INTRODUCTION

In 1957 there were no analyzers, most of the equipment was made ‘in-house’ and often relied on knowledge being ‘handed down’ and mouth pipetting was the norm. Although a few textbooks were available they were very limited in their methodologies and interpretation of tests. Clinically the range of routine biochemistry tests was limited and the interpretation was similarly limited. The majority of ‘routine’ biochemistry tests evolved from a chemistry origin and was published in mainly chemistry-orientated journals. The routine pathology laboratory would not normally have access to the majority of these journals and frequently relied on a single textbook for biochemical analysis with the methods being transcribed on to laboratory method cards. It is important to consider that at the time the concept of requesting tests e.g. liver function tests (LFTs) or electrolytes was not known and individual tests were most frequently requested depending on the clinical decision. Most individual tests used one to two ml of either serum or plasma. In this article a retrospective look at the some important biochemical methods available in 1957 and their interpretation is presented with the principles of the methods. For ease of considering the use of tests in a modern context, they have been grouped in to a modern view of their use.

Electrolytes

Although the importance of plasma sodium was understood, the relevance of potassium was doubted, as it was primarily intracellular and therefore of ‘academic’ interest only. Sodium was of clinical interest as it had been recently described as being low in Addison’s Disease (Loeb, 1932). A linkage with chloride and bicarbonate was considered and the use of sodium, potassium and chloride were considered ‘useful’ in surgical emergencies.

Analytically flame photometry was still in its infancy and the chemical method of Trinder’s was most frequently used (Trinder 1951). Briefly, sodium was precipitated with magnesium uranyl acetate as sodium uranyl acetate and proteins precipitated with alcohol. Excess uranyl acetate in the supernatant was read against a blank and calculated using sodium standards. This was a modification of the method by McCance and Shipp (1931) who precipitated proteins using trichloracetic acid and then precipitated the supernatant sodium as sodium zinc uranyl acetate. The precipitate was treated with uranyl ferricyanide and a plum-red colour (uranyl ferricyanide) was compared using a colorimeter against a blank and sodium standards. Results for both methods were reported as mgm/100ml. Potassium similarly relied on a precipitation method but was precipitated directly from the serum using sodium cobalt nitrate giving potassium sodium nitrate, which was washed then dissolved in hot water and choline chloride and sodium ferricyanide added with a resulting emerald green colour, which was proportional to the concentration of cobalt and hence potassium (Jacobs and Hoffman, 1931). Results were calculated using standards and a blank and reported as mgm/100ml. Blood chloride was not considered important unless it was used in conjunction with sodium to establish how much sodium chloride solution should be given in treating burns, vomiting and severe diarrhea.

When assayed it was usually using a titrimetric technique such as the one summarized here (Whitborn, 1921). Blood proteins were precipitated using sodium tungstate and sulphuric acid and the chloride in the supernatant precipitated using silver nitrate. Excess silver nitrate was back titrated using potassium thiocyanate with iron-alum as the indicator and calculated as mgm/100ml of chloride or mgm/100ml sodium chloride.

Liver function tests

In 1957 the concept of using a set of analytes and enzymes was not known and tests of ‘liver efficiency’ were most frequently used often as single tests. Typically the most common tests were: bilirubin, alkaline phosphatase, zinc sulphate turbidity test and bromosulphalein test. Of these, bilirubin was considered an important analyte as there was a good understanding of jaundice. Urinary bile salt detection (Fuchet’s test) was a commonly used technique as it was widely believed that they were formed in the liver, therefore an efficient test of ‘liver efficiency’ (Kawerau, 1953).

Bilirubin

The most commonly used method was that of Van den Bergh (1916), which used diazo reagent (two solutions: A, comprising of sulphanilic acid, concentrated hydrochloric acid and distilled water; B, sodium nitrate and distilled water). These were mixed 1:3 of A and B on the day of the test. Serum was added to the diazo reagent and the colour allowed to develop. If there was no colour within 30 minutes the direct bilirubin was negative. For total bilirubin, serum was added to diazo reagent then alcohol and saturated ammonium sulphate added. The resulting flocculent was centrifuged and the colour of the supernatant compared with a set of cobalt sulphate standards using a Lovibond comparator to give mgm/100ml bilirubin. Each test i.e. direct and total bilirubin required one ml of serum.

Alkaline phosphatase

At the time the origin of alkaline phosphatase was unknown and was thought to be ‘leakage’ from bones or similar tissues. Elevation of activity was established in obstructive jaundice and bone disease as well as diseases of the liver (Kawerau, 1953). An assay in common use (King and Armstrong, 1934) had serum added to phenyl phosphate (substrate) in an alkaline buffer and incubated with a control which had the proteins precipitated prior to substrate addition. After incubation a phenol reagent (Folin and Ciocalteux reagent) was added to test, control standard and blank, mixed centrifuged and sodium bicarbonate added to the supernatant and the resulting colour read in a colorimeter.

