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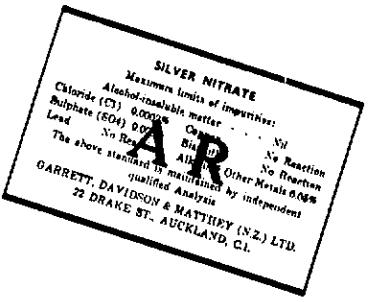

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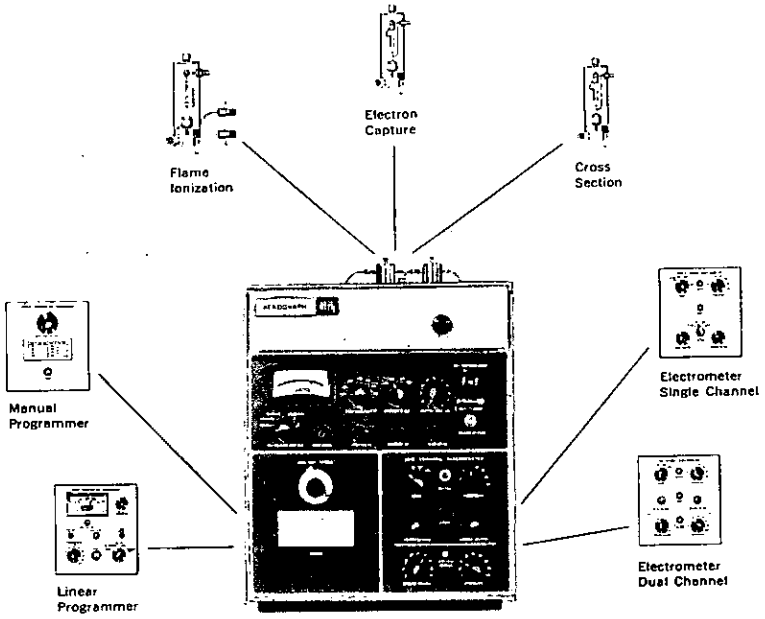

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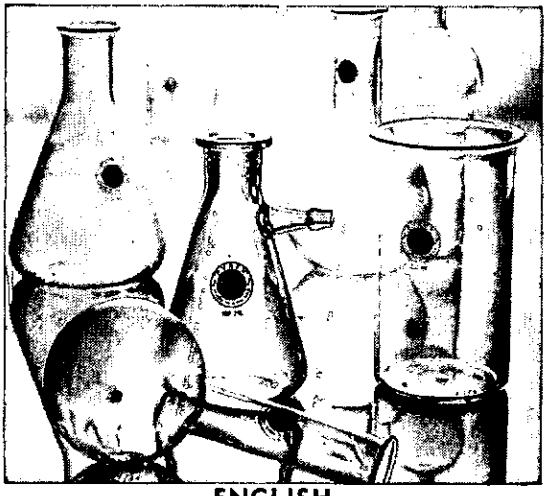

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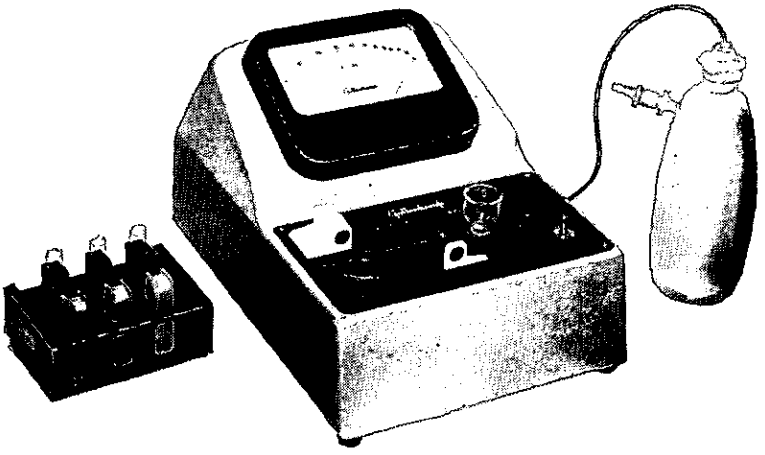

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2. Nemodruk, A. A. and Kochetkova, N. E., *C.A.*, 1962, 57, 11855h
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A very reactive and versatile intermediate, readily forming polymers and copolymers. With Grignard reagent 1:2- or 2:2-diphenyl-ethanol is produced[1]. The coloured reaction products from styrene oxide and pyridine or quinoline derivatives provide a method of colorimetric assay for this class of compound[2].

1. Karasch, M. S. and Clapp, M., *J. Org. Chem.*, 1938, 3, 355
2. Roth, H. J. and Schrimpf, H. O., *Arch. Pharm.*, 1960, 293, 22-8

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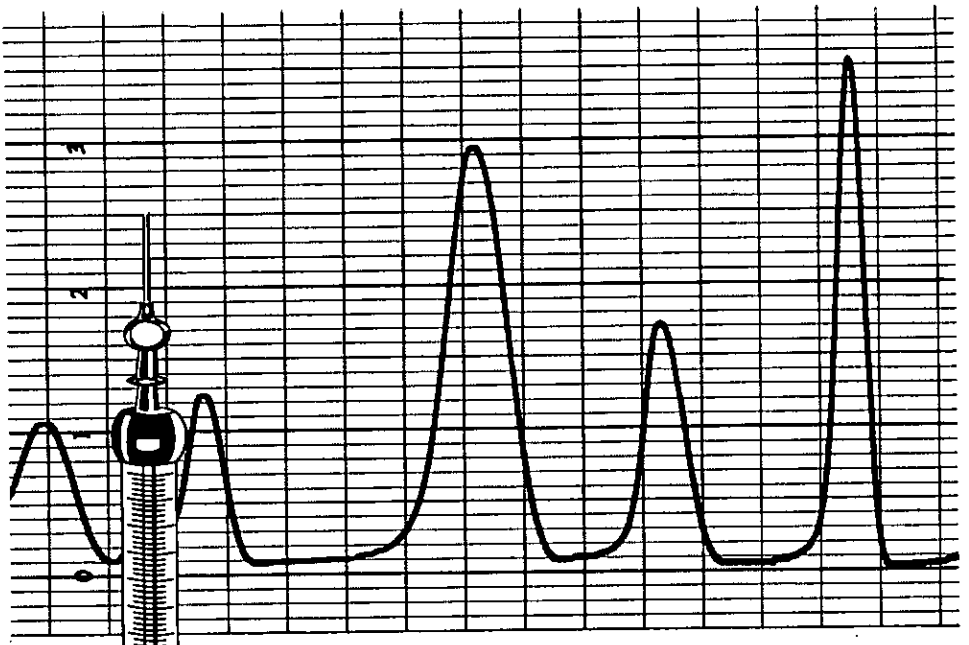

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TECHNICIAN TRAINING

In this issue are published the names of the first students to complete the examinations for the Science Technician's Certificate. The main subject for all the successful candidates was chemistry, the first subject in which the higher level courses were organized, but courses leading to certificates in other science disciplines have started.

Also recorded in the Registry in this issue are the names of technicians who were awarded the Laboratory Assistant's Certificate as a result of the 1963 examinations conducted by this Institute. It is intended that the L.A.C. examinations will cease when those applicants now enrolled have qualified. During its nearly twenty years' operation, the L.A.C. has served a useful purpose in encouraging technicians to continue studies, but it has became redundant with the development of both training and certification on a national basis under the Technician Certification Authority.

This does not mean that the Institute as a body and its members individually should not continue to concern themselves with technician training and qualifications. As the employers and directors of chemical technicians, chemists must inevitably be interested in the scope and standard of training. One major problem with the Authority courses at present is that adequate tuition, especially at the higher levels, is available only in the larger centres. Technicians in smaller towns are therefore handicapped and the efforts being made by a few Institute members to assist local technical schools with evening classes are no adequate substitute for professional teaching. It is particularly important, too, to ensure that technician training does not duplicate or imitate university degree courses. What is required is a training with a strong bias to the practical carrying out of chemical techniques, with sufficient theoretical knowledge to understand general principles, especially those involved in operation and maintenance of laboratory instruments, but not necessarily the more abstruse concepts of chemical theory.

A technician can make an essential contribution to a research project because he possesses skills which his graduate colleagues who are responsible for experimental design and interpretation do not have. It is better for both if the training of the technician is not merely an inferior parallel to a B.Sc. course.

