneration with mental changes were examined, and the effects of the disease upon the brain determined. Three changes were seen in the white matter: (1) a diffuse proliferation of microglia without pallor of myelin staining; (2) pallor of myelin staining associated with ballooning of myelin sheaths (no products of myelin breakdown accompanied 1 or 2); (3) focal areas of complete breakdown of myelin with sudanophil products in amoeboïd microglia and light fibrous gliosis (cf. Adams and Kubik, 1946).

Muscle biopsy (including vital staining with methylene blue and histological demonstration of acetyl cholinesterase) was found of value in demonstrating the involvement of the lower motor neuron. This investigation had the advantage over cutaneous nerve biopsy (Greenfield and Carmichael, 1935) in that it demonstrated the changes in the nerve endings as well as in the nerve fibres. The changes most closely resemble those seen in cases of the Guillain-Barré syndrome, and may have relative diagnostic value, e.g., in their distinction from the appearances seen in diabetic neuropathy (Malins and Woolf, 1956).

REFERENCES


Symposium on the Estimation of \(\alpha\)-Keto Acids and Abnormalities of Keto-acid Metabolism in Disease

In his opening remarks Professor R. H. S. Thompson (Guy's Hospital, London) (chairman) referred to the early work in Sir Rudolph Peters's laboratory at Oxford on the changes in pyruvate metabolism in thiamine-deficient pigeons. Much of that early work had been done with relatively unspecific methods, such as the determination of the level of bisulphite-binding substances in the blood, and later the 2:4-dinitrophenylhydrazone method. These relatively primitive methods did, however, serve to reveal the increased level of pyruvate in the blood in thiamine deficiency.

Using a "loading" dose of glucose to increase the sensitivity of the test, it was shown that many patients with polyneuritis of different types showed high blood \(\alpha\)-ketoc acid levels (Joiner, McArdle, and Thompson, 1950). In only a proportion of these, however, were these high blood levels restored to normal by parenteral administration of thiamine. To go further with this problem, therefore, it was necessary to use a more specific method to determine whether the accumulating keto-acid was in fact pyruvic acid. Cavallini, Frontali, and Toschi (1949) introduced the next important step by using paper chromatography for the separation of the 2:4-dinitrophenylhydrazones of \(\alpha\)-keto acids, and methods were soon devised for the quantitative elution and estimation of these separated derivatives.

The joint meeting of the Association of Clinical Pathologists and the Association of Clinical Biochemists was opened by Professor Sir Rudolph Peters, who gave a masterly survey of the work done in his laboratories on keto-acid metabolism and the citric acid cycle.

Sir Rudolph Peters's paper was followed by a symposium on the estimation of \(\alpha\)-keto acids.

Recently, interest has been taken in pyruvate metabolism in diabetes mellitus. Since in this condition we are concerned also with the question of insulin sensitivity and of the availability of glucose for conversion into pyruvate, the interpretation of blood pyruvate levels is particularly difficult. Also the possibility of an accompanying ketonaemia stresses the need for highly specific analytical methods.

REFERENCES


B. McArdle (Guy's Hospital, London) then described a method for the quantitative determination of pyruvic and \(\alpha\)-ketoglutaric acids employing the paper chromatographic separation of their 2:4-dinitrophenylhydrazones. The main differences from previous methods were the use of alcohol to prevent the butyryl emulsions which hindered separation during the ethyl acetate extraction of blood filtrates, and secondly the use of a small amount of ammonia for extracting the hydrazones from the ethyl acetate. This last procedure considerably reduced the number of extractions required in most existing methods. Results were accurate to within ±5% for pyruvate and ±3% for \(\alpha\)-ketoglutarate.

He had found only minute amounts of \(\alpha\)-ketoglutarate in the cerebrospinal fluid, whereas in urine it was usually in greater concentrations than in pyruvate.
This was especially so in childhood and in women, particularly during pregnancy. The α-ketoglutarate excretion in women varied during the menstrual cycle in much the same way as citrate, being highest about the time of ovulation and during the second half of the cycle and falling immediately before or with the onset of the period. The excretion of pyruvate, related to that of creatinine, was unaffected by age and sex; it was often increased in hepatic and renal failure and in certain endocrine disorders. In neurological diseases, in which the excretion of α-ketoglutarate was little affected, the pyruvate was found increased in about half the cases of polyneuritis, in muscular disorders, in subacute combined degeneration, and occasionally in disseminated sclerosis and motor neuron disease.

