The Association of Clinical Pathologists
(Including joint meeting with Association of Clinical Biochemists)

The 67th general meeting was held at the Royal College of Surgeons, London, from 21 September to 22 September 1961. On 23 September the joint meeting with the Association of Clinical Biochemists was held. Summaries of papers given at both meetings follow.

THE OSMOTIC FRAGILITY OF LEUCOCYTES IN LEUCOPENIC DISORDERS

H. G. H. and D. L. RICHARDS (Winchester) described again their technique for the estimation of leucocyte osmotic fragility reported earlier. Aliquots of blood are added to varying strengths of saline (from 0·85% down to 0·20%) and after 24 hours at 4°C, the saline-blood suspensions are converted to leucocyte counting preparations by the addition of acetic acid. Counts are then performed on the leucocytes in each saline concentration and graphs drawn to incorporate two curves, namely, 1, the total cell count for each tube, and 2, the count of cells which remain refractile, i.e., undamaged by the effects of osmosis. The median leucocyte fragility is defined as that concentration of saline in which half the leucocytes originally added remain refractile (resistant to osmosis).

The authors described 17 cases of leucopenia covering a wide variety of causes from toxic to myeloproliferative, and showed that in 14 of these the fragility was very markedly increased while in three it was significantly decreased below normal. The possible significance of these results in the mechanism of leucopenia was discussed, and the view put forward that the osmotic fragility of leucocytes is a measure of their average length of life.

FIBRINOlySIS RELATED TO AGE IN MEN

H. T. SWAN (Sheffield) said that fibrinolysis was assayed by the time taken for sterile plasma clots to lyse at 37°C. Clots were prepared from serial dilutions (100% to 10%) of ice-cold fresh plasma in veronal buffer at pH 7·4. Three different patterns of results were obtained and this prevented easy comparison of experiments. Most comparable results were obtained by noting the lysis time taken by the 50% plasma clot. All the results presented were based on this figure alone.

Assays were performed on 192 men whose ages ranged from 21 to 87 years and who were at the time in surgical and radiotherapy wards. Days of stress were avoided as far as these could be recognized. Blood was drawn about 10 a.m. by the same person, using an identical technique.

The mean age within each decade was calculated, together with the mean lysis time of the same group of patients. When these values were plotted against each other it became obvious that there was a faster rate of lysis with increasing years. The slope was sufficiently obvious not to require significance testing. By the method used, the lysis time at the age of 40 was approximately halved by the age of 80. The conclusions for cases grouped because of known malignancy were identical with those where no malignancy was recognized and for the group as a whole. The individual factors responsible for the change with increasing years were not investigated.

THE SECRETORY PATTERN OF THE GASTRIC MUCOPOLYSACCHARIDES USING HISTAMINE AND INSULIN AS STIMULANTS

J. SCHRAGER (Wigan) described his attempt to study the secretory patterns of the gastric mucopolysaccharides. The chemical structure and composition of these substances is as yet unknown. It was therefore decided to estimate their carbohydrate components. Methods were adopted for the estimation of protein-bound hexoses, hexosamines, fucose, sialic acid, and bound sulphate. The data provided by these estimations enabled quantitative description of the gastric mucopolysaccharides to be obtained.

Two hundred fractional gastric analyses were carried out. Two fasting aspirations were obtained at 10-minute intervals, followed by intramuscular histamine and three aspirations each at 20-minute intervals. Intravenous insulin was then given and a further three specimens were obtained, each at 20-minute intervals, aiming at a blood sugar between 20 and 30 mg. %. In each specimen the pepsin, free acid, protein-bound hexoses, hexosamines, sialic acid, fucose, and bound sulphate contents were estimated.

In health the fasting specimens are rich in all the carbohydrate components. With an increase of acid after histamine, there is a simultaneous fall in the secretion of the carbohydrate components; their secretion recovers within an hour after histamine but falls again with the response of pepsin to the hypoglycaemia. This depressant effect of acid and pepsin secretion on the mucopolysaccharides is a constant and characteristic feature and was found in all the fractional gastric analyses carried out.

