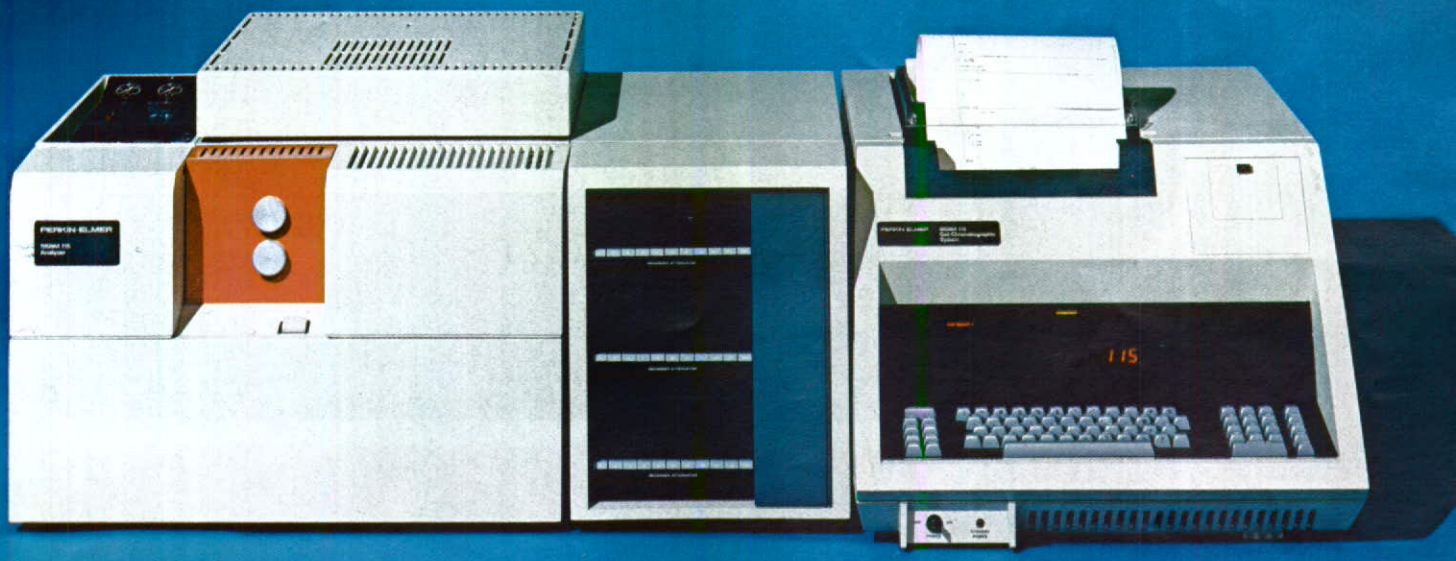
June 1981 Volume 45 No. 3

Chemistry in new zealand

Official Journal of the New Zealand Institute of Chemistry, P.O. Box 1926, Christchurch.

50 yrs of Teamwork in Chemistry

Inside: Abstracts of Conference Papers; Magnetic Circular Dichroism; Century of Closed Carbon Chains; National Hormone Laboratory; Cellulose, Fuel of the Future; Industrial Potential of Enzymes.



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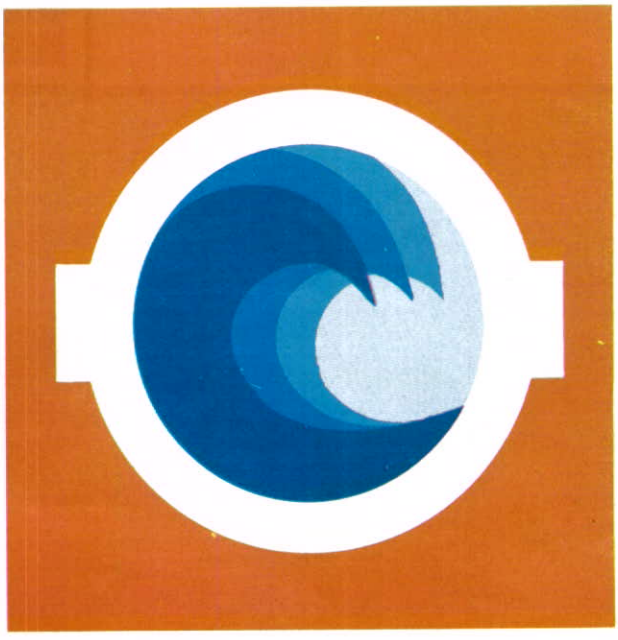

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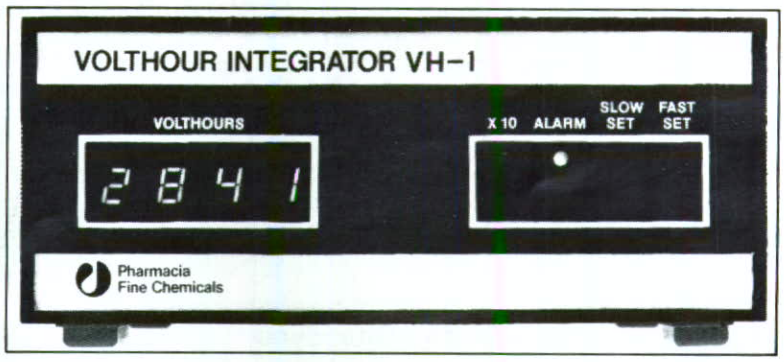

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50 yrs of Teamwork in Chemistry