CHEMISTRY IN THE HOSPITAL LABORATORY

JOAN MATTINGLEY

Senior Hospital Scientific Officer, Wellington Hospital

The hospital laboratory is also called the Pathology Department. Pathology is the branch of medicine which studies structural and functional changes in tissues and organs in various diseases. In the past the work of the pathologist was concerned mainly with morbid anatomy and histology. Thirty years ago most doctors themselves did analyses of their patients' specimens. Requirements were simple. Urine was boiled to detect protein, indicating a kidney fault, or boiled with Benedict's solution for sugar, to complete a diagnosis of diabetes. With microscopes they did simple cell counts to diagnose anaemia, or searched stained smears of pus to identify the infective bacteria. The pathologist was consulted rarely, and then usually to conduct the post-mortem to determine the cause of death.

Today the situation is quite different. The pathologist is head of a department covering many aspects of pathology in its modern form. The enormous advances in science during the last thirty years are reflected nowhere better than in the phenomenal growth of the medical laboratory. The application of many of the modern methods of the basic sciences to problems of medicine has resulted in a striking increase in medical knowledge. Much more is now known of the finer structures of the body organs and tissues, how they work, their inter-relationships with one another, and consequently a better understanding of how and where they go wrong. For example, the "kidney disease" of the past, today is diagnosed more specifically. The kidney is a complex organ, and the trouble can often be pinpointed to a particular part of it. This is done by the clinician assessing all the evidence derived from numerous laboratory examinations of the patient's blood and urine, and his own direct observations of the patient.

Today no general hospital is without a laboratory. Hardly a patient is diagnosed and treated without having submitted blood or other specimens for various analyses and other investigations. At present such laboratory work falls into four main categories:

(1) Morbid anatomy and histology. This involves post-mortems and the microscopic examination of sections of tissue — *e.g.*, to decide the malignancy or non-malignancy of a cancer.

(2) Haematology, determining numbers and types of various blood cells, as in anaemias and leukaemias, and the classification of bloods into their respective blood groups.

(3) Bacteriology, identifying by special growth media, etc., the infective agents from pus and other sources, for correct diagnosis and treatment.

(4) Biochemistry, the application of chemistry to the study of the components of living matter and their reactions in living cells. These components are broadly classified as carbohydrates, proteins, lipids, enzymes, minerals and hormones. Hospital biochemistry consists mainly of quantitative and qualitative analyses of these in blood, urine, cerebro-spinal fluid, gastric contents, or any other part of, or product of body fluids and tissues. Drugs, poisons and other "foreign" substances may also be involved. In health, the amounts of such components occur within narrow limits, and a balance within such narrow limits is maintained despite the daily intake of all sorts of conglomerations in the form of food. In many diseases this balance is upset and one or more may be present in greater or lesser amount than is normal. Their estimation can thus contribute to diagnosis and be helpful in following the course of treatment which is designed to restore the normal chemical state and hence, normal health. For example, blood sugar normally ranges from 65 to 120 mg per 100 ml. The classical diabetic may have a level of 300 mg or more. He may well be comatose with this amount. On the other hand, with an overdose of insulin his level may drop to 30 mg, when he is again comatose, but for the reverse reason. A blood sugar estimation is therefore of much importance, and incidentally of much urgency, if the correct treatment is to be given.

The results of most hospital laboratory analyses are required the same day as the specimens are received. Some of them are urgently required, which means within half an hour. This means that the methods used must be fast and accurate. Accuracy can, however, be sometimes sacrificed for speed, when the question is whether there is an excessively abnormal amount present, rather than whether 1.1 or 1.2 mg are present. Some estimations, of course, are not urgent, and may take a week for the actual analysis. Always, great care must be taken with the specimen. There is little opportunity for repeating estimations, and "bucket"

chemistry scarcely exists. Most estimations are done with microgram amounts. Many specimens are precious because they can be obtained only once. No patient, no matter how tough, cares for a lumbar puncture to be repeated just because someone botched his analysis! Half a millilitre of blood from a baby may have to suffice for six quite different analyses. Loss or wastage of such a specimen can produce crises of no small order. Similarly, five millilitres of blood from an adult must be enough for many of the analyses that may be required. Some specimens are taken at a particular time, for example, a certain number of hours after a suspected heart attack, when the level of serum lactic dehydrogenase is of diagnostic value. This enzyme is present in heart tissue in comparatively large amounts. When the heart is damaged by an infarct, much of this dehydrogenase is released into the bloodstream. The increased blood level is only transitory and the timing of the taking of the blood specimen important. Thus the laboratory must not spill, spoil, lose or confuse with another, this unrepeatable specimen.

In any one day many different types of analyses are done. Sodium and potassium are estimated by flame photometry, and, in some laboratories, calcium and magnesium too. In Wellington Hospital, calcium and magnesium are done by atomic absorption, and in certain specimens by volumetric or colorimetric analysis. Electrometric apparatus is used for rapid chloride estimations. Colorimetry is used for sugar, cholesterol, phosphorus, iron, iodine, certain proteins, urea, steroids, bilirubin, and certain enzyme estimations. Some of these, particularly blood sugar and blood urea, are done by an auto-analyser, because so many specimens are received each day. Fractionation of proteins in body fluids is usually accomplished with electrophoretic apparatus. Methods of chromatography, column, paper and thin layer, are used for separating amino acids, sugars, indoles, steroids, porphyrins. Fluorimetry is used for adrenalins and some steroids, and gas analysis for blood oxygen, carbon dioxide and sometimes carbon monoxide. Old-fashioned methods of nephelometry still have a place in the hospital repertoire, and inorganic group separations are occasionally needed. In fact, there is scarcely a field of chemical analysis which has not some application in medical chemistry. Some examples of the particular applications of certain analyses will illustrate their use.

In many cases of prolonged kidney and/or heart failure, or after prolonged post-operative vomiting, the levels of serum sodium, potassium, chloride and bicarbonate are

altered beyond their normal limits. Vomiting results in a loss of gastric juice containing much chloride. This is replaced from the blood, resulting in a blood chloride deficiency. The bicarbonate level in the blood rises to compensate for the lost chloride ions. In the meantime, because the total anions must equal total cations, the blood sodium is reduced to equalize the loss of chloride. The level of blood potassium rises to compensate the sodium loss. The resulting severely ill patient is having a hard time maintaining his blood pH within the narrow normal limits of 7.35 to 7.45. When the clinician is supplied with the details of blood analysis, he can decide what treatment to begin, which intravenous solutions will help to correct the chemical imbalance, and how much of them. Rarely, these patients develop complete but temporary kidney failure, during which time the blood urea climbs to dangerously high levels, when death ensues. If the excess urea can be removed before its toxicity overwhelms him, and the electrolyte imbalance corrected quickly, the patient can be kept alive until his kidneys begin to function again. He can be restored to reasonably normal health provided his kidneys have suffered no permanent damage. The artificial kidney is used for these patients. This apparatus consists of a large bowl filled with gallons of solution of salts in the same concentration as in normal blood. A coil of dialysing membrane many feet long is suspended in the solution. The patient's blood is led from one of his arteries into this membrane and back into his body through one of his veins. This circulation system is maintained for approximately five hours usually. By simple dialysis the normal concentration of blood electrolytes is restored and urea largely removed. Regularly, a sample of the blood is removed for analysis, to check progress. The analytical requirements of this work were a stimulus to the manufacturers of flame photometers.

Successful heart surgery, a recent development of great interest, requires fore-knowledge of the position and extent of the defect in the patient's heart. This knowledge is partly obtained from estimations of oxygen content of blood samples obtained by cardiac catheterization. A narrow tube is passed into a vein in the patient's arm and pushed along inside the vein until it passes into the heart. Small samples of blood are removed from various regions within the organ, the position of the tip of the catheter being observed with X-rays. The oxygen content of each sample is measured in Van Slyke's blood gas apparatus.

Blood from the left side of the heart, having just returned from the lungs where it is oxygenated, contains normally much more oxygen than that from the right side. A hole in the wall between, as is found in some heart defects, allows mixing of the two bloods. The abnormal oxygen levels thus found provide the surgeon with evidence for the position and size of the defect to be repaired.