The ratio of sulphate to hexosamine in the fasting specimen is about 2 mEq. to 3 mEq., that is to say, out of three molecules of hexosamine two are sulphated. The sulphated mucopolysaccharide is thus a major component of the gastric mucopolysaccharides.

The gastric mucosa is rich in sulphatase enzymes A and B which regulate the sulphation of the mucopolysaccharides. In some fasting specimens (with about 15 mEq. of H+), the sulphated mucopolysaccharides contribute as much as 40 to 60% of the H+ concentration,
thus controlling to a certain extent the acidity of the resting gastric secretion. There is evidence that the sulphated mucopolysaccharides have antipeptic activities.

The investigations reported showed a constant quantitative relationship between all the carbohydrate components and it has been observed in all the fractional gastric analyses carried out.

One of two alternative explanations is possible:—1 The carbohydrate components belong to one chemical substance, the gastric mucosa secreting only one mucopolysaccharide. 2 The gastric secretion contains several mucopolysaccharides and the constant quantitative relationship is due to a common stimulatory secretory mechanism.

To differentiate between glucosamine and galactosamine, the total hexosamine in every specimen was estimated and the glucosamine and galactosamine separately. Glucosamine and galactosamine do not belong to the same chemical compound as they do not show a constant quantitative relationship. Work on electrophoresis confirmed this conclusion.

Phosphate buffer at pH 7.2 was injected into the stomach before aspirating the content, thus inhibiting peptic activity and preventing the formation of proteolytic products. The strips were stained with B.P.B., P.A.S., and alcian-blue-P.A.S.-positive and alcian-blue-negative bands and alcian-blue-positive and P.A.S.-negative bands were obtained. These findings confirmed that the gastric secretion contains several mucopolysaccharides.

Patients with duodenal ulcer show a distinctive secretory pattern. There is a very low mucopolysaccharide content in all the specimens. In some the mucopolysaccharides disappear completely and the degree of sulphation is lowered and instead of two out of three molecules being sulphated one out of six is sulphated. The mucosa is deprived of the protective shield of the mucopolysaccharides.

Patients with carcinoma of the stomach show a disturbed secretion of the mucopolysaccharides and the quantitative relationship breaks down completely. The disorganized cellular topography of the gastric mucosa is reflected in the disturbed secretion. There is no relationship whatsoever between the sulphate and hexosamine nor between any other carbohydrate components. The disordered gastric mucopolysaccharide secretion has been encountered in all the 50 patients with gastric carcinoma investigated and may well have a diagnostic significance.

Cystadenoma and Cystadenocarcinoma of the Pancreas

J. A. Campbell and A. H. Cruickshank (Liverpool) reported their observations on 14 cases of cystadenoma and three cases of cystadenocarcinoma of the pancreas. All the cases were in women whose ages ranged from 13 to 90 years. Four tumours measuring 2 to 4 cm. in diameter had not caused symptoms or signs. Tumours from 7 to 12 cm. in diameter were palpable in the upper abdomen and half the patients with such tumours had complained of pain. In two cases with large tumours there had been severe bleeding into the alimentary canal.

The cystadenomas were of two types: 1 Tumours with small loculi lined by cubical epithelium in which the epithelium had secreted little mucus but mucoid stroma was abundant. There were six tumours of this type. No evidence of malignant change was found in this type of tumour. 2 There were eight tumours with large cystic spaces filled with mucus and lined by tall mucus-secreting epithelium.

Signs of recent or old haemorrhage were common in both types of tumour. In the three cases of cystadenocarcinoma at least two appeared to be the result of malignant change in pre-existing cystadenomas of the mucus-secreting type.