CONTENTS

SPECIAL INSERT

Abstracts Of 1981 Conference Papers

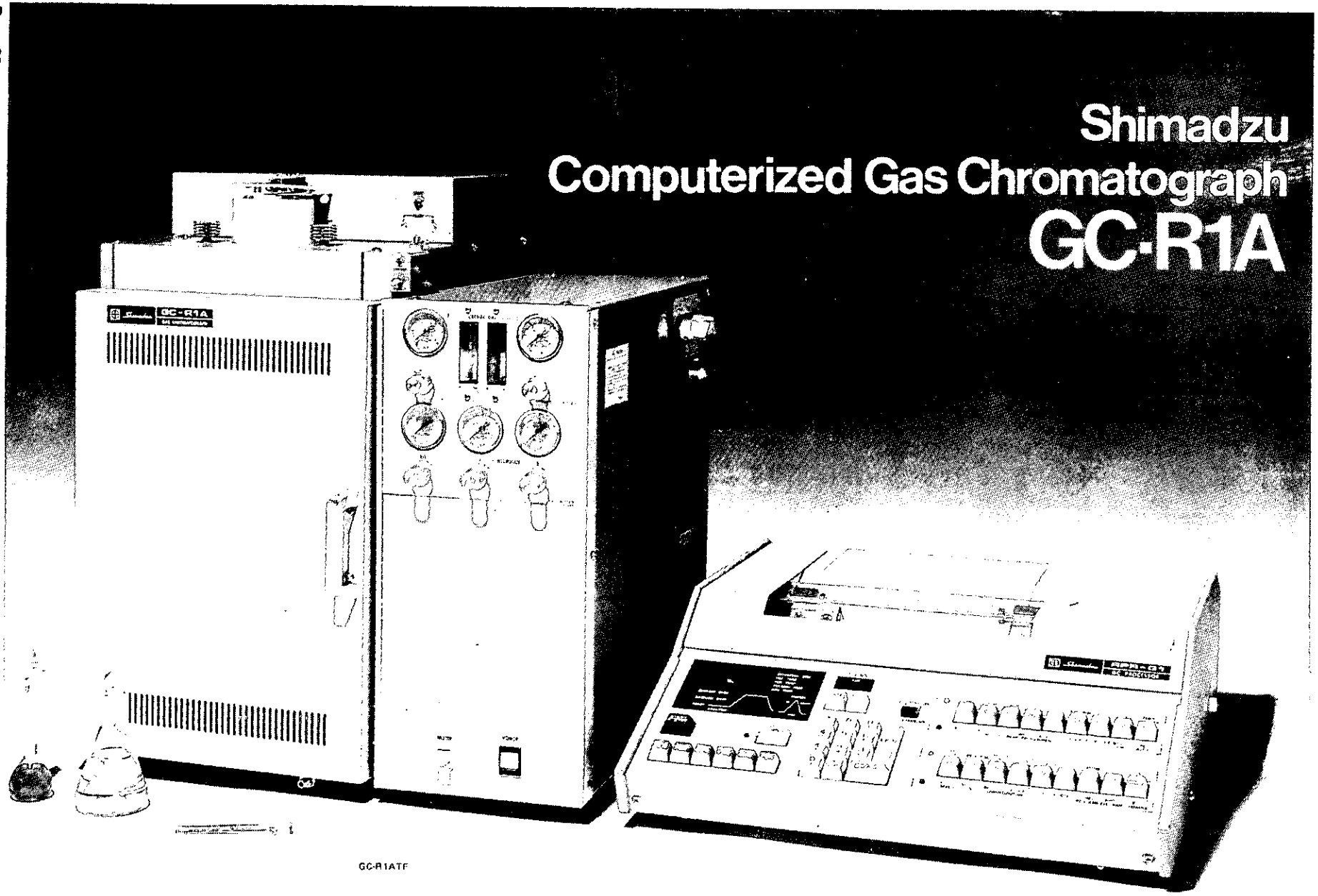
Cover: Perkin Elmer's Sigma 115, the new sophisticated Data Handling/Gas Chromatography system.

STOP PRESS

REMINDER!

Closing date for NZIC Golden Jubilee Conference registrations is Tuesday, June 30. A late fee will apply to registrations received after that date.

REVISED CLOSING DATE JULY 15



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Guest Editorial

New Zealand Futures

According to that distinguished physicist P.S. Blackett "a long term vision is the best guide out of any confused situation". In 1961 when he said that, futures studies had not been thought of, but the quotation could well have been a justification for the Commission for the Future. Another comes from the historian J.S. Roberts, who has recently said that "there is no need to conclude that [technology] will not still provide mankind with the tool-kit he needs to survive ...", but there are two dangers: (1) acceleration of change may outpace the capacity of the human race to produce answers to its problems, and (2) the international power structure may become so unstable as to be a major threat to humanity. CFF's role is to try to prepare New Zealand society to meet such eventualities. It does this in two ways, by professional and dispassionate research, and by public education and participation. These are characteristics of a professional chemist, who is trained to identify problems, understand them and then communicate his/her findings. Indeed the work has been done using a similar technique to chemical research:

(a) The field was reviewed and the problem areas identified, as with the literature review of a thesis. This was published as "New Zealand in the Future World" in 1980.

(b) The relationship between the problems and the known facts was explored, equivalent to the exploratory work of a thesis. There have been five parts to this —

  (i) The contexts, in which the alternative futures for New Zealand based on distinctly different value judgements for society are explored. These were released briefly in late 1980. As a result of the public response they have been modified, and quantified. They will be publicly issued in final form in July.

  (ii) The modelling work, notably by Prof. B. Philpott, which helped identify the essential choices available to us. This work continues.

  (iii) The policy research groups, studies in depth of areas of major importance. 'Communications' is nearly finished; 'Disasters' is midsteam; and 'Work' is just starting.

  (iv) The Decision Analysis Group, in which present-day decision makers and interested groups give us their views of the future. NZIC is a member of this group.

  (v) Public opinion, including two polls, and several workshops, quizzes and many letters. CFF considers this activity to be vital, for anything not acceptable to the public must be a non-starter.

(c) Then we draw all this, the first stage of our work, together in a statement of conclusions, equivalent to the discussion section of a thesis. Work on this is well advanced. It will be made public early in 1982. Like any concluding section it will be emphatic and clear cut and will propose new urgent work which needs to be done.

Associated with this programme has been public education. This has included all the mass media, together with widespread seminars, lectures, workshops etc. in most centres from Kaitaia to Bluff.

And what has so far been achieved? I believe we can claim to have influenced the following significantly.

- The nuclear power debate in which the Royal Commission on Nuclear Power Generation asked us for a submission on matters of principle. Their findings were very close to our own conclusions.
- The formation of the Committee on Genetic Engineering.
- The microprocessor debate which we started.
- The debate on the use of alcohol fuels for transport.
- The recognition that the rising price of oil is likely to cause severe balance of payments problems and would force us to change our lifestyles unless we become energy self-sufficient.
- A recognition that New Zealanders regard economic growth and social harmony as two sides of the coin of equal importance.
- Transformation of the gloom and doom of the late 1970's to one of cautious optimism as the public realise that we have a lot going for us.