Some disorders are not acquired through the normal wear-and-tear processes of living but appear to be due to defective genetic material. The theory that each metabolic step in a living process requires a particular enzyme whose presence depends on the presence of a particular gene, suggests that a number of diseases called "inborn errors of metabolism" could be due to the hereditary lack of a single gene. Consider the consequences of a simple block; normally substance A is converted into substance B which in turn is converted into substance C, and so on. If the enzyme converting A to B is missing, A may accumulate to toxic levels. The deficiency of B leads to a deficiency of C. C may be an essential metabolite, without which growth or development cannot take place. Meantime, A may be metabolized along an alternative path which leads to the formation of substances which are highly toxic. Rarely, A and B are not important and C can be obtained in another way, so that no harm results.

Disorders such as phenylketonuria and galactosemia are explained in this way. Absence of phenylalanine hydroxylase leads to a high blood level of phenylalanine which is partially metabolized by an alternative path producing toxic end-products. The detection of this condition in the first few weeks of life enables the child to be fed a diet low in phenylalanine. This diet keeps the blood levels of phenylalanine and its toxic products low, reducing the damage they may do to the developing brain. This could mean the difference between a moderate mental handicap and hopeless imbecility. Similarly, galactosemia, a disease with severe mental and physical defects due to a block in the metabolism of galactose, can be modified by the complete removal of galactose and its precursors from the child's diet from birth. Milk is poison to such children. The excess metabolites in these and similar diseases are usually detected with paper chromatography. As speed in diagnosis is essential, thin layer chromatography is better for some.

As medical knowledge develops and changes, diagnosis and treatment demand more and more assistance from biochemical analyses. Newer, faster, better, more accurate methods are constantly replacing older ones, or are added to the already wide range. Chemistry has an increasingly important contribution to make to modern medicine.

ANALYSTS AND ENZYMES

D. E. WRIGHT
Ruakura Agricultural Research Centre, Hamilton

INTRODUCTION

No matter to which branch of science we belong, all of us depend upon some form of measurement to provide experimental data. As chemists, we all appreciate the need for analytical methods which are accurate, sensitive, specific, simple and preferably cheap. All of us have used methods involving volumetric or gravimetric measurements or spectrophotometry; most of us have used one or more of the numerous applications of chromatography or electrophoresis, but few chemists have used enzymes as analytical reagents.

The first recorded use of enzymes was the detection of hydrogen peroxide with the enzyme peroxidase in 1845. Another enzyme, urease, was used in the early part of this century for detecting and estimating urea. Thus, while enzymatic analytical methods are becoming more widely used as a result of increased knowledge of enzymology and the ready availability of purified enzymes from commercial sources, the technique cannot be considered new.

The use of living cells (*e.g.*, bacteria) in analysing proteins, vitamins, amino acids, etc., has, of course, contributed greatly to our knowledge of nutrition. This use of intracellular enzymes is termed biological assay rather than enzymatic analysis. This latter term is generally used for determinations involving enzymes which have been isolated from cells and at least partly purified.

The great advantage of enzymes as analytical tools is their high degree of specificity. The need to remove interfering substances is reduced, thus simplifying the method. Since enzymes are used under mild conditions of pH and temperature, labile compounds, difficult to measure by other methods can be estimated.

The characteristic feature of an enzyme is its ability to catalyse a chemical reaction. The main factors affecting the rate of enzyme catalysed reactions are the concentration of enzyme [E], the concentration of substrate [S], the presence of activators, coenzymes, and inhibitors, the pH and the temperature. The mechanism of an enzyme reaction can be described by the following equation where an enzyme-substrate complex is formed which breaks down to the enzyme plus product:

$$\underset{\text{(Enzyme)}}{E} + \underset{\text{(Substrate)}}{S} \longleftrightarrow ES \longleftrightarrow \underset{\text{(Product)}}{E + P}$$

GENERAL PRINCIPLES

Enzymatic analysis can be used to determine the concentration of a substrate [S] or the concentration of an enzyme [E]. Since the estimation of [S] will be of more general interest, estimation of [E] being mainly used as an aid to diagnosis in clinical medicine, most of my comments and illustrations will be confined to the former method.

The concentration of substrate can be measured by two techniques which are basically quite different.

(1) The first method measures either the concentration of the product formed or of the residual substrate. Usually sufficient enzyme is added to the mixture to enable the reaction to proceed as rapidly as possible, within, say, five minutes.

(2) Since the rate of an enzyme reaction is dependent, within certain limits, on the concentration of S or the presence of activators, etc., this principle can be used quantitatively. In this method, [S] and [E] are so arranged that the rate of the reaction, defined as the amount of substrate reacting in unit time, is not so fast that it cannot be measured accurately.

METHOD 1: MEASUREMENT OF PRODUCT CONCENTRATION

The proportion of [P]/[S] will depend on the equilibrium constant of the particular enzyme reaction being studied. Where the substrate is completely changed into the product, then from measurements of [P] by chemical or physical means it is simple to determine [S]. Most enzyme reactions, however, do not proceed to completion. In these circumstances, it is often possible to drive the reaction by removing the product. For instance, if the product is a carbonyl-containing compound — *e.g.*, pyruvic acid — then a carbonyl reacting agent such as hydrazine can be added. The hydrazine will complex with the carbonyl thus effectively removing P.

Either chemical or physical methods can be used to estimate [P].

(a) If P is acidic while S is neutral, then the reaction can be followed either by titration of P with dilute alkali, or by incubating in a Warburg manometer flask containing $NaHCO_3$ buffer. As the acid is formed, CO_2 is released from the buffer and the increase in pressure can be readily measured and converted to the volume of CO_2 formed.

(b) Often P can be estimated colorimetrically — *e.g.*:

$$\text{L-arabinose} \xleftarrow{\text{L-arabinose isomerase}} \text{L-ribulose}$$

The product, L-ribulose, is a ketopentose sugar which can be estimated by the cysteine carbazole reaction, L-arabinose not interfering with the method.

(c) One of the most frequently used methods uses the coenzymes nicotinamide adenine dinucleotide (NAD^+) or nicotinamide adenine dinucleotide phosphate ($NADP^+$). These coenzymes act as hydrogen acceptors for many dehydrogenase enzymes. Reduction of the oxidized form (NAD^+) liberates one H^+/mole, another H reducing the pyridine ring at the *para* position.

Reduction of the coenzyme leads to the formation of a spectral peak at 340mμ. Since the pyridine nucleotides act as coenzymes in a wide variety of biological oxidations, they can be used to estimate many substrates either directly or by coupling a dehydrogenase enzyme to measure the product indirectly. An example would be the estimation of phospho-enol-pyruvic acid (PEP).

1. $CH_2{=}C(OH)-O-P(=O)(OH)-OH$ (PEP) + Adenosine diphosphate (ADP) $\xleftrightarrow{\text{Pyruvic Kinase}}$ $CH_3\text{-}C(=O)\text{-}COOH$ (Pyruvic Acid) + Adenosine triphosphate (ATP)

2. Pyruvic Acid + NADH + H^+ $\xleftrightarrow{\text{Lactic dehydrogenase}}$ Lacid acid + NAD^+

Thus, by following the change in absorption at 340mμ it is possible to estimate PEP.

METHOD 2: MEASUREMENT OF REACTION RATE

1. *Substrate Measurement*

As mentioned previously, the rate of a reaction will be proportional to the concentration (within limits) of the substrate. Since so many factors (*e.g.*, pH, temperature, etc.) can influence the rate, the conditions of the assay have to be very exact. Usually because of these problems, this technique is used only when a substrate cannot be estimated by Method 1.

This method is useful in measuring compounds which are utilized in reaction 1, then regenerated in a subsequent reaction 2. In these cases the concentration of the compound remains constant. An example is the determination of coenzyme (CoA) with α-oxoglutaric oxidase and deacylase.

$$\text{(i)}\ \alpha\text{-oxoglutaric acid} + \text{CoA} + \text{NAD}^{+} \xrightarrow{\text{oxidase}} \text{succinyl}-\text{CoA} + \text{NADH} + \text{H}^{+} + \text{CO}_2.$$

$$\text{(ii)}\ \text{Succinyl}-\text{CoA} \xrightarrow{\text{deacylase}} \text{succinic acid} + \text{CoA}.$$

The CoA is consumed in reaction 1 and regenerated in reaction 2, its concentration thereby remaining constant. Since reaction 1, the oxidative decarboxylation of α-oxoglutaric acid, is dependent on the concentration of CoA, the rate of reduction of the participating NAD^+ will be a measure of the amount of CoA present.

2. *Activator and Inhibitor Measurements*

Many enzyme reactions require not only a substrate and corresponding enzyme (and coenzyme), but also activators such as inorganic ions (Mg^{++}, Mn^{++}, Ca^{++}) or sulphydryl groups as in glutathione.