Acute Laryngitis and Obstructive Emphysema in the Newborn

G. R. Osborn and R. L. Flett (Derby) Obstructive atelectasis and obstructive emphysema are frequently found in the lungs of the newborn and full microscopic examination often reveals the local valvular mechanism responsible. In an important group, examination from the trachea to the alveoli fails to reveal the cause of the condition. Most and probably all of these residual cases are due to acute laryngitis which may develop very soon after birth. Histologically the lesions in the larynx are similar to those that follow indwelling tubes in the oesophagus, trachea, and urethra. In the case of the larynx they are not due to mechanical factors such as intubation nor are they primarily bacterial in origin but are caused by laryngeal spasm. During this the inferior folds (vocal folds or cords) are damaged by pressing against each other; the superior folds (ventricular bands) may be similarly affected. Spasm is explained by the evolution of the larynx which was not evolved for speech but as an inlet and outlet valve. Its maximum development for running is found in the horse and deer, and for an arboreal existence in the lemurs and some monkeys. The larynx of man, like that of the baboons and gorillas, is regressive. It is less regressive and therefore a better valve in the infant than in later life. Alveoli are not filled with fresh air during inspiration but filling largely depends on expiration against the resistance of the contracted bronchial musculature and partly closed larynx. Excessive resistance will produce obstructive emphysema which may progress to interstitial emphysema and pneumothorax. Complete failure of this resistance at birth could be the cause of the formation of hyaline membrane in the alveolar ducts; whether this is so or not can only be proved by the laryngologist.

Swelling of the Brain Associated with Minor Burns

John L. Emery and C. Campbell-Reid (Sheffield) Six children were described between the ages of 3 and 13 months who developed cerebral symptoms within two to eight days after a relatively minor burn.

All six children died with gross swelling of the brain and pressure cones. One child who had similar symptoms but did not die later developed a spastic hemiplegia.
The necropsy technique, by which such cases can be diagnosed at necropsy by approaching the foramen magnum from the back of the neck, was described in detail.

**GENERAL ASPECTS OF AUTOMATION**

A. Ferrari (New York) described the AutoAnalyzer and its use in the laboratory. The AutoAnalyzer, with its new components and using newer techniques, covers a much broader spectrum of analytical procedures today than it did a year or two ago.

**PRACTICAL EXPERIENCES OF AUTOMATION IN HAEMATOLOGY**

M. G. Nelson (Belfast) presented results of an attempt to apply elementary principles of automation to some of the routine procedures required in a large hospital haematology department. The annual work load for this type of test was 75,000: 38% haemoglobin estimations, 13% erythrocyte counts, 22% leucocyte counts, and 13% peripheral blood and bone marrow smears. Automatic haemoglobin estimation was achieved by using the AutoAnalyzer, the technique employed being the colorimetric estimation of oxyhaemoglobin with the manifold and flow circuit arranged to make a production rate of 60 an hour possible. The manifold finally adopted could be used equally well to estimate cyanmethaemoglobin, but the reagent for this was a little less simple to make up and comparisons had shown that there was no significant error in estimating haemoglobin as oxyhaemoglobin by this method.

The pre-dilution necessary for cell counting on blood specimens has been simplified by the use of an automatic dispensing pipette to deliver accurate reproducible volumes of diluent with the blood being added manually. For the occasional leucocyte count by the visual method the Baird and Tatlock peristaltic pump diluting apparatus has been found useful.

The EEL and Coulter cell counting machines were both exhaustively evaluated for both erythrocyte and leucocyte counting. It was found that both instruments were perfectly satisfactory for red cell counting and that accuracy to within ± 2% could be routinely achieved. For leucocyte counts, the EEL machine proved unsatisfactory in routine use, but with the Coulter machine, after some study of the most suitable diluting fluid, it had been found possible to perform accurate counts on a routine basis at any level of leucocyte concentration to an accuracy ± 2.5%.

The production of stained blood and marrow films has been greatly facilitated by using the automatic slide staining machine produced by Elliotts of Liverpool. Using a staining cycle with a relatively short fixing time this apparatus produces high quality stained films, the main advantage of which is in their absolute reproducibility which greatly improves the ease of visual differential counting.

The results of this approach to routine haematology have been to provide accurate laboratory data for the clinicians. It has not been found that the apparatus required has made undue demands on the work area. Trained technical staff have been freed for more specialized work, although training facilities in manual methods for student technicians have inevitably been sacrificed. Economically, the capital cost of the equipment involved has been less than £4,000, but it has been found that the work is now carried out by four technicians who thus process about 19,000 tests each per year. This trend is most dramatically shown when the annual work load of the laboratory for the past 10 years is plotted on the same graph as the changes in technical establishment over the same period. The latter curve shows a tendency for the formation of a plateau coincident with the introduction of automation to the laboratory, and provides eloquent proof that the methods adopted have achieved economy in man-power.