α-Keto Acids in Diabetes

M. J. H. Smith (King’s College Hospital) said that the use of paper chromatographic methods of analysis had made it possible for the α-keto acids in body fluids to be easily identified and their concentrations measured with reasonable accuracy. In human blood the two most prominent α-keto acids are pyruvate and α-ketoglutarate. Glucose, by the processes of glycolysis, is a major source of pyruvate in the body, and pyruvate may give rise to α-ketoglutarate via the citric acid cycle. The investigation of abnormalities in the metabolism of these acids is therefore of interest in diabetes mellitus because of the underlying disorder of carbohydrate metabolism.

The published work on the blood concentrations of pyruvate and α-ketoglutarate in diabetic patients has been conflicting, and the discrepancies may be due either to analytical errors caused by the presence of varying amounts of aceto-acetic acid or by other factors, such as previous exercise, which particularly alters blood pyruvate levels. Using a more specific reagent (1:2-diamino-4-nitrobenzene) for the α-keto acids (Taylor and Smith, 1955) no difference in the blood concentrations of the α-keto acids was observed between normal subjects and ambulant diabetics. The work was extended to a study of the effects of the oral administration of glucose on the fluctuation of blood pyruvate in normal subjects and two groups of diabetic patients. The diabetic patients were divided into two groups according to the clinical classification of Lawrence (1951). In the normal subjects and one of the diabetic groups (obese type) the administration of glucose caused a significant elevation of the blood pyruvate level, but not in the other diabetic group (thin, insulin-deficient type). The significance of these changes was discussed and the changes in the blood α-keto-glutarate levels were also described.

References


The Sweat in Fibrocystic Disease of the Pancreas

P. T. Flute, B. W. Webb, and M. J. H. Smith (King’s College Hospital, London) reported that the sweat of 99% of children with fibrocystic disease of the pancreas showed persistently raised levels of sodium and chloride (Di Sant’Agnese, 1956). Under controlled conditions of sweat collection similar values were found only in primary renal or adrenal disorders.

Using a simple method of sweat collection (Webb, Flute, and Smith, 1956) analysis of sweat from 12 cases of fibrocystic disease gave levels of sodium of 72 to 157 mEq./litre of sweat and chloride 68 to 145 mEq./litre. Sweat from 20 children with various other diseases and with no signs or symptoms referable to the gastro-intestinal tract showed levels of sodium of 4 to 52 mEq./litre and chloride 9 to 40 mEq./litre. Thus, using the method described, values for either sodium or chloride greater than 70 mEq./litre, in the presence of the typical clinical symptoms and signs, strongly support the diagnosis of fibrocystic disease.

References


Serum Transaminase in Coronary Thrombosis and Other Conditions

D. N. Baron, Joyce Bell, and Celia Oakley (Royal Free Hospital, London) said that glutamic-oxaloacetic transaminase was estimated in serum by the technique of Karmen (1955) in a “unicam” SP 500 spectrophotometer, using glass cells. Correction was made for variation in room temperature (transaminase activity 15°/20°/25° = 0.6/1.0/1.38). The normal range in serum (at 20°C) was 7–25 units. In a normal individual the serum transaminase does not vary from day to day and is unaffected by food, normal activity, and venous constriction. The level is unaltered when serum is kept for one week at room temperature or for at least two weeks frozen.

Transaminase has been reported in high concentration in cardiac muscle, skeletal muscle, brain, liver, and kidneys.

In patients with myocardial infarction the serum transaminase rises above normal three to nine hours after the onset and returns to normal within three to eight days (unless there is a further infarction). The extent and duration of the rise depend on the size of the infarct, but usually exceed 75 units; the highest level in our series was 570 units. A rise was found in 21 clinical cases of infarction proven by E.C.G. or necropsy findings, and in one clinically probable case; there were no false negatives. No rise was found in patients presenting with the differential diagnostic problems of pulmonary embolism (seven cases) or acute abdomen, with or without peritonitis, except in the one patient with acute pancreatitis in whom a rise to 600 units in the first 24 hours from the onset was recorded.

Slightly raised levels were found in two cases of severe polymyositis, and borderline values in milder cases and in rheumatoid arthritis with muscular wasting. Serum transaminase was unaltered by major muscle trauma in an orthopaedic operation.
A value above 1,000 units was found in acute hepatic necrosis, high values in infective hepatitis and active alcoholic cirrhosis, and a normal value in chlorpromazine jaundice.