An example is the activation of iso-citric dehydrogenase (ICDH) by Mg^{++}.

$$\text{Isocitrate} + \text{NADP}^{+} \xrightarrow[Mg^{++}]{\text{ICDH}} \alpha\text{-oxoglutarate} + \text{NADPH} + \text{H}^{+} + \text{CO}_2.$$

The reaction, which is very sensitive to low concentrations of Mg^{++}, is followed by plotting the absorption at 340 mμ. Ca^{++} ions interfere but can be removed with oxalate, while Mn^{++} produces an even greater activation than Mg^{++}. However, since Mn^{++} usually is present in much lower concentrations than Mg^{++} this is not always a problem.

Similarly, the inhibition of an enzyme by an inhibitor can be measured. An example is the inhibition of carbonic anhydrase by the insecticide DDT.

$$H_2CO_3 \xrightarrow{\text{carbonic anhydrase}} H_2O + CO_2$$

The enzyme catalyses the hydration of carbon dioxide to carbonic acid, the equilibrium favouring carbon dioxide release. By measuring the carbon dioxide manometrically, the activity of the enzyme and its inhibition by DDT can be estimated.

EXPERIMENTAL METHODS

Since enzymes are proteins and thus can be denatured by extremes of pH, temperature, etc., it is necessary to handle them carefully. However, the belief that all enzymes are very unstable is not true and with the appropriate careful handling they can be most reliable stable reagents. Many enzymes can be bought from commercial sources at reasonable cost and in various degrees of purity. Sometimes it is more convenient to prepare the enzyme by established methods such as those found in *Methods in Enzymology.*

To illustrate that some enzymes can be prepared easily, the procedure for preparing alcohol dehydrogenase from yeast is outlined below. This enzyme oxidizes ethanol to acetaldehyde, NAD^+ being the hydrogen accepting coenzyme.

Yeast is crumbled and dried between sheets of paper at room temperature for 5 days. The yeast is ground in a ball-mill at 0°C and extracted with dilute sodium phosphate for 2 hours at 37°C and 3 hours at room temperature. The residue is removed by centrifuging. The supernatant liquid is kept at 55°C for 15 minutes, cooled and centrifuged. To each 100 ml liquid, 50 ml of cold acetone is slowly added, the temperature being kept at −2°C. The precipitate is discarded and another 55 ml/100 ml yeast extract added. After centrifuging, the precipitate is suspended in cold water and dialysed against running tap water. To the clear supernatant liquid 36 g of ammonium soulphate is added per 100 ml of solution. The precipitate is collected, dissolved in 20 ml water and 4 g of ammonium sulphate added. The resultant precipitate is discarded. To the liquid further ammonium sulphate is added to 60% saturation. The alcohol dehydrogenase precipitate is collected by centrifuging and stored at −20°C in 50% saturated ammonium sulphate (*Methods in Enzymology*, Vol 1, p. 500).

METHODS OF MEASUREMENT

(1) Spectrophotometry is used where possible.

(2) Manometric methods measuring changes in gas pressure are also suitable for some assays.

  (i) Gas production — *e.g.*, CO_2 from decarboxylase reaction.

  (ii) Acid formation in presence of $NaHCO_3$ buffers.

Tissue Extraction

Enzymatic analysis is used mainly to estimate the levels of metabolites in biological fluids and tissues. In some cases, such as in blood, the only treatment required is to deproteinize the blood. Frequently, where tissue is used — *e.g.*, muscle, liver — the compound to be estimated has to be extracted. The disintegration of a tissue is governed by two considerations; first, precautions to prevent the breakdown of labile metabolites and, secondly, maintenance of intracellular concentration gradients. The former is very important with metabolites such as the coenzyme adenosine tri-phosphate (ATP) which is readily hydrolysed to ADP + inorganic phosphate. The latter consideration applies to studies with mitochondria which contain much higher levels of certain metabolites, coenzymes and enzymes than the rest of the cell.

These precautions include working at temperatures just above 0°C or in some cases quick freezing a tissue in liquid nitrogen to prevent any undesirable enzyme action, using buffers of appropriate pH and controlling the osmotic nature of the extracting solution so as to preserve intracellular structures.

ESTIMATION OF SUBSTRATES

Some examples of substrate determination are given below.

Determination of D-glucose in Blood

$$\text{(1) D-glucose} + \text{ATP} \xleftarrow[Mg^{++}]{\text{Hexokinase(HK)}} \text{D-glucose-6-PO}_4 + \text{ADP}.$$

$$\text{(2) ADP} + \text{phospho-enol pyruvate (PEP)} \xleftarrow[\text{Kinase (PK)}]{\text{Pyruvic}} \text{ATP} + \text{Pyruvate}$$

$$\text{(3) Pyruvate} + \text{NADH} + H^{+} \xleftarrow[\text{dehydrogenase (LDH)}]{\text{Lactic}} \text{Lactate} + NAD^{+}.$$

Procedure

Mix 0.8 ml H_2O+0.1 ml blood+0.1 ml trichloracetic acid. Centrifuge off protein precipitate. Pipette into cuvette 2.53 ml pH 7.6 buffer, 0.04 ml PEP, 0.06 ml NADH, 0.1 ml KCl, 0.1 ml $MgSO_4$, 0.1 ml ATP, 0.01 ml LDH, 0.01 ml PK,

0.05 ml HK. Mix, stand for 5 minutes and read optical density E_1 at 340 mμ. Add 0.1 ml sample and after 10 minutes read E_2. From the differences in optical density it is possible to calculate the amount of glucose in the sample by the following formula:

$(E \times 180)/(6.22 \times 10^{n}) = $ mg glucose/ml of mixture.

Fructose, glucosamine and mannose can interfere. However, when analysing glycogen these sugars are not present. Since these sugars are usually found in only very small amounts, their presence seldom interferes with the method.

Determination of Lactate

(1) Lactate + NAD^+ + hydrazine $\xleftrightarrow{\text{Muscle LDH}}$ pyruvate hydrazone + $NADH + H_2O + H^+$.

(2) Lactate + Ferricyanide $\xleftrightarrow{\text{Yeast LDH}}$ pyruvate + Ferrocyanide.

The decrease in colour on reduction of ferricyanide can be measured at 405 μ.

Determination of L-amino acids with decarboxylases

Certain bacteria when grown under suitable conditions, produce specific L-amino acid decarboxylases. The reaction releases CO_2 which can be measured manometrically.

$$R \cdot CH(NH_2) \cdot COOH \xleftrightarrow{\text{decarboxylase}} R \cdot CH_2 \cdot NH_2 + CO_2.$$

Using this method, the L-isomers of lysine, arginine, ornithine, tyrosine, histidine, glutamic and aspartic acids can be estimated.

Determination of the coenzymes NAD^+ and NADH

$$\text{Ethanol} + NAD^+ \xleftrightarrow[\text{dehydrogenase}]{\text{alcohol}} \text{Acetaldehyde} + NADH + H^+$$

This reaction can be used to estimate either NAD^+ by having a high ethanol concentration or NADH by adding acetaldehyde. In tissues the oxidized and reduced coenzyme occur together so one portion of tissue is extracted in cold perchloric acid which destroys NADH and another portion is briefly heated in dilute KOH, destroying NAD^+. After the extracts have been neutralized the appropriate solutions are placed in cuvettes and the E_{340} calculated.

DETERMINATION OF ADENOSINE TRIPHOSPHATE (ATP)

One of the neatest methods for estimating ATP uses the enzyme luciferase. This method is based on the observation that aqueous extracts of firefly lanterns which were no longer luminescent emitted light on the addition of ATP.

(1) ATP + luciferin $\xrightarrow[\text{luciferase}]{Mg^{++}}$ adenyl-luciferin + pyrophosphate.

(2) Adenyl-luciferin $\xrightarrow{O_2}$ adenyl-oxyluciferin + H_2O + light.

Using a fluorimeter, 1μg ATP/ml can easily be measured. If a quantum counter is available, the sensitivity is increased 100 to 1,000 times.

DETERMINATION OF ORGANOPHOSPHORUS INSECTICIDES

Organophosphorus insecticides such as parathion inhibit the enzyme cholinesterase. The extent of inhibition can be measured by incubating the sample with a known excess of cholinesterase (CHE). After 30 minutes, a known excess of acetycholine (ACH) is added. One hour later the acetic acid released is measured by titration.