**THE ABO AND RHESUS BLOOD GROUPS IN PERTHES' DISEASE**

J. Malcolm Cameron and Marian M. Izatt (Glasgow) Determinations were carried out of the ABO blood groups and Rhesus phenotypes of 142 children from a series of 185 suffering from unequivocal Perthes' disease. Where possible their parents and sibs were also grouped. The results showed no apparent relationship between the development of Perthes' disease and the distribution of the ABO groups in the children concerned.

There was the suggestion, however, of an increase in the incidence of the Rhesus-negative phenotype, rr (cde/cde), among the affected children. Unfortunately the series was too small in number to reveal if this was indicative of a significant association between Perthes' disease and the Rhesus blood groups or if it had been due to chance alone. At least, parental and sibship studies show that an association, if one does exist, is unlikely to be due to genetic stratification within the population.

Sera from the mothers of affected children were examined for the occurrence of Rhesus antibodies. The conclusion was reached that maternal antibody, acting through the agency of haemolytic disease of the newborn, plays no appreciable part in the aetiology of a subsequently developing Perthes' disease in the child.

**ESTIMATION OF TOTAL MARROW CELLULARITY IN MAN BY THE RADIOACTIVE IRON METHOD USING BOTH RIB AND ASPIRATED MARROW SAMPLES**

W. J. Harrison (Westminster Hospital, London) said that the method was based on that proposed by Suit (1957) in which Fe** has been used as a marrow tag. Total marrow cellularity (TMC) was estimated in duplicate on 10 haematologically normal patients undergoing thoracotomy, rib marrow and aspirated marrow being used in each case; the results averaged 11.1 and 10.4 × 10⁶ nucleated cells/kg. body weight respectively. Calculations of total marrow cellularity based on erythrokinetic considerations by Osgood (1954) and Patt (1957) agreed well with these figures when the marrow reticulocyte stage is allowed for in the case of Osgood's work. Donohue,
Gabrio, and Finch (1958) used rib marrow; their higher result (18 × 10⁶/kg) is probably due to their neglecting radioactivity which escapes from cells during the preparation of cell suspensions.

In seven cases of disseminated malignant melanoma, using only aspirated marrow, the mean was 11·2 × 10⁶/kg. This was probably an overestimate since in disease there are considerable difficulties in assessing the proportion of the injected Fe⁴⁺ in the total marrow at the time of sampling.

At multiple marrow puncture (Pegg and Kemp, 1960) approximately 1% of the total body marrow is obtained.

REFERENCES


AN IMPROVED RAPID HEPARIN TOLERANCE TEST AND ITS USE IN THE POST-OPERATIVE PERIOD

R. D. EASTHAM (Frenchay Hospital, Bristol), said that the plasma recalciﬁed clotting test had been modiﬁed with reduction of the normal time from the quoted range of 90 to 180 seconds to 35 to 45 seconds, with greatly improved reproducibility. (The method is published on page 86.)

The improved plasma calcium clotting test and its modiﬁcation as a heparin tolerance test were used in an attempt to detect either those patients particularly liable to develop post-operative thrombosis or those who had very recently developed thrombosis. Preliminary results showed that there was a deﬁnite shortening in the clotting times, both with and without added heparin, after operation. Blood from patients treated with phenylindandione gave times prolonged above normal and these results showed only rough direct correlation with either the Quick one-stage prothrombin time or the Thrombotest results.

RELATIONSHIP OF COAGULASE ACTIVATOR TO PROTHROMBIN

E. W. BALL (Stoke-on-Trent) After paper electrophoresis coagulase activator and prothrombin each showed a single peak of activity. The peaks coincided with each other and with alpha-2 globulin of normal serum. During electrophoresis factor VII separated from prothrombin, necessitating the addition of factor VII to achieve accurate estimation of prothrombin.