Zinc sulphate turbidity test

Zinc sulphate in a barbitone buffer will precipitate gamma-globulin and was used to establish whether protein electrophoresis was necessary (Kawerau, 1953). The resulting turbidity was compared with a series of protein standards treated identically (10mgm protein equaled one turbidity unit).
Bromosulphthalein (BSP) test
This was an assessment of the liver’s ability to clear a foreign substance. A known dose of BSP was injected in to a vein. At 30 minutes post injection, blood was taken from the opposite arm and serum put in to test tubes. One or two drops of sodium hydroxide were added to the first tube and water to the second tube. Any remaining BSP would colour red and could be compared to BSP standards. Normally BSP is cleared within 30 minutes and BSP retention (high colour) was taken to indicate liver disease (Kawerau, 1953).

Pancreatic efficiency
Amylase was a well-recognized test of pancreatic function, although urine amylase appeared to be preferred over blood amylase. Irrespective of whether urine or blood was used the most frequently used method was that based on Wohlgemuth’s method whereby starch was digested by urine or blood amylase and the remaining undigested starch was detected with iodine solution. The amylase activity was calculated in arbitrary units (Wohlgemuth’s units) relating to the colour development from the addition of the iodine solution (Stocks, 1914)

Renal efficiency
Generally renal efficiency investigations would be triggered by the presence of protein in the urine as well as other indicators such as cells and casts. If the urine protein screen was negative then it was regarded as having no further need to proceed with blood urea or other tests of renal function such as concentration test (McLean and de Wesslow, 1920).

Blood urea
The urease-nesslerization method progressively became the method of choice over other methods (Archer and Robb, 1925). Blood was incubated with a urease suspension (this was usually ‘a knife point’ [0.1g] of soya bean meal), following incubation; proteins were precipitated using sodium tungstate and sulphuric acid. After centrifugation Nessler’s reagent (a solution of potassium and mercuric iodide in sodium hydroxide) was added to the supernatant, standard and blank and the resulting colour read in a colorimeter. The test was highly sensitive to ammonia and could be invalidated if ammonia was present in the laboratory.

Blood creatinine
This was not regarded as being as reliable as blood urea for the general diagnosis of renal disease, however, there is evidence that some clinicians used it as a prognostic aid i.e high creatinine concentrations usually had a poor patient outcome. At the time however, little value was attached to any practical use for blood creatinine assays.

Blood uric acid
This is believed to be the first blood analyte to be determined in 1848 by Sir Archibald Garrod using sheep blood. The most frequent use of uric acid was in the diagnosis of gout and notable increases were observed in leukemia, renal disease, and certain cases of eclampsia. Clinical and radiological examination was considered to be generally more important than uric acid in the diagnosis of gout. Blood proteins were precipitated using sodium tungstate and sulphuric acid (Folin, 1939). Supernatant, standard and blank were prepared and 40 per cent urea added followed by 15 per cent sodium cyanide, then the uric acid reaction solution (mixture of phosphoric acid, water, sodium tungstate and lithium carbonate). After incubation the developed colours were read using a colorimeter and uric acid concentration calculated using the uric standard.

OTHER ROUTINE BLOOD TESTS
Glucose (blood sugar)
Blood glucose assays were well established although they were based on the detection of reducing sugars in the blood (glucose being the principle sugar). The use of the blood sugar tolerance tests was well established for the diagnosis of diabetes and for the monitoring in pregnancies when severe glycosuria was identified. At the time there were a number of methods for glucose measurement. The method of Folin and Wu (1920) was frequently used. Blood was deproteinised with sodium tungstate and sulphuric acid, following deproteinisation the supernatant was mixed with an alkaline copper solution (sodium carbonate, tartaric acid and copper sulphate), standards and blank were prepared also. Following incubation at 100°C the tubes were cooled and phosphomolybdc acid solution added. The developed colour was read in a colorimeter after allowing the carbon dioxide generated by the reaction to be liberated.

Cholesterol
Cholesterol was considered most useful for the diagnosis of xanthomatosi (deposits of cholesterol in skin around the body) and hypothyroidism, otherwise it was not regarded of much use in clinical work. The method of Myers and Wordell (1918) was considered the most appropriate technique at the time. Blood was mixed with plaster of Paris and dried. The dried blood was extracted with chloroform then with acetic anhydride and sulphuric acid. A green colour develops and compared with similarly treated standards and read using a colorimeter.

Acid phosphatase
The use of this enzyme in the diagnosis of prostate cancer was well recognized as was the stability of prostate acid phosphatase in the presence of formalin. Usually the alkaline phosphatase method was used with a change of incubation buffer pH to 4.5, the addition of formalin and the increase in the incubation time from 30 to 60 minutes (Gutman and Gutman, 1938).