(1) CHE + Inhibitor ⟷ Inhibitor-CHE + Excess CHE.

(2) Excess CHE + ACH ⟶ HAc + Choline + unhydrolized ACH.

The above examples are only a few of the substrates which can be measured with enzymes. It is inevitable that, with an ever-increasing knowledge of biosynthesis and breakdown of natural products, more analytical techniques will be developed using enzymes.

This brief description of enzymatic analysis has dealt only with the determination of [S]. The measurement of [E] has been an established part of clinical diagnosis for many years.

Measurements of enzyme activity have shown that different organs differ quantitatively and also qualitatively in their enzyme composition. By estimating changes in the levels of enzymes — *e.g.*, in serum — it is often possible to locate tissue damage elsewhere in the body. An example is the greatly increased (up to 150-fold) glutamic-oxaloacetic transaminase in serum from patients with viral hepatitis.

It is hoped that this outline of enzymatic analysis will provide stimulus for some readers to use or even develop methods for enzymatic analysis. An excellent book, *Methods of Enzymatic Analysis* (Academic Press) edited by H. U. Bergmeyer is recommended for further reading.

SCINTILLATION AUTOGRAPHY

Easterfield Address, 1963

A. T. WILSON
Chemistry Department, Victoria University of Wellington

INTRODUCTION

Hydrogen is an element of fundamental importance in biological processes. It is therefore important to have a method for studying its pathway through living systems. Paper chromatography is an extremely powerful technique for the separation of complicated mixtures of compounds and has found much application in the field of biochemistry. One of the greatest problems of paper chromatography is the detection of the individual compounds on the paper. This has been done by spraying the paper with suitable chemicals which develop colour reactions with the compounds of interest.

Unfortunately, this usually destroys the compounds and is not of general application. With radioactive materials, a film may be laid on the chromatogram which on development gives dark areas corresponding to the radioactive compounds. This technique works well for ^{14}C, and ^{32}P, but for ^{3}H the maximum energy of the β-particle (18 keV) is too low for it to leave the paper and enter the emulsion of the film. My work involved the development of a technique whereby this difficulty is overcome.

The technique involves soaking the chromatogram in a liquid scintillator so that the energy of the disintegrating tritium atoms is converted into light quanta which can travel into the film to produce an image.

In practice the chromatogram is attached, with wire staples, to some convenient backing material (used X-ray film is suitable). Marker segments, which are pieces of filter paper on which have been placed tritium labelled compounds, are attached to the corners of the backing sheet with staples. These later provide reference points on the backing sheet and also on the film with which to line up the film and the original chromatogram, thus identifying a spot with a definite area on the paper chromatogram. The chromatogram sheet thus prepared is laid in the bottom of

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This article is based on the Address delivered by Dr A. T. Wilson at the Institute of Chemistry Conference at Palmerston North, August, 1963, following receipt of the Easterfield Medal awarded by the Royal Institute of Chemistry.

a shallow tank and covered with a scintillating liquid (see below for discussion of scintillation liquids). A sheet of screen type X-ray film (see below for discussion of film) is next placed on top of the chromatogram, taking care to eliminate any bubbles. The tank is then covered with an airtight top. After a few days, the film is removed from the tank, drained of scintillating liquid, and allowed to dry in the air of the dark-room. When all the toluene has drained or evaporated from the film, it is placed in the wash water and wiped with a soft cloth to remove any terphenyl which may have remained. The film is then developed and fixed in the normal manner. Thus, as in ^{14}C radioautography, compounds can be tentatively identified by their position and this identification confirmed by removal from the paper and co-chromatography with known compounds, or by chemical treatment and re-chromatography.

DETAILS OF METHOD

The tanks are made by soldering ¼ in. square extruded brass rod on to a heavy gauge brass sheet of suitable size, taking care to keep the top as flat as possible. Another piece of brass plate serves as a lid which is kept in place with a lead brick. The tanks are kept in a dark-room cupboard.

The classical scintillation solution, namely, 3 g/l *p*-diphenyl benzene (terphenyl) in toluene, which is used for scintillation counting is quite satisfactory. The presence of sulphur compounds in toluene may cause some quenching of the light but this is a relatively small effect and can be overcome by the use of "sulphur-free" toluene which is commercially available, or by passing the toluene down an alumina column. Since commercial medical "screen type" X-ray is made to be used with a calcium tungstate fluorescent screen, the sensitivity of the method can be improved about 40% by adding a suitable "wavelength shifter" (*e.g.*, diphenyl hexatriene 0.01 g/l) to the scintillating solution. This makes the fluorescent spectrum of the solution match more closely the wavelength response of the film.

Marker segments are small pieces of filter paper which have been impregnated with some tritium-labelled compound. They are attached to the corners of the backing sheet along with the chromatogram and serve as markers, which enables the developed film to be lined up with the chromatogram for spot location. They are prepared by placing a spot of labelled-tritium compound (*e.g.*, algae or yeast which has been treated with tritiated water) on a piece of filter paper which, after drying, is cut into segments.

For maximum sensitivity it is advantageous to have as fast a film as possible. For practical purposes, it is also an advantage to have the film in reasonably large sheets. The manufacturers of film for normal photographic purposes sacrifice speed (even in the very fast films) in order to keep the grain size down. Screen type X-ray film, on the other hand, is much faster to light than even the fastest of normal photographic films. That it has a large grain size is not important in this application. Screen type X-ray film (such as Kodak "Royal Blue") has the added advantage that it is readily available in large (14 in.×17 in.) sheets which are used for conventional chest X-rays. It also can be used with a safety light since it is relatively insensitive to light of the longer wavelengths.

The developer and fixer as supplied by the film manufacturers when used according to their directions will produce the optimum image. Care should be taken, however, to dip the film in water and wipe it with a soft wet cloth to remove any terphenyl before placing in the developer.

A question that immediately presents itself is whether this technique can be applied to very low level ^{14}C chromatograms which take a very long time to expose in the normal manner. Unfortunately, this technique is of no help in this problem. The technique is more sensitive for short exposures (approx. 24 h) than is conventional radioautography, but for longer exposures it is progressively less sensitive. This is in the nature of the photographic process itself. In conventional radioautography one is developing the actual β-particle tracks and the density of these is to a first approximation independent of the exposure time. In the photography of light, however, which is the principle of the scintillation autography technique, there exists for a given film an optimum time during which a given quantity of light will produce the greatest effect. This period is much less than a day for any film. Thus for longer exposures the effect of a given quantity of light decreases as the length of time over which it is received increases. Thus, if long exposures (many weeks) are necessary for weak ^{14}C chromatograms then conventional radioautography is the best approach. Similarly, for tritium experiments enough isotope should be used to get the desired exposure in less than a week.

Sensitivity depends on the length of exposure time, but if 3 g/l terphenyl and 0.01 g/l disphenyl hexatriene in toluene are used with Kodak "Royal Blue" film, then a spot whose area is one square centimetre, and whose activity is 0.1 μC can be easily detected after an exposure of 50 h.

The quality and sharpness of a scintillation autograph compares favourably with radioautograms produced by the β-particles from the ^{14}C. Because of the universal occurrence of hydrogen in biological systems, and the availability, low health hazard and cheapness of tritium (10^{-4} that of ^{14}C) coupled with the ease of preparation of ^{3}H compounds, this technique should find wide application.

Tritium can be introduced easily into many organic molecules, either by hydrogenation of the corresponding unsaturated compound or by exposure to tritium gas. This latter method of preparation (the Wilzbach synthesis) seems to be of a very general application, but suffers from the fact that it yields a product contaminated with small amounts of ^{3}H material of high specific activity. With the technique described in this paper, however, it is possible to prepare chromatographically pure ^{3}H compounds.

APPLICATION OF THE TECHNIQUE

This experimental technique has been used at Victoria University to investigate biochemical problems which are not accessible to conventional biochemical techniques.

Tritium in the form of tritiated water behaves unlike any other radio-tracer. This is because the protons from the water present in a biological system enter into many of the biochemical reactions which together constitute the metabolic pathway in a living system. Thus in the presence of tritiated water ^{3}H protons are incorporated and eliminated at many points in the network of biological reactions with the production of labelled metabolites.

The tritium atom may be incorporated into the molecule of a biological metabolite in three days:

(1) *Rapidly exchangeable hydrogen:*

e.g., $-OH$ & $-SH$; $-NH_2$ & $>NH$; $-COOH$

These functional groups exchange hydrogen very rapidly (in a very small fraction of a second) because they become ionized and can pick up a triton.