Contrary to previous reports, coagulase activator and prothrombin behaved similarly during Seitz ﬁltration and during incubation with calcium ﬂuoride. It is believed that this result was achieved by the addition of factor VII during the estimation of prothrombin. It is concluded that the identity of prothrombin and coagulase activator is now established, and that the estimation of activator may be employed in place of that of prothrombin in investigating blood coagulation.

RELIABILITY OF BIOPSIES IN ASSESSMENT OF MUSCLE POTASSIUM IN PATIENTS WITH ALIMENTARY DISEASE

C. T. G. FLEAR,1 A. O. CAMPANA,2 and IRENE FLORENCE (Department of Experimental Medicine, Cambridge, and the General and Queen Elizabeth Hospitals, Birmingham) Methods are available for analysis of small samples of skeletal muscle which can yield answers within a few hours of taking a biopsy. Technically, therefore, it should be possible to make clinical use of muscle biopsies, the desirability of which is emphasized by the unreliability of serum levels as indices of body and cellular content. This should not be difﬁcult as cell potassium makes up some 99% of that in a sample and therefore affords an index of cellular content to be evaluated against suitable standards. Difficulties arise, however, in the choice of suitable standards. In spite of the very considerable variability in composition of human skeletal muscle, a biopsy can afford a useful index of cellular potassium throughout the muscle sampled. In view of the negative correlation between sodium and potassium, it is essential, when evaluating the potassium content of a biopsy, also to take into account its sodium content. It is also necessary to evaluate a biopsy against normal values obtained from the same skeletal muscle. The interpretation was discussed of ﬁndings from patients with various alimentary disorders.

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SOME METABOLIC ASPECTS OF HUMAN OBESITY

G. L. S. PAWAN (Department of Medicine, Middlesex Hospital, London) In the obese, daily calorie intake is sometimes no greater, and may even be less, than that of persons who are not obese. Total energy expenditure, efﬁciency of energy utilisation, and the response to calorie reduction may be different from that in normal individuals. Fasting blood levels of non-esteriﬁed fatty acids, pyruvic, lactic, and citric acids are higher than normal and respond differently to glucose administration. Normal persons given a 1,000-Kcal. diet, in which 90% of the calories are supplied as fat, exhibit within a few days marked hyperketonemia, hyperketonuria, hypoglycaemia, and marked negative nitrogen balance. The obese appear to tolerate this diet better than the non-obese, show little or no ketosis, maintain blood sugar within normal levels, and exhibit little body protein loss. In the obese, there is some evidence of an increased use of the pentose phosphate pathway of carbohydrate metabolism; liver function is often abnormal; and phosphorylase activity of subcutaneous adipose tissue and the urinary excretion of certain steroids is higher than that of normal persons. It is suggested that in the study of obesity attention should be directed not only to appetite regulation and calorie intake but also to energy expenditure and to the metabolic factors influencing synthesis, mobilization, and utilization of body fat stores.
The Association of Clinical Pathologists

RED CELL ANTIGENS AND ENZYMES AFTER STORAGE AT -196°C, -100°C, AND -78°C.

R. G. HUNTSMAN (Memorial Hospital, Peterborough), B. A. L. HURN (Lewisham Hospital, London), ELIZABETH W. IKIN (M.R.C. Blood Group Reference Laboratory, London), H. LEHMANN (St. Bartholomew's Hospital, London), and J. LIDDELL (Radcliffe Infirmary, Oxford) reported that storage of red cells frozen rapidly in liquid nitrogen was a convenient alternative to preservation of red cells with glycerol for serological purposes. Blood anticoagulated with 1 mg. E.D.T.A. per ml. is mixed with half its volume of 40% sucrose (Huntsman, Hurn, and Lehmann, 1960). The mixture is added drop by drop to the liquid nitrogen through a fine needle connected either to a syringe or, for larger quantities, to a plastic transfusion bag compressed by weights. The frozen pellets are stored in a Linde liquid nitrogen refrigerator (LNR-25-B). This refrigerator costs approximately £200 to purchase and 8d. a day to run.