Calcium
Calcium was considered important in the diagnosis of tetany and for monitoring parathyroid hormone treatment using parathyroid extracts. Although it was recognized that calcium could be analysed by flame photometry, the method of Kramer and Tisdall (1921) was frequently used. Calcium was precipitated from serum using saturated ammonium oxalate. After centrifugation the tubes were drained and the precipitate washed with dilute ammonia solution followed by 0.1N sulphuric acid, boiled then titrated immediately after cooling with potassium permanganate until a pale pink colour lasting one minute is achieved. Calcium concentration was calculated from the titration. This method uses two ml of serum per test.

Phosphate
Phosphate analysis was considered most helpful in rickets. Proteins were precipitated using trichloroacetic acid. An aliquot of supernatant was treated with molybdc acid and phosphomolybdate, followed by hydroxyquinone in the presence of sodium sulphate. The resulting blue reaction colour was read in a colorimeter compared with standards and a blank (Briggs, 1922).

Acid base
The ratio of carbonic acid to bicarbonate was well recognized in controlling blood pH. However, the principle causes of acid base disruption were considered to be ketosis via the detection of urinary ketones, renal insufficiency, and fever. The determination of acid base balance was difficult with no suitable pH, PCO2 and PO2 electrodes.

Bicarbonate
It was widely considered that bicarbonate gave comparable results for alkali reserve, which was similar to total carbon dioxide. Blood was collected under paraffin oil and stirred thoroughly then centrifuged to obtain plasma under oil. Two 100ml conical flasks were prepared (‘Reference’ and ‘Test’). The ‘Reference’ contained phenol red (indicator) plasma and physiological saline. In the ‘Test’ phenol red, plasma and 0.01N hydrochloric acid were added. Both flasks were ‘whirled’ vigorously and incubated for 30 minutes at 37°C. Physiological
saline was then added to both flasks, and the solutions covered with paraffin oil then titrated with 0.01N sodium hydroxide until the ‘Test’ colour equaled the ‘Reference’ colour. Plasma bicarbonate concentration was calculated from the titration (Van Slyke, 1922).

**Blood proteins**
Measurement of plasma or serum proteins was based on the micro-Kjeldahl technique, however, the biuret method was gradually replacing the micro-Kjeldahl technique due to its simplicity. Total protein was precipitated with trichloroacetic acid and the precipitate was dissolved in sodium hydroxide followed by crystalline copper sulphate solution. The resulting colour was read in a Lovibond Comparator. For albumin, ammonium sulphate was added to either plasma or serum and the solution filtered. The filtrate then had trichloroacetic acid added mixed then treated as for total protein (Howe, 1923; Fine, 1935).

**Protein electrophoresis**
Protein electrophoresis was not a routine technique and was generally indicated by a positive zinc sulphate turbidity test or proteinuria. Electrophoresis tanks were usually made ‘in house’ to a common design (Flynn and de Mayo, 1951). A barbitone buffer was used and the support medium was Whatman number 1 filter paper strips. The origin was marked with pencil and serum loaded at the origin then the current applied for 16 to 18 hours. The filter paper strips were then removed, dried and stained (often with brom-phenol blue in alcohol and mercuric chloride), rinsed then dried. Five main bands could be located and interpretation related to well defined clinical conditions (Hardwicke, 1954; Flynn and de Mayo, 1951; Flynn, 1954).

**CONCLUSION**
Over the last 60 years there have been dramatic developments in laboratory medicine and clinical biochemistry is a sharp reflection of such change. As clinical demands evolved new approaches to diagnostic testing were required such as the development of flame photometry for sodium and potassium and the concept of diagnostic test profiles e.g. electrolytes, liver function tests etc. With the advent of biochemistry analysers, especially the Technicon® continuous flow auto-analysers the single biochemical test taking hours to perform with little or no quality control system rapidly disappeared. Acid-base balance also reflected such changes with the development of gas and pH electrodes providing rapid and accurate patient acid-base status. These changes were reflected in a change in the scientific and medical literature with increasing numbers of journals dedicated to the use of the newer technologies and the ability to obtain detailed biochemical analysis of disease states. The rapid availability of biochemical testing and the deceasing volume of blood and plasma to undertake such tests allowed for improved patient management, accurate diagnosis and significant changes in surgical procedures. Analytical techniques have made significant progress since 1957 and today modern medicine is on the cusp of a new generation of technologies and diagnostic power.

**AUTHOR INFORMATION**
Michael Legge, PhD MRSB FIBMS FNZIMLS FFSc(RCPA), Honorary Associate Professor
Department of Biochemistry, University of Otago, Dunedin

Author for correspondence: Michael Legge.
Email: michael.legge@otago.ac.nz

**REFERENCES**

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