(2) *Slowly exchangeable hydrogen:*

This is a special case rarely met with in biological metabolites, where a hydrogen is involved in a keto-enol type system.

$$R-\underset{\underset{O}{\|}}{C}-CH_2-R \quad \rightleftharpoons \quad R-\underset{\underset{OH}{|}}{C}=CH-R$$

The hydrogens on the carbon atom adjacent to the keto group will exchange slowly with water.

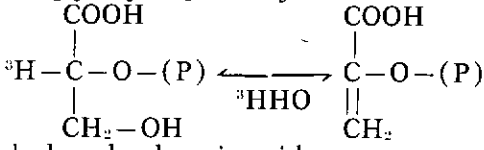
(3) *Non-exchangeable hydrogen:*

This occurs with hydrogens bound directly to a carbon atom and not involved in a keto-enol type system. The tritium of tritiated water can only be incorporated into this position via a biochemical (or chemical) reaction.

If tritiated water is added to a biological system, tritium is incorporated into a great number of metabolites. This is because many biochemical reactions involve the addition of a hydrogen derived from water to a carbon — *e.g.*, the addition of water across a double bond. It should be noted that, since most enzymatic reactions are reversible, this can occur in the forward or backward reaction.

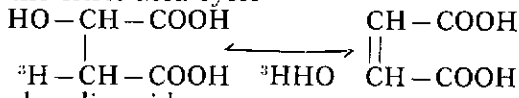
Some typical examples are:

(1) From the glycolytic pathway



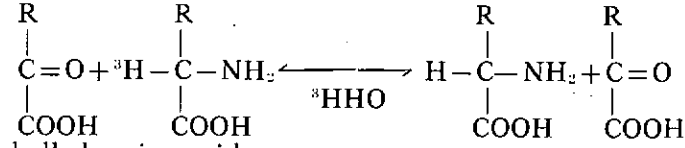
labelled phosphoglyceric acid.

(2) From the citric acid cycle



labelled malic acid.

(3) Transamination reactions



labelled amino acids.

Having become labelled, a metabolite can then pass the label on to the succeeding components in the biochemical pathway.

USE TO DETECT BIOCHEMICAL PATHWAYS

The addition of tritiated water to a biological system, the extraction of the metabolites and their separation and identification by paper chromatography is, as has been described above, a very rapid, simple and cheap procedure.

Clearly, if some material, say citric acid, becomes labelled, it must be involved in some metabolism. It may or may not be Krebs cycle metabolism; if, however, other Krebs cycle intermediates have also acquired label, the evidence is stronger. Conversely, if citric acid is present and has not become radioactive, it is good evidence that it is not involved in metabolism and that the pool containing this citric acid is not involved in Krebs cycle metabolism. Similar arguments apply to the glycolytic pathway, hexose monophosphate shunt, transamination reactions, fat metabolism, etc.

The advantages of such a technique are:

(1) It can be used on *in vivo* systems and there is no question of the water not pentrating the cell wall.
(2) It is quite simple and does not require complicated or expensive equipment.
(3) It can be used to study biological reactions to which conventional biochemical techniques are not applicable.
(4) Conventional *in vitro* biochemistry on cell-free systems assumes that the biochemical pathways studied are the same as in the intact organism. The *in vivo* technique above is a useful check on such an assumption.
(5) Such a technique may lead to the discovery of unknown metabolites and as yet undiscovered metabolic pathways. For example, if a radioactive material on the chromatogram proved to be a hitherto unknown metabolite, it would serve as a starting point for more conventional biochemical studies.

The technique is complementary to and not competitive with conventional biochemical techniques. Once the technique indicates the possibility of certain biochemical reactions, the next step is to attempt to isolate and study the enzyme systems using conventional techniques. The procedure is so simple it can always be done as a preliminary experiment to a more detailed conventional study.

At Victoria University of Wellington we have used the approach described above to study some biochemical problems which are not accessible to conventional biochemical techniques. For example, John Spedding, for his Master's degree thesis, studied the early reactions (first few minutes) in a germinating seed. Miss Shirley Edwards, for her Master's thesis, studied the early reactions in germinating fungal spores, and at present Robert Mann, for his thesis, is studying the reactions taking place in seeds at 0° C to attempt to determine why they will not germinate at this temperature. He is also studying the reactions taking place when seeds, spores and pollen are exposed to low relative humidities of ^{3}HHO vapour.

CONFERENCE 1964 — HAMILTON

The venue of 1964 Conference, August 25–28, will be the University of Waikato, in Hillcrest Road, Hamilton, about three miles from the centre of Hamilton. Special buses will be arranged to coincide with lecture sessions. Unfortunately, the conference cannot be "residential" but ample accommodation has been booked in hotels and motels.

As a result of recent illness, Mr R. J. Lancaster has resigned from the Chairmanship and Mr J. E. Allen has taken over this responsibility. The Conference Secretary is Dr R. Locker (Meat Industry Research Institute, Hamilton).

The Committee has arranged for Dr J. S. Shannon, who is in charge of the organic chemistry group of the Coal Research Division, C.S.I.R.O., Sydney, to visit New Zealand as a Guest Lecturer. In addition to two lectures by Dr Shannon and an opening address by Dr D. R. Llewellyn, who was recently appointed Vice-Chancellor of the University of Waikato, the programme provides for four Review Lectures, two of which will be given by visitors from overseas. Professor R. M. Noyes, of University of Oregon at present working at Canterbury University College, will deal with chemistry of free radicals in solution. Dr K. Sutherland, from Marrickville, N.S.W., will speak on industrial aspects of alginate chemistry. Other reviews will be presented by Mr G. Beavis from the Oil Refinery, Marsden Point, and by Dr G. B. Peterson, Plant Chemistry Division, D.S.I.R. The remainder of the programme contains 40 papers by Institute members and will be arranged in concurrent session throughout.

As the Conference coincides with the celebration of the centenary of the founding of Hamilton, there should be something of a festive air about the city at this time.

AUSTRALIAN SPECTROSCOPY CONFERENCE

The fifth Australian Spectroscopy Conference will be held in Perth, Western Australia, from May 31 to June 2, 1965. This Conference is sponsored by the Spectroscopy Committee of the Australian Academy of Science. The chairman of the Organizing Committee is Dr A. R. H. Cole and the General Secretary Dr A. J. Parker (*Department of Chemistry, University of Western Australia, Nedlands, Western Australia*).

CONFERENCE 1963 — REPORT

The following items of general interest are taken from the report of the 1963 (Palmerston North) Conference Committee.

Programme

In addition to offered papers, it was decided to have two sessions of invited papers only (Industrial development in N.Z., and Diversification in the Food Industries) and four invited major review papers; Inorganic, organic, biochemistry, and teaching of biochemistry. With the first three of these latter papers it was aimed to follow the main paper with a session of specialized research papers in the same general field. The main speaker was permitted to exercise some control, if desired, over the papers included in these sessions; of 14 papers in these sessions, 6 were invited in this way.

Excluding these invited papers, 23 papers were included from 33 offered papers. Of the total papers given, 39, 16 were from Government research institutes, 22 from university and 1 from industry. In order to leave as much programme as possible for visitors, members living locally were not encouraged to give papers; apart from 2 invited ones in the food session, only 3 such were included. The 10 papers rejected were excluded on the grounds of space, more than one paper offered by the same author, or irrelevance.

One set of three concurrent sessions was run and proved successful. The time devoted to annual meetings, Easterfield Addresses, Presidential Addresses, etc., is serious in a conference of short duration. It would have helped considerably if we could have known the name of the recipient of the Easterfield award during the planning stages.

The excellent attendance at the conference was probably due in part to the number of invited papers of potentially high standard and the availability of the programme to members at the time of enrolment (April-May). It is pointless arranging special papers to suit the needs and interest of members if these cannot be announced until after most members have had to decide whether or not to enrol.

Trade Exhibition

The financial success of the conference was in large measure a result of the support given by commercial interests. About 100 firms were invited to participate in a trade exhibition. Of these, 13 did so and paid 3s. 4d. a square foot for exhibition space with a minimum charge of £5. In addition, the trade representatives were asked to pay a conference fee of £1 10s. which entitled them to participate in all Conference activities on the same footing as N.Z.I.C. members. This participation in Conference activities was much appreciated by the representatives and several firms wrote after the Conference expressing their appreciation of the way in which the exhibition was handled.