These are some of the things which have been achieved, or become clear over the last four years. Others will emerge as the findings of our summary paper on 'New Zealand Futures' crisp up. Some are already evident. For instance, here are just two:

- If we do nothing to ensure that adequate numbers of chemical and electronic engineers are available in the next 10 years, our exploitation of the wood bonanza in the 1990's and of microelectronics, will be in jeopardy.
- Either we take steps to educate for parenthood (in the schools or elsewhere) or we will continue to have violence in our society.

These then, are the sort of contributions you can expect from CFF. To do this kind of work, skills in research and education are needed, but above all the findings must be presented without fear or favour, and equally important, without any attempt to say what ought to be done, or to be involved in pressure group politics. I think my professional training as a chemist has helped to sustain that position and to present the work with integrity. I hope and expect that my fellow chemists will support this activity and I invite all to contribute to it as you consider appropriate. Please write or telephone, or set up a study group or workshop in your region, with which CFF will assist.

James F. Duncan
Chairman
Commission for the Future

James Duncan is Professor of Inorganic and Theoretical Chemistry at Victoria University of Wellington.

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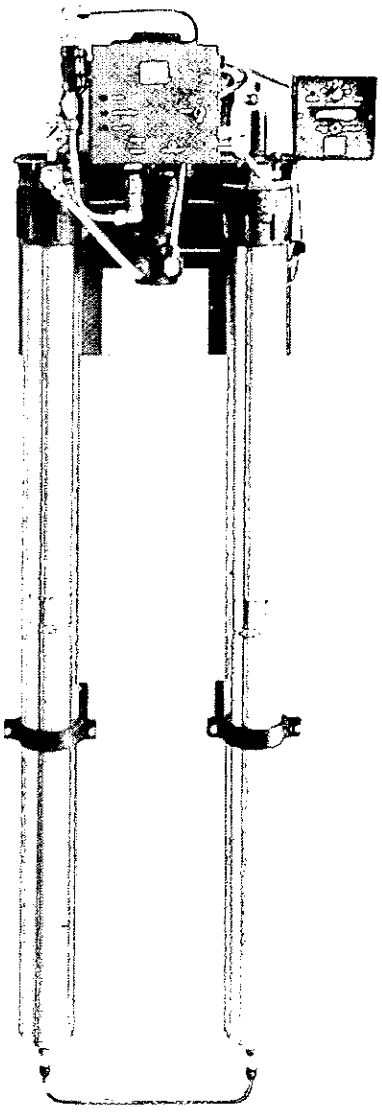

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What's Happening

"Chemistry in Canada" paints a gloomy picture of ageing faculties in the chemistry departments of Canadian Universities, the average age having risen from 42 in 1970, 45 in 1975 to 48 in 1979-80. This coincides with an increase of people with full professorial status from 30% in 1970 to 55% today. At the same time student populations are declining.

* * *

Sir Frederick Dainton, FRS, has associated himself with the new Social Democratic party in Britain. Another facet of British politics is that despite the fact that **Mrs Thatcher** trained as a chemist, there is no spokesman for science in her cabinet.

* * *

The DSIR has set up a steering committee under **Dr Ray Bailey** FNZIC, Applied Biochemistry Division, to initiate studies on the composition of NZ foods. Dr Bailey would welcome comments from people or organisations interested. Address: Private Bag, Palmerston North.

* * *

The Oil and Colour Chemists' Association is holding its annual convention at the Rotorua International Hotel from July 30 - August 2. The theme is "Overview Concerning Cladding Alternatives", which also has the acronym OCCA. Enquiries to Box 5192 Auckland.

* * *

Council is expected to approve at its meeting on August 23 a new grade of Student membership. All enquiries to the Registrar, Box 1926, Christchurch.

* * *

From "Nature" for 1931, the year of the founding of the NZIC, we culled the following items:—

The Hawkes Bay earthquake was recorded at the Kew Observatory over a period of 4 hours; **Ernest Rutherford** (erstwhile temporary master at Christchurch Boys' High School) was created a Baron; 300th anniversary of the death of **Henry Briggs** who calculated logs of the first 30 000 numbers to 14 decimal places; in its Golden Jubilee year the Society of Chemical Industry was concerned with energy and the hydrogenation of petroleum; Massey Agricultural College opened; **Elsdon Best** died; centenary of the start of the voyage of the "Beagle"; use of woad officially discontinued.

* * *

Any old Conference photos? **Miss J. Watson** of Nelson has sent us some and they will be shown at Conference.

This year Du Pont became the first US chemical company to have an R & D budget of more than $500 million.

* * *

The 2nd International Conference on Chitin and Chitosan will be held at Sapporo, Japan, July 12-14, 1982. Enquiries to **Prof. Ferrier,** Victoria University of Wellington. The 9th International Conference on Atomic Spectroscopy will be held September 4-8, 1981. Enquiries to the Japan Society for Analytical Chemistry, Gotanda; Sanhaitsu 26-2 Nishigotanda 1-chome, Shinigawa-ku, Tokyo 141.

* * *

We were privileged to attend the official opening of **Dr Jim Sprott's** new laboratory building in Carlton Gore Road, Auckand, by **Mr Doug Antony,** Deputy Prime Minister of Australia, on May 13. We found that Mr Antony is, like ourselves, interested in the growing of oilseeds, but after unsatisfactory results with soybeans, he has turned to cotton.

* * *

CHEM NZ, the interesting little publication put out by the Chemical Education Division of the NZIC asks its readers, "What is the brown substance formed on the skin after contact with silver nitrate? How is it removed?" So far we haven't got the answers.

* * *

Australia will have lead-free petrol by 1985, and all vehicles sold after January 1, 1986, will have to be designed to run on this fuel. A test car running on it and fitted with a catalytic exhaust converter used 13% less petrol than an identical vehicle running on leaded petrol.

* * *

Sir Charles Wheatstone, who gave his name to the Wheatstone bridge, also invented and patented the concertina.