High values had been found in patients with ruptured kidney and with hemiplegia due to cerebral embolism. A normal value was found in the argentaffin carcinoma syndrome and in chronic nephritis with uraemia.

The estimation had been useful in investigating suspected myocardial infarction with equivocal clinical and E.C.G. findings, and might be of value in assessing certain diseases of the liver and skeletal muscle.

REFERENCE

Turbidity Measurements of Cerebrospinal Fluid and Urine Proteins, Zinc Sulphates, Thymol Turbidities, and Fibrinogens with the Grey Wedge Photometer

PATRICIA KEND and R. D. ROSEFIELD (Postgraduate Medical School, London) said that Yeoman (1955) described the use of the Grey wedge photometer for measuring the turbidity produced by C.S.F. proteins with sulphasalicylic acid.

Comparisons were made between the results obtained on reading turbidities, produced with C.S.F. and urine protein, in the Grey wedge photometer and by visual comparison with artificial protein standards. The yellow-green (Ilford 625) eye-piece was used, and as shown experimentally, using prepared protein standards and artificial vimanul standards (King and Wiggins, 1952), gave readings with slightly more linear relationship to protein concentration than with the red (Ilford 608) eye-piece. Agreement between the two methods was within ±3%.

The photometer may also be used to read zinc sulphate and thymol turbidities, in which case the red eye-piece was used to cut out any interference due to haemoglobin in haemolyised sera. A series of normal controls fell within the expected range for both zinc sulphate and thymol turbidities, and among patients studied the result obtained was that expected for the particular disease. Good correlation was obtained when the turbidities were read in the Grey wedge photometer and against the artificial standards.

The photometer is also useful in turbidity measurements to determine fibrinogen when it is salted out with 12.5% sodium sulphate (King and Wootton, 1956; Campbell and Hanna, 1937). Good agreement between fibrinogen determined by this method and by Kjeldahl nitrogen estimation was obtained.

REFERENCES

Comparative Studies on the Dyeing of Albumin and Globulin in Paper Electrophoresis

D. J. R. LAURENCE and E. M. ABDEL-WAHAB (Postgraduate Medical School, London) said that serum albumin was a robust molecule and specific loss of albumin in an acid dye bath occurred unless the strip was heated for 18 hours before staining, or a dye used forming a very insoluble complex with the protein. Relative dye uptakes by albumin and globulin vary according to the dye and solvent used in the dye bath, but differences become less after prolonged preheating and approach the theoretical ratio 1.4 : 1 for the total cationic groups. Certain dyes give ratios close to the theoretical value after short heating times, as the dye-protein complex is insoluble. The high albumin concentration in the centre of a protein band leads to decreased stability and a "hollow" band is obtained after staining.

No hollow band has been observed with amidoschwarz staining and the effect of oven heating time is slight. A similar result was obtained for lissamine green staining as long as a sufficiently large dye bath was used. Losses of protein with bromphenol blue or bromoresol green staining are large unless prolonged oven heating is employed, but are less in methanolic acetic acid or HgCl₂-ethanol than in ethanolic or aqueous acetic acid. It is difficult to retain the yellow monovalent ion of these indicators during staining, and the standard methods result in staining almost entirely with the blue divalent ion. With ion exchange mixtures, albumin causes a greater indicator shift than globulin.

Different albumin-globulin factors have been found for elution and scanning, and it is likely that albumin is denatured nearer the paper surface than is globulin.

Coacervation in the Thymol Turbidity Test

V. ANNE L. BREWS (Farnborough Hospital) said that coacervation was thought to be due to the formation of liquid droplets, or coacervates, during the settling out of sols. Within appropriate ranges of the sol and electrolyte concentrations, the sol, instead of being precipitated, forms regions of high concentration or viscous droplets, which may coalesce to form a separate liquid phase. In the phase of coacervation the droplets may possess anisotropic optical properties.

During the past eight years, and the analysis of over 2,000 sera, the phenomenon of coacervation has been observed nine times while carrying out the thymol turbidity test. The sera were from cases of aleukaemic monocytic leukaemia (1), anaemia of unknown origin (1), myelomatosis (3), lymphoid leukaemia with cryoglobulinaemia (2), and macroglobulinaemia (2). A high gamma globulin peak was found in the electrophoretic patterns of these sera.

The phenomenon is detected by reading the optical densities of the turbidity produced on mixing the serum with the buffer reagent, after five minutes, one hour, two hours, and 18 hours' incubation at 37°C. When turbidity gradually disappears over this period the presence of a coacervating globulin is indicated.