Recovery of intact cells, grouping properties, and four intracellular enzymes were measured after storage at -196°C. (liquid nitrogen), -100°C. (electrical refrigerator), and -78°C. (CO₂ snow). Over a period of two months at -196°C recovery remained at 90 to 95%, which is the same as obtained immediately after freezing. There was no deterioration in grouping properties. Aldolase and glutamic oxaloctic transaminase had fallen by 14% and 17%, respectively. Glucose-6-phosphate and 6-phosphogluconic acid dehydrogenase activities appeared to be enhanced.

Present results suggest that storage at -100°C and -78°C is less satisfactory.

REFERENCE


BIOCHEMICAL EXPERIENCES IN PARTIAL HEPATECTOMY

H. CLEEVE (St. George's Hospital, London) said that three cases of partial hepatectomy performed by Mr. Rodney Smith had been studied post-operatively. The estimated bulk of liver tissue recovered was 75% in Case 1, 90% in Case 2, and 50% in Case 3. Among the points of interest uncovered by the biochemical findings was a drop in blood urea from 23 mg./100 ml. pre-operative level to 9 mg./100 ml. on the second day, and a drop in prothrombin level from 466 mg./100 ml. to 266 mg./100 ml. on the first day rising to 692 mg./100 ml. on the ninth day in Case 2, suggesting that the removal of nine-tenths of the liver was near to the limits of tolerance.

Of the serum enzymes studied, the glutamyl pyruvic transaminase showed a rapid rise in the first three days in all cases reverting to normal over a varying period up to 20 days. This was assumed to reflect the necrosis of some of the residual tissue. The lactic dehydrogenase, on the other hand, rose after operation and maintained a fluctuating high level for 14 days. Experimental studies suggest that this activity may represent the initial stages of liver rejuvenation.

The albumin levels showed an immediate post-operative fall with a corresponding rise in total globulins, and were recovering only slowly during the patient's stay in hospital.

In all patients the histological appearances of the residual liver were normal.

MONOAMINE OXIDASE ACTIVITY IN TOXAEMIA OF PREGNANCY

M. SANDLER, JENNIFER COVENFY, and ELIZABETH BALDOCK (Queen Charlotte's Hospital, London) A small but significant decrease in monoamine oxidase activity was detected in placentas from patients with toxaemia of pregnancy when compared with placentas from normal pregnant subjects. This change tended to be greater in patients with more severe manifestations of the disease.

Although the recovery of 5-hydroxyindoleacetic acid after 5-hydroxytryptamine ingestion was significantly decreased in toxaemia, suggesting the possibility of a generalized monoamine oxidase defect, this interpretation was not confirmed when 24-hour excretion of tryptamine was used as an index of enzyme inhibition. There was a significant increase in tryptamine output in normal pregnancy but, instead of the further increase which might have been predicted in the presence of a generalized enzyme defect, toxaemic patients showed a decrease to non-pregnant levels.

Further work to attempt to elucidate these findings is in progress.

HIGH-VOLTAGE ELECTROPHORESIS AND ITS CLINICAL APPLICATIONS

GEOFFREY FRANGLEN (St. George's Hospital Medical School, London) described an apparatus for high-voltage electrophoresis capable of applying up to 25,000 v. and 0-1 A. across strips of filter paper 150 cm. long and 15 cm. wide. Many substances of clinical interest can be analysed, including sugars, indoles, the mandelic acids, and Figlu. Attention has been concentrated on the separation of peptides from enzymic protein hydrolysates, and on the quantitative estimation of amino-acids by means of the ninhydrin-cadmium acetate reagent. Recently the analysis of amino-acids has been extended to ultramicro levels by use of the dye, 5-diethylamino naphthalene 1-sulphonyl chloride; this forms highly fluorescent complexes with amino-acids, separable only by high-voltage electrophoresis. The analysis of the allo-forms of amino acids was described, and their presence and significance in biological material discussed.