* * *

The Chemistry Section of the Canterbury Science Teachers' Assn. had a session on the use of computers in the teaching of chemistry on April 23, with **Prof. B. Penfold** in the chair.

* * *

The report "Atomic Weights of the Elements 1979" has now been published in Pure and Applied Chemistry **52** 2349-2384 (1980). The changes in the atomic weight and/or uncertainty for 12 elements are detailed. The report includes a new definition of atomic weight, a complete review of the natural isotopic composition of the elements and a review of stable isotopic abundances of elements from nonterrestrial sources.

* * *

NZ Forest Products Ltd and Tasman Pulp & Paper Co Ltd will form a joint venture company to establish and operate a plant to fractionate crude tall oil and crude sulphate turpentine and manufacture pine oil. Costing about $12 million, the plant sited at Mt Maunganui is expected to earn over $9 million annually in domestic and export sales.

Food For Thought

"You are not stretching your (secondary) students sufficiently, either in breadth or depth!" This, and other searching statements were made by **Gordon Livingstone** when he and **Jim Gardiner** addressed the Canterbury Branch in May. Jim (who is from New Jersey, and on exchange with **Allan Wooff** of Christchurch Boys' High) and Gordon (who is from Norwich City College, and on exchange with **Selwyn Malster** at Christchurch Polytechnic) spoke on "Chemical Education in the US, UK and NZ". From Jim one heard of the pressure imposed by senior high students on their teachers as students seek optimum grades to secure placement in prestigious universities. Gordon presented the view that **energy** is the key concept in chemistry and should be the theme running through our teaching. He also said "Chemistry (education) is nothing if it can not be **applied**" — food for thought.

People

Mr R.W. Cawley, FNZIC, has won the NZ Institute of Food Science & Technology J.C. Andrews Award for eminence in food technology. Bob is currently Director of the Wheat Research Institute, Christchurch.

Mr Hec. Orchiston, who will be well-known to older members of the NZIC as an agricultural chemist has been awarded the D.Sc. by Lincoln College in what was described as a "most prestigious award for his studies in the application of chemistry and mathematics to soil moisture and nutrition".

Mr John Beck, FNZIC, a past Chairman of the Wellington Branch, who was appointed General Manager of Lion Breweries in 1978, has been appointed a Director.

Dr David Rands, FNZIC, Operations Director of Taubmans International (NZ) Ltd, has been transferred to the Sydney office of the company. Taubmans are now disputing second place among surface coating manufacturers in NZ and is an example of a successful company run by chemists.

Visitors to the Chemistry Department of the University of Auckland this year include **Prof. R.J.H. Clark,** University College, London, **Prof. E.S. Hansen,** Acadia University, Nova Scotia, **Dr J.J.P. Stewart,** University of Strathclyde, Glasgow; **Prof. D.L.H. Williams,** Durham, **Dr M. Zvagulis,** McMaster University, Ontario and **Mr P. Raut,** Dijon, France.

Mr J.L. Wakeman of A.C. Hatrick Ltd, is now with Chemby Chemicals, Takapuna; **Dr D.M. Collins** is now with the Wallaceville Animal Health lab; **Mr R.U. Roy,** Regional Supt. of Education Christchurch has retired; **Dr C.H. Sissons** has moved from Ruakura to the University of Waikato; **Miss A.M. Thomson** from Tamatea High School, Hawkes Bay to Tauranga Girls'; **Dr Gail Irwin** has been promoted to Senior Tutor at the Wellington Polytechnic; **Miss**

Beck

Rands

A. Stanley-Hunt has been appointed Chemist with Unilever, Petone, and **Mrs L.M. Ball** is now with the Chemistry Division at Gracefield; **Mr H. Green** has resigned from the Auckand Industrial Development Divn; **Mr John Parnell** has gone from Warburton Franki, Auckland, to Philips, Wellington; **Dr R.F. Gerlach** has left the University of Michigan at Ann Arbor and joined Exxon Research Laboratories, Linden, N.J.

Mr Dennis Nelson, a PhD student at Victoria University has followed in the footsteps of **Dr John Featherstone** in winning the Edward Hatton Award of the International Association for Dental Research, which was presented at its recent annual meeting in Chicago. After he has completed his Doctorate, Mr Nelson will join the Wellington Dental Research unit with an MRC post-doctoral research fellowship.

Mr Roger Perkins has been named managing director, BJN Holdings (NZ) Ltd, manufacturers of British Paints, Berger Paints and the Selleys range of products.

Mr Zosim Demchenko, Chief Chemist at Griffin & Sons Ltd, the biscuit and confectionery manufacturers, retired recently. Zosim arrived in NZ in October 1950 as one of a contingent of displaced persons from Europe. He soon joined Lever Brothers NZ Ltd (now Unilever NZ Ltd) as a bench chemist and spent 11 years there, becoming laboratory superintendent in the process, before joining Griffins in 1962. Zosim was one of the first members of the NZ Institute of Food Science and Technology. He, along with his wife, **Zina,** is a life member of the NZIC.

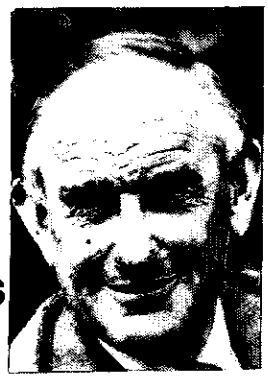
Dr Milton T.W. Hearn of the Otago University Medical School Autoimmunity Research Unit has been invited to speak at the plenary session of the 5th International Symposium on Chromatography at Avignon later this year. He has also been invited to act as chairman at the first international symposium on HPLC of Proteins and Peptides in Maryland next November. A further honour has been his appointment as editor-in-chief of a review journal which has an international board of eminent protein chemists and biochemists.

Southampton University continues to host NZ chemists as visiting research workers. **Drs Mark Daroux** (electrochemistry), **Stuart Heron** (high temperature liquids) and **Graham Wright** (electrochemistry) are currently pursuing research projects, and recent visiting lecturers have included **Prof. Digby Macdonald** (Director, Fontana Corrosion Centre, Ohio State University) and **Dr David Williams** (Materials Development Division, Harwell).