BRANCH NEWS AND NOTES

AUCKLAND

Early in March, those members not deterred by heavy rain met in the Lecture Room, Shell House, to hear Mr S. G. Brooker smoothly deliver his presidential address on "Margarine".

Later in March there was a special meeting of the branch at which members discussed the proposed affiliation of the N.Z.I.C. with the Royal Society of New Zealand, changes in the Rules concerning the office of Vice-President, and changes in the Rules governing Associateship by examination.

"The Theory of Resonance In Chemistry" was the subject of a lecture to members and science students at the April monthly meeting. The speaker was Professor C. A. Coulson, Rouse Ball Professor of Mathematics at the University of Oxford and Fellow of Wadham College, Oxford.

As this issue goes to press in May, members will meet to hear three speakers cover different aspects of chemistry teaching. Mr R. Scott, Inspector Post-Primary Schools, is expected to outline some changes in the teaching of chemistry abroad, Mr H. S. Maslen, Senior Lecturer, University of Auckland, is listed to discuss some changes occurring in the teaching of chemistry in New Zealand, and Mr K. Buckley, Senior Science Teacher, Westlake Boys' High School, is to discuss how the CHEM study course is working out in New Zealand's classrooms.

WELLINGTON

A postgraduate course in theoretical chemistry has been inaugurated at Victoria University of Wellington.

The Chemistry Department of Victoria University of Wellington in conjunction with the Regional Council of Adult Education has held a course for post-primary school teachers on the CHEM Study method of teaching chemistry at the secondary school level.

In April, Professor C. A. Coulson, F.R.S., presented a lecture on the uses and abuses of the concept of resonance in chemistry to members of the local branch, and, at the University, he addressed members of the Chemistry Department and visitors from the D.S.I.R. on the xenon fluorides.

Mr H. Whitfield, who was previously on the staff of the Lucas Heights Research Establishment of the Australian Atomic Energy Commission, has received a Research Fellowship to work with Professor J. F. Duncan at Victoria University of Wellington on the Mössbauer Effect.

A symposium entitled "Some Theoretical Aspects of Transition Metal Ions" was held at the Chemistry Division of D.S.I.R. on May 11 and 12. The programme organizer was Dr R. M. Golding.

A party of seven scientists connected with the work of the Australian Atomic Energy Commission paid a visit to the Departments of Chemistry and Physics at Victoria University of Wellington on May 19. The leader of the party was Mr M. C. Timbs, General Manager, Australian Atomic Energy Commission.

CANTERBURY

Professor C. A. Coulson, Rouse Ball Professor of Mathematics at Oxford University, recently visited Christchurch. His lecture to the Institute, on the contribution of quantum theory to chemistry, was attended by an appreciative audience of 103 members.

Another highlight of the recent institute programme was a refresher course on selected topics in organic chemistry. Individual lectures were given as follows: Professor J. Vaughan, "The Methyl Group"; Dr A. Fischer, "Acids, Bases and Reactivity"; Dr D. N. Kirk, "Hydroboration".

Professor J. Vaughan has been appointed Head of the Chemistry Department of the University of Canterbury, to succeed Professor Packer on his retirement in January of next year. Professor Vaughan is shortly to spend seven weeks overseas on an Erskine Fellowship, visiting in particular the English universities of Sussex, Essex, East Anglia and York.

On May 4, Mr A. H. Horn gave a talk to the Institute on "Safety in the Laboratory". The I.C.I. film *Black Monday* was also shown. The climate of opinion with regard to laboratory safety rules is reflected in the recent revision of safety regulations of the Chemistry Department of Canterbury University. Amongst other things, the new rules limit the hours during which research students are permitted to do experimental work and forbid students to work alone — *i.e.*, without someone within earshot.

The United States Air Force office of Scientific Research has given technical approval for a two-year grant of $25,000 to Dr L. F. Phillips, of the Canterbury University Chemistry Department, for research on gas-phase reactions. This is a renewal of a previous two-year grant of $23,900.

ASSOCIATESHIP CERTIFICATES

The Registrar has pointed out that a number of newly elected Associates may be concerned at not receiving their Associateship certificate and has asked that the reason for this delay be explained. When supplies of the certificates became low last year, it was decided that the opportunity should be taken to revise the design of the certificate. Some difficulty has been encountered in obtaining an aesthetically satisfactory layout into which the necessary wording can be inserted and printing of the new certificate has been held up to obtain advice from a person with expert knowledge of design. New Associates will receive their certificates as soon as the revised form is available.

N.Z. CERTIFICATE IN SCIENCE

Passes in Chemistry Option

In 1963, the first students completed their examinations for the New Zealand Certificate in Science, Chemistry Option. The successful candidates were:

Mr P. F. Thompson, Howick.
Mr R. G. Ditchburn, Wellington.
Mr W. R. Owers, Lower Hutt.
Mr H. A. Polack, Wainuiomata.
Mr D. W. Rolls, Wellington.

These are the first Certificates to be awarded in Science, and the Institute, which has throughout encouraged the developing of these courses in Chemistry extends congratulations to these students. Congratulations are due also to the Technician Certification Authority, which has organized the courses and examinations, on these first products of its efforts to improve the quality and status of chemistry technicians.

THE REGISTRY

Fellows

(Elected April 21, 1964)

ADAMS, Arthur Francis Reginald, M.Sc., Lincoln College.
PANCKHURST, Max Humphrey, M.Sc., Ph.D., D.Phil., University of Otago, Dunedin.
PENFOLD, Bruce Russell, M.Sc., Ph.D., University of Canterbury, Christchurch.

(Elected May 19, 1964)

CAWLEY, Robert William, B.Sc., Wheat Research Institute, Christchurch.

Associates

(Elected April 21, 1964)

CARR, Malcolm David, M.Sc., Ph.D., Victoria University of Wellington.
DAVIES, Janet Ruby, M.Sc., 19 Ranui Tce., Linden, Wellington.
McCORT, John Graham, B.Sc., 117 Roberta Drive, Christchurch 2.
RUMSBY, Martin Gregory, Ph.D., Massey University of Manawatu, Palmerston North.
WHIMP, Peter Olaf, M.Sc., Victoria University of Wellington.

(Elected May 19, 1964)

ALEXANDER, John Keith, B.Sc., B.E.(Chem.), Chief Mechanical Engineer's Office, N.Z.R., Wellington (Chemical Engineer).
CARTER, Garrick Neville, B.Sc., Chemistry Dept., Auckland University (Radio-chemical Technician).
DOLLIMORE, Annette Florence, B.Sc., Shell Oil (N.Z.) Ltd., Wellington (Technical Assistant).
EMERSON, George West, M.Sc., Biochemistry Dept., Medical School, Dunedin (Lecturer).
EVANS, Albert Arthur, B.Sc., B.E.(Chem., Hons.), Dept. of Chemical Engineering, University of Canterbury (Assistant Lecturer).
FITZSIMONS, Desmond, B.Sc., Abels Ltd., Auckland (Works Manager).
FOURIE, Ray Michael, M.Sc. (Natal), Auckland Technical Institute (Science Teacher).
GALLAGHER, Ian Henderson Christopher, M.Sc., Biochemistry Dept., Medical School, Dunedin (Ph.D. Student).
GIRVEN, Richard James, M.Sc., Mobil Oil N.Z. Ltd., Petone (Manufacturing Chemist).
HOGG, John Shone, M.Sc., Waimea College, Richmond (Head of Science Dept.).
JACKSON, William James, B.Sc., Hons. (Bristol), A.R.I.C., Southland Girls' High School, Invercargill (Head of Science Dept.).
JAMESON, Michael Bruce, M.Sc., Chemistry Dept., University of Canterbury (Ph.D. Research Fellow).
JONES, Richard Glyn, M.Sc., Dept. of Physical Chemistry, University of Leeds (Research Student).
MADLE, David Victor, B.Sc., Hons. (London), I.C.I. (N.Z.) Ltd., Seaview Factory, Lower Hutt (Chief Chemist).
NOTTINGHAM, Peter Maxwell, M.Sc., Ph.D. (Aberdeen), Meat Industry Research Institute, Hamilton (Microbiologist).
Oo, Khaik Cheang, B.Sc., Hons., Biochemistry Dept., Medical School, Dunedin (Ph.D. Student).