ISO-ENZYMES OF ALKALINE PHOSPHATASE

A. L. LATNER and A. W. HodSON (King's College and Royal Victoria Infirmary, Newcastle upon Tyne) Using starch-gel made up with 0-05 M Tris-HCl buffer at pH 8·8 and, after electrophoresis, a substrate of Ca-naphthyl-phosphate, it had been possible to demonstrate separate bands of alkaline phosphatase activity in various tissue
extracts by coupling the liberated naphthol with 5-chloro-2-amino-toluene. When the extracts are mixed their bands of activity appear in their separate specific positions. Liver, bone, and intestinal alkaline phosphatases were separated and a probable difference in migration behaviour of alkaline phosphatase activity in kidney extract demonstrated.

Using the migration data obtained, it had been possible to recognize liver phosphatase activity in the sera of patients with liver disease, and bone phosphatase activity in specimens from patients with bone disease. Judging from the migration behaviour, it appeared that the enzyme activity commonly present in normal serum was derived from liver. Intestinal and bone alkaline phosphatases apparently occur only occasionally.

A possible diagnostic application had arisen in the differential diagnosis of jaundice. Moreover, metastases occurring simultaneously in liver and bone give rise to a serum activity pattern corresponding to bone disease, and so jaundice resulting from metastases can be differentiated from hepatocellular disease.

**THE USE OF ION-EXCHANGE PAPERS AND REVERSED PHASE PAPER CHROMATOGRAPHY FOR THE SEPARATION OF BARBITURATES**

H. V. STREET (University of Edinburgh) The behaviour was described of mixtures of barbiturates when subjected to chromatography on modified cellulose ion-exchange papers using aqueous and organic solvents. With horizontal circular chromatography on diethylaminomethyl cellulose ion-exchange paper (DE20) and a solvent of tertiary amyl alcohol saturated with 0.1 M-ethylene-diaminetetra-acetic acid (di-sodium salt), a mixture of phenobarbitone, barbitone, butobarbitone, and quinalbarbitone could be clearly resolved in four hours. It was shown that a mixture of phenacetin, salicylic acid, p-acetylamino phenol, and phenobarbitone could be separated by chromatography (15 min.) followed by ionophoresis (60 min.) on DE20 paper using 0.2 N-ammonia solution as solvent for both chromatography and ionophoresis. The technique has been successfully applied to extracts of blood containing these four compounds.

The effect of variation in temperature on the separation of mixtures of barbiturates by reversed phase chromatography was also discussed. It was shown that with tributyrin-impregnated Whatman No. 1 paper, chromatography at 86°C. gave good resolution in 15 minutes of a mixture of barbitone, phenobarbitone, butobarbitone, and quinalbarbitone. The solvent was M/15 phosphate buffer, pH 7.4. By extending the running time to 60 minutes, a mixture of 13 different barbiturates could be separated with the exception that resolution of pentobarbitone and amylobarbitone was not achieved by this technique.

The application of this latter technique to blood extracts was discussed and it was suggested that because of the good resolution obtained within 20 to 30 minutes the procedure could be of particular value to clinical chemists and to clinicians.

**SOME MODIFICATIONS TO THE AUTOANALYZER METHOD FOR SERUM ALKALINE PHOSPHATASE**

K. B. COOKE and V. J. PATSTON (Westminster Medical School, London) The existing AutoAnalyzer method for serum alkaline phosphatase estimation can only be used on undiluted sera with activities below about 35 K.-A. units although experiments with varying times of incubation indicate that the technique could estimate activities up to 100 K.-A. units. Two methods were described which allow sera with activities below 100 K.-A. units to be estimated without pre-dilution.

In the first the original King-Armstrong barbitone buffer (Armstrong and King, 1934), its concentration suitably adjusted, replaces the carbonate buffer.

In the second technique the carbonate buffer is retained. The sample is diluted, and then divided between two streams, one of which is used for the test estimation in the normal way. The second stream is immediately mixed with a carbonate-4 aminoantipyrine reagent for the blank estimation. With this technique alkaline phosphatase is estimated on only 0.3 ml. of sample. Inclusion of a dialysing stage also allows other estimations, such as serum phosphatase, on the same sample. Finally the addition of Tween 20 to the water washes between samples eliminates 'noise' which is a troublesome feature of the original method.

**REFERENCE**