The Chemistry Department has grown rapidly to become one of the leading science departments in UK, writes Prof. Wright, who was NZIC President in 1978. It has an annual intake of 85 undergraduates, selected from about 500 applicants, for a 3-year B.Sc. honours degree. In addition there are two specialist M.Sc. courses, and 96 post-graduates working on their Ph.D. projects. There are 47 academic staff, about 50 technicians, some 53 temporary research fellows, and about 10 visitors.

"It is not surprising that conditions are sometimes cramped," he comments "but there is a good deal of novel research in progress including synthesis, photochemistry, spectroscopy of surfaces, liquid crystals, electrochemistry and electron spectroscopy. The two Wolfson Units on Chemical Entomology and Electrochemical Science have been particularly successful in applied fields; their inventions range from cockroach traps to trickle-tower electrochemical reactors for effluent purification."

The Rogers Report



At its April 2 meeting Council resolved to review in 5 years the status of the NZ Diploma of Science. The Diploma was introduced in 1979 by the Authority of Advanced Vocational Awards as a post NZCS qualification. Council noted that holders of the Diploma as well as NZCS with the requisite years of professional experience can apply for membership of the Institute under the existing rules. Council also asked the Membership Committee to update the 1976 commentary on rules of admission in the light of subsequent policy decisions.

* * * * * *

At the 1980 AGM **Godfrey Husheer** requested Council to investigate financial assistance available from Government for the training of science technicians.

Dr Ellis has explored with the Department of Labour the additional apprentice incentive scheme. While designed to promote trade training and trade skills this also applies to a limited range of technician cadets in building and dentistry but not to scientific technicians. However, the Additional Jobs programme may benefit chemical companies who take on additional staff to expand business and production. Members interested are recommended to discuss the conditions with local officers of the Labour Department.

* * * * * *

Mr Hugh Templeton, Minister in charge of the Inland Revenue Department has replied to a request from the Institute that the limit which can be claimed as a tax deduction on any one book be raised from $20 to $50 or more. The Minister states that the Institute's letter will be considered in pre-budget deliberations. He also notes that the legislation never intended that the limit of $20 on books, journals and periodicals should cover the total cost.

* * * * * *

Dr Ian Shearer, Minister of Science and Technology and Minister of the Environment referred to an OECD report on Science and Environmental Policy in NZ when he spoke to Council. Dr Shearer invited the Institute either directly or through its Public Affairs, Hazardous Chemicals and Environmental Committees to comment to him on this report and other matters. The Minister said he wished to raise the public's appreciation of the scientific work of DSIR, MAF and industrial and academic staff.

* * * * * *

We were fortunate to meet **Dr William Horwitz** during his visit to NZ just before Easter. He is present Acting Director, Science Policy Staff, Bureau of Foods in the Food and Drug Administration, Washington, D.C., and is a past Editor of the well-known and authoritative AOAC methods.

Magnetic Circular Dichroism

T. Brittain, Department of Biochemistry, University of Auckland, Auckland, New Zealand.

Introduction

It was in 1845 that Michael Faraday discovered the phenomenon which forms the basis of magnetic circular dichroism (mcd); namely when any light absorbing material is placed in a magnetic field which has a component parallel to the direction of light propagation, it becomes optically active. Unlike the phenomenon of natural circular dichroism, in which the optical activity is a consequence of the presence of an asymmetric-centre, mcd derives from the imposition of asymmetry on an absorbing centre, which may or may not have previously been chiral. Mcd thus immediately has an advantage over natural cd in that all light absorbing media are open to study by this technique.

In order to understand the mechanism whereby absorbing centres become optically active when placed in a magnetic field, it is necessary to consider the electronic energy levels of the molecule in question. Although a full understanding of the phenomenon requires a knowledge of quantum mechanics [1,2] an insight can be gained from a simpler standpoint.

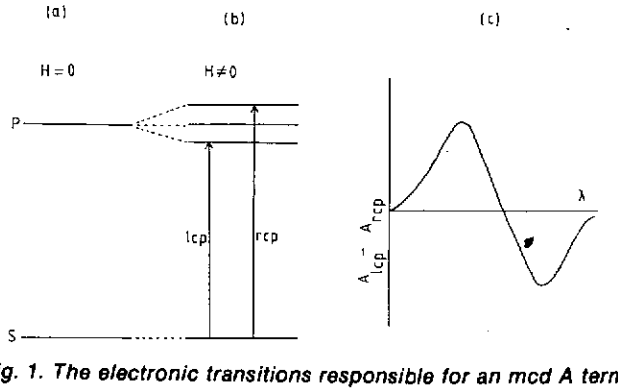


Fig. 1. The electronic transitions responsible for an mcd A term.

Consider a non-optically-active centre in which, in the absence of a magnetic field, excitation occurs from one energy level (a singlet, S) to another (a triplet state, P) (Fig. 1a). In this situation there is no preferential absorption of either left circularly polarised (lcp) or right circularly polarised light (rcp). On the application of a magnetic field (H) the upper triplet state is split by the field into three sub-levels as the degeneracy is lifted (Fig. 1b). In this situation a differential absorption of lcp and rcp light is observed, which follows well defined quantum selection rules. As mcd data is normally presented as the difference in absorption of lcp-rcp light versus wavelength this situation yields an mcd curve (Fig. 1c), which has come to be known as an A term. In the opposite situation, that is excitation from a ground state triplet to an excited singlet state the converse argument applies and is depicted (Fig. 2a, b, c). In this case a C term is obtained. It should be noted that, in the presence of a magnetic field, the populations of the split triplet ground state follow a Boltzman distribution. It follows that the observed intensity of a C term is characterised by its expected temperature dependence. One other case is possible, in which the various excited and ground states may be mixed in the presence of a magnetic field. This

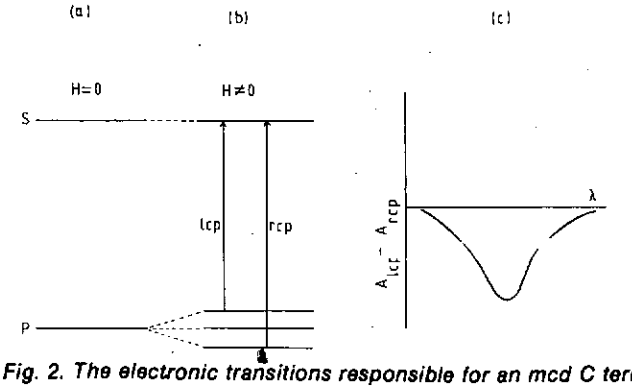


Fig. 2. The electronic transitions responsible for an mcd C term.

leads to a B term, which is similar in shape to a C term but shows no temperature dependence in its intensity.