OSBORNE, Kenneth John, A. G. Healing & Co. Ltd., Auckland (Industrial Chemist).
RANSON, Thomas, B.Sc. (Birm.), Freyberg High School, Palmerston North (Teacher).
RICHARDS, Peter Russell, M.Sc., Christchurch Boys' High School, Christchurch (Assistant Master).
RODLEY, Gordon Allen, M.Sc., Ph.D. (Lond.), Chemistry Dept., University of Canterbury (Lecturer).
SHEARER, Barry James, M.Sc., Dept. of Organic Chemistry, University of Adelaide, S.A. (Demonstrator).
WILSON, Robin Stewart, B.Sc., Hons. (Otago), Glaxo Laboratories Ltd., Palmerston North (Asst. Pharmaceutical Production Manager).

Resignations

JOHNSON, A. F.; STANSFIELD, R. H. J.

Laboratory Assistant's Certificate

(*Awarded April 21, 1964*)

CALVERT, David John, 31 Vermont Road, Birkenhead, Auckland.
CHILD, Robyn Gayle, 60 Glendinning Ave., Dunedin.
GIBSON, Margaret Evelyn, 137 Barrack Road, Panmure, Auckland.
SARGENT, Margaret Helen, 215 Wainoni Rd., Wainoni, Christchurch.

REVIEW

THE CHEMISTRY OF BERYLLIUM. By D. A. Everest. Published by Elsevier Publishing Company, Amsterdam, London and New York. 1964.

This is Volume 1 of a collection of monographs edited by Professor P. L. Robinson. It contains a clearly written, well referenced 150-page account of the chemistry of beryllium; the approach is thoroughly modern and the literature is covered up to 1962 in over 500 references.

Technological aspects (which have been the central feature of recent research on this element and are well documented elsewhere) are only briefly alluded to, and the volume provides a short, readable account of an element whose chemistry, except in some restricted fields, is rather undeservedly out of fashion nowadays.

W.E.D.

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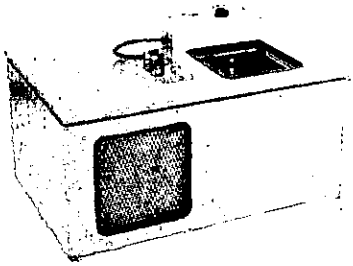

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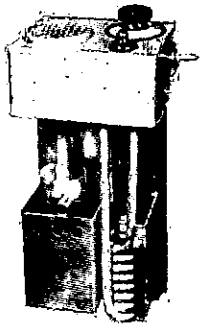

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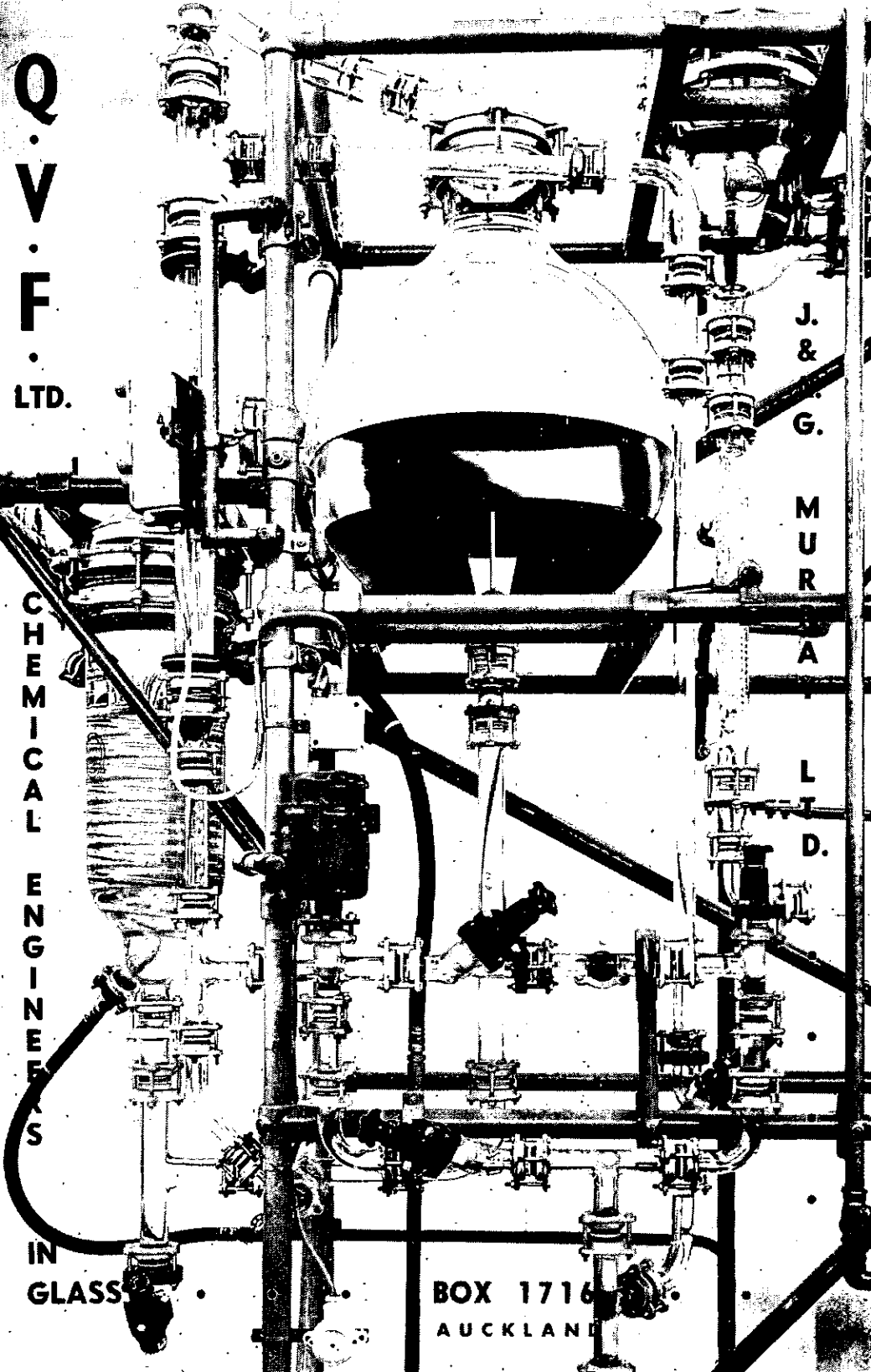
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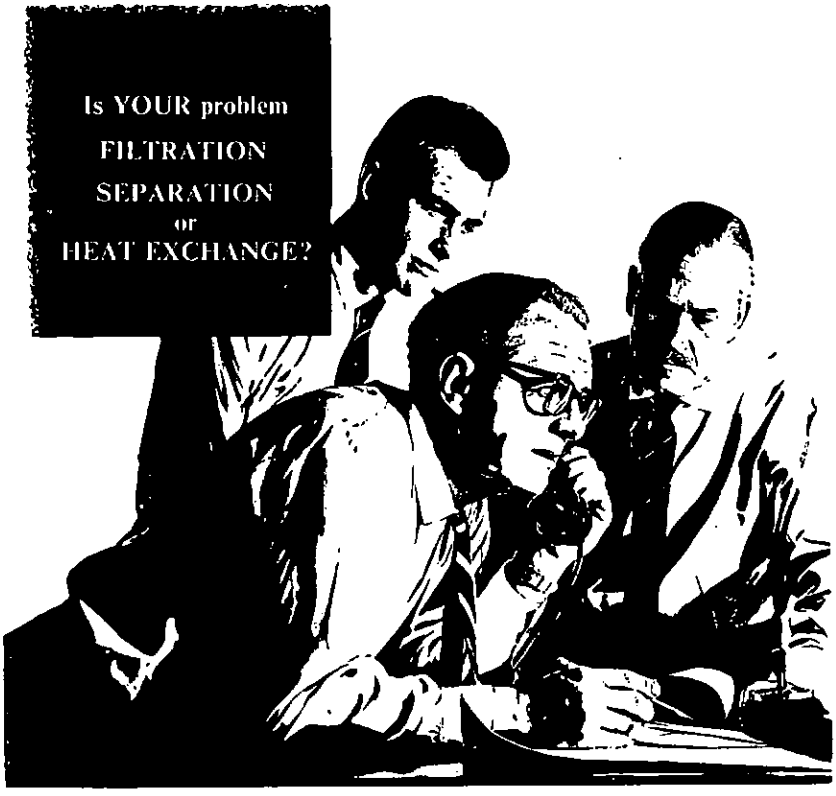

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