Mcd Measurements

But what of the practical side of mcd? Although the phenomenon is now nearly 150 years old it was not until the 1960s that mcd became a promising tool for the chemist. This was mainly due to limitations in the apparatus available to the experimenter. Today, to quote from a recent biochemical review, 'Mcd has now been developed to the point where it should be one of the several tools at the disposal of all scientists who use visible, u.v. and near i.r. spectroscopies to probe the structure and function of molecules[3].'

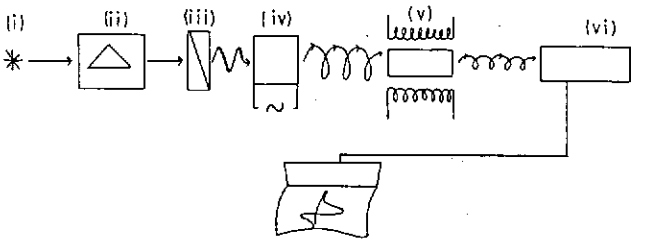


Fig. 3. Schematic diagram of an mcd spectrophotometer.

The apparatus which is necessary to obtain a spectrum, whether it be home made or commercial, consists of the following components (Fig. 3).

(i) A light source, which is usually a stabilised xenon arc.

(ii) A monochromator, usually of the grating type, used to select the measurement wavelength.

(iii) A polariser which produces linearly polarised light.

(iv) A device to convert the linearly polarised light alternately to lcp and rcp light. In the past this has often been an electro-optic modular (a Pockel's cell) composed of ammonium dihydrogen phosphate but, due to the wavelength limitations this type of device imposes, recent apparatus ulitise photoelastic modulators. These new alternatives can be made of a wide range of materials, appropriate to the wavelength range of interest. They represent perhaps the most important recent advance in the measurement of mcd data, allowing investigations from 135 nm to 11,000 nm to be made.

(v) A magnet which can produce a field parallel to the light path and also contain the sample. As the intensity of the mcd effect is proportional to the applied field, super-conducting magnets, capable of producing fields in excess of 70,000 Gauss (7 Tesla), have recently become popular. However, because of the initial cost and liquid helium requirements of these magnets many workers prefer to use electro magnets. Permanent magnets have also been used but suffer the disadvantage of relatively low field strengths.

(vi) A photomultiplier detector and associated electronics and recorder.

The combination of all these components has most commonly been achieved simply by the addition of a magnet system to an existing dichrograph.

For the more specialised characterisation of the temperature dependence of C terms a cryostat is also included in the apparatus. The samples for measurement are most often crystals or solutions, although thin films and glasses in glycerol or sucrose have been used for low temperature measurements.

Uses

Although mcd is of great use to both chemists and biochemists alike, I will restrict the following survey essentially to the field of biochemistry, as it is in this area that I have experience. Even so I hope that this survey will allow the reader to appreciate the wide range of possibilities and, perhaps, see for himself uses of these techniques in his own field.

The scattering of light from particles in solution is not a function of the polarisation of the incident beam. Thus as mcd is a technique in which the ratio of the intensity of the transmitted light is measured, rather than the absolute intensity, mcd measurements are not adversely affected by turbid solutions. This aspect has been of particular advantage in the identification and characterisation of components such as the cytochromes present in suspensions of mitochondria[4].

Due to the high mcd activity and spectral resolution of the amino acid tryptophan, this form of spectroscopy has been used as a means of non-destructive determination of the tryptophan compositions of proteins and recently has become the method of choice[5].

From a purely spectroscopic standpoint, mcd has been used to great advantage in the resolution of otherwise unresolved transitions in a number of compounds[6]. As a high extinction coefficient in an absorbing species is not necessarily associated with a high mcd activity, this technique can be used to detect the presence and concentration of high mcd activity components in a mixture dominated by highly absorbing species. Mcd has also played a role parallel to that of natural cd in differentiation between structures of different symmetry[3].

But undoubtedly the major recent development in this field has been in the study of haem proteins. Using mcd it has been possible to characterise the oxidation and spin state of a wide range of proteins[7-12], and use of variable temperature spectroscopy has made possible the demonstration of electronic site-site interactions in multi-haem proteins.

The use of mcd, however, has not been solely restricted to the research area. It has been shown that this form of spectroscopy can also have value in the clinical area. The detection of myoglobin in serum, for example, can be a useful probe for the detection of otherwise undiagnosed myocardial infarct. The normal level of myoglobin in serum is 0.1 ug/ml, but this rises to 50 ug/ml immediately after a heart attack, then returns to normal after $\sim$ 2 days. The usual means of detection for myoglobin is by immunoassay or radio-immunoassay, both of which require highly skilled operatives. If mcd is employed, as little as 0.02 ug/ml can easily be detected in 1 ml of a serum sample by unskilled operatives in just a couple of minutes.

But perhaps an even more striking example of the possible use of mcd in the clinical area is in the determination of urinary porphyrins. In hereditary diseases, such as porphyria, the levels of urinary copro- and uro-porphyrin are raised. In such cases as heavy metal poisoning, hepatitis or leukemia only the level of the copro- isomer is increased. The levels of the two isomers in urine is therefore a useful diagnostic test. In the past the porphyrins have been determined by a process involving chromatography of relatively large volumes followed by fluorescence spectroscopy. This method is not optimal, due to the large volume requirement and photosensitivity of the porphyrins. Because of the characteristic mcd band shape of the two isomers and the large signal amplitudes relative to the other usual contaminants in urine, mcd is capable of determining the concentration of each isomer at levels of 10 ug/l, even in the presence of an excess of 90% of the other isomer. Apart from the numerical advantages of this approach it should also be pointed out that mcd requires only 1 ml of whole urine (a great advantage for the newly born), no prior preparation and yields accurate data in only a few minutes[13].

Conclusions

I hope that the above survey, if not covering the entire range of application of mcd, has at least given the reader a 'taste of the flavour' of the topic. As pointed out this form of spectroscopy has recently made marked strides forwards, thanks to recent advances in technology and theoretical interpretation, particularly in the region of absorption bands.

Although the optical analogue of the Zeeman effect, mcd can easily be used for the study of liquids and solutions. It has already been used with effect in the study of solids, metal complexes, aromatic and heterocyclic compounds, ketones, amino acids, porphyrins, haems, nucleotides and iron-sulphur centres. Due to its unique information content and fields of application I am quite sure that within the near future, with sufficient exposure, this form of spectroscopy will become known to and used by all chemists and physical biochemists alike.

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Stress And Strain: A Century Of Closed Carbon Chains*

*** A summary of the 1980 ICI Lecture delivered to the Wellington Branch on September 10, 1980**

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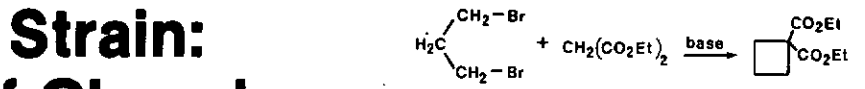
Summary
The early history of small-ring alicyclic chemistry is surveyed to provide an appropriate background to recent developments in the area of cycloproparene chemistry.

In the 15 years following the imaginative formulation of the constitution of benzene by Kekule developments in organic chemistry were restricted to the "fatty" series and the "aromatic" series of compounds. The concept of closed carbon chains (carbocycles) with fewer than six members was dismissed on the basis of bond angle deformation from the tetrahedral value.

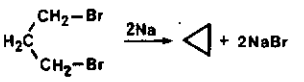
Despite advice to the contrary, W.H. Perkin Jr. began in Munich in 1882 a series of experiments deliberately aimed at the construction of closed carbon chains with 3-, 4- and 5-members[1]. In the following two years papers were published[2] delineating the syntheses of the cyclobutane and cyclopropane diesters shown in Scheme 1 and their mono-carboxylic acid derivatives by what we now regard as a 'classical' malonic ester synthesis. This and the subsequent work of Perkin attracted considerable attention and provides the foundation of alicyclic chemistry.

Although Perkin's work was the first to gain the attention of the scientific community at large, his preparation of the 3- and 4-membered ring systems depicted in Scheme 1 were not the first. In 1882 Freund[3] reported on the treatment of 1, 3-dibromopropane with sodium metal (Scheme 2) whereby a gas was obtained which was later shown to contain cyclopropane



(W. H. Perkin Jr 1883-4)

Scheme 1

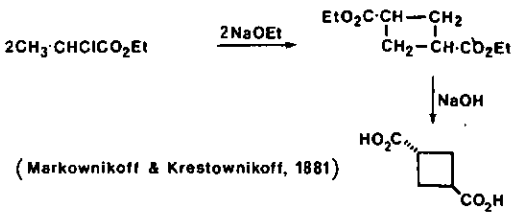


(Freund, 1882)

[Sodium was replaced by zinc in 1887 - Gustavson]

Scheme 2

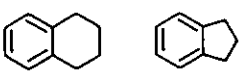
contaminated with propane and propene. However, the earliest[4] authenticated[5] claim to a small carbocyclic ring-system appeared one hundred years ago. The Russian chemists Markownikoff and Krestownikoff[4] isolated small quantities of the cyclobutane-dicarboxylic acid shown in Scheme 3 while attempting to effect the self-condensation of ethyl 2-chloropropanoate. As a result of the studies outlined above von Baeyer put forward his well known strain theory[6] and predicted that 5-membered rings should be very stable — a feature established soon afterwards.



(Markownikoff & Krestownikoff, 1881)

Scheme 3

While a cyclobutane derivative was the first small ring compound to be synthesised, the parent hydrocarbon was not obtained until 1907 and then by way of the more strained cyclobutene which was prepared by the Hofmann elimination procedure[7]. The even more strained cyclopropene, with angle deformation from 120° to 60° about the unsaturated centres, was finally obtained in 1923, again by way of the Hofmann procedure[8]. Even though these simple cycloalkenes were unknown, derivatives fused into the benzene ring were proposed as early as 1888. In fact, Perkin[9] obtained tetralin and indan (the latter before indene was known) but noted that cyclobutabenzene and cyclopropabenzene were yet to be obtained. As Scheme 4 implies, this family of compounds was not completed until Vogel[10] obtained cyclopropabenzene quite recently.



(Perkin, 1888)

R=Br (Finkelstein, 1909) (Vogel, 1965)
R=H (Cava, 1956)

Scheme 4

In 1930 Mills and Nixon[11] proposed that the strain caused by fusion of a small ring across the **ortho**-sites of an aromatic molecule should manifest itself by favouring one Kekule structure over the other. The argument has

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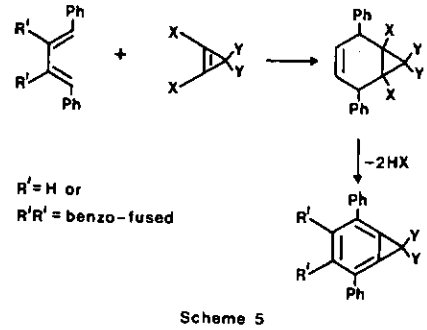
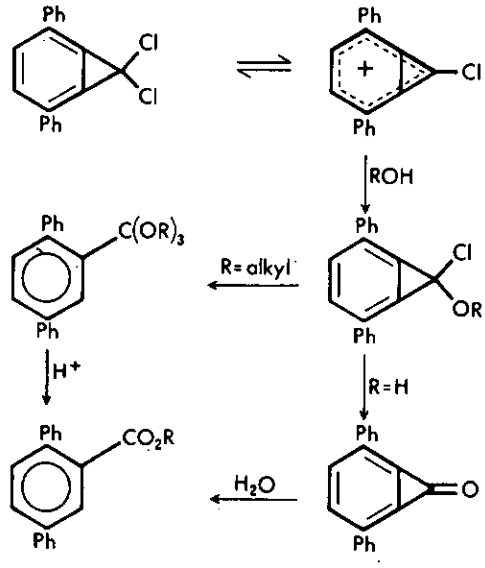
(1a) (1b)

been supported by recent theoretical studies. Since cyclopropabenzene is the most strained member of the cycloalkabenzene series (strain energy ~285 kJ mol $^{-1}$), evidence for bond fixation, **viz.** (1a) **vs** (1b), was thought more likely to be found in this compound and its derivatives than in its higher homologues. Considerable attention has been focussed on these molecules in recent times because of the desire to establish the limits of stress, strain, and distortion that can be imposed on the benzenoid framework and to delineate the consequential influences on bonding, structure and chemical reactivity; a detailed review of the field has appeared elsewhere[12].



Scheme 5

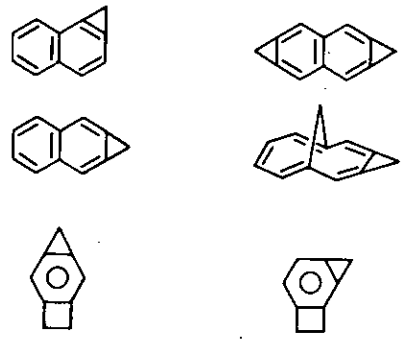
The early work in these laboratories had, as its goal, the synthesis of a **gem**-dihalocyclopropabenzene since ionization and hydrolysis should yield the novel cation and the derived ketone respectively (see Scheme 6 below). The benzene derivatives[13] were obtained in almost quantitative yield by the pathway shown in Scheme 5; similar results were obtained with napthalene derivatives[14]. After considerable effort the cyclopropabenzenyl cation (Scheme 6) was obtained under conditions conducive to long life and the spectroscopic data recorded are fully consistent with the proposed σ-bridged, charged delocalized structure[15]. Although the **gem**-dihalocyclopropparenes are stable in the solid state, the high internal energy is released almost instantaneously in protic solvent resulting in the formation of an aromatic carboxylic acid derivative (Scheme 6)[13]. When water is involved, the decomposition proceeds by way of the derived ketone but the molecule has thus far eluded



Scheme 6

isolation and characterization[15]. The synthetic potential of the **gem**-dihalocyclopropparenes has been exploited; with Grignard reagents the halogen substituents are replaced to yield other cyclopropparene derivatives[14 16].

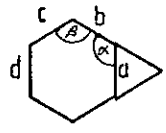
Concomitant with our own studies has been the development of other viable routes to the parent hydrocarbons, and the molecules depicted in Scheme 7 are now known[12]. The strain energies of cyclopropa-benzene and -naphthalene are comparable (~285 kJ mol^{-1}) but that of the bis-cyclopropanaphthalene (Scheme 7) is more than doubled (~700 kJ mol^{-1}) because of added distortion to the δ -framework. Thermochemical studies on the remaining hydrocarbons have yet to be performed.

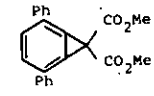
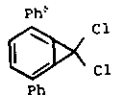
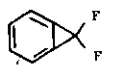
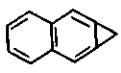


Scheme 7

With the range of derivatives now available it is not surprising that a number of valuable physicochemical measurements have been made. Thus characteristic infrared, ultraviolet and nmr parameters have emerged which allow for the assignment of structure relatively easily[12]. Unfortunately only a few derivatives have been found amenable to x-ray analysis (Table). As can be seen from the data in the Table no evidence for bond localization is observed. What is found is a shortening of bond b when compared with benzene (1.395 Å) and dramatic angle deformation in the 6-membered ring. Consequently, we must conclude that the excessive strain in the ring system is, in large measure, accommodated by severe distortion in the 6-membered ring and not by bond fixation. This is supported by the uv and nmr data of the compounds which imply typical aromatic character. It is clear that further studies with even more strained derivatives are needed and perhaps by the centenary of Perkin's proposals[9] 'cyclohexatriene' character will have been established.

TABLE: STRUCTURAL PARAMETERS OF SOME CYCLOPROPARENES *



	a	b	c	d	α	β
	1.333	1.387	1.419	1.392	126.5°	109.4°
	1.35	1.405	1.39	1.39	126	109
	1.339	1.355	1.423	1.411	126.1	111.5
	1.368	1.337	1.437	1.439	124.9	114.7

*** Data taken from Ref. 12: bond lengths in Å units and angles in degrees.**

Acknowledgements

It is a pleasure to acknowledge the able assistance of my co-workers.

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The National Hormone Laboratory of New Zealand

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The human pituitary gland is about the size of a pea and it sits in a bony box immediately under the brain to which it is physically attached and by which it is largely controlled by chemical means. The gland consists of two distinct parts, anterior and posterior, and while both secrete hormones that are vital for the maintenance of physiological function, we are primarily concerned with the anterior pituitary gland, because of its role in a wide range of processes that include growth, development and reproduction.

Three of the six anterior pituitary hormones so far recognised are peptides, the others are glycoproteins. Failure of secretion of one or more gives rise to serious clinical disorders of which one of the most dramatic is seen in the Growth Hormone-deficient child. Without this peptide, longitudinal growth is slow, the child may suffer frequent fits and brain damage because of abnormally low concentrations of blood glucose, and in the absence of treatment he or she will reach adult life as a "pituitary dwarf", with all the attendant physical, social and economic disadvantages that such a diagnosis entails.

When Growth Hormone was first isolated from animal glands, hopes were high that it could be used therapeutically, like insulin from animal pancreas, but clinical trials were unsuccessful. The hormone was found to be almost species specific and the only effective treatment is replacement with human Growth Hormone (hGH)[1].

Mode Of Action, Substrate Specificity And Properties Of Cellulases

Cellulases are acidic proteins with isoelectric points between 3 and 6 and molecular weights between 5600 and 85,000. The fungal cellulases have a pH optimum in the region 4 to 6 and many of these enzymes are stable over extremes of pH and temperature. The majority of cellulases are glycoproteins containing up to 40% carbohydrate. The cellulases from thermophiles, with the ability to operate at temperatures above 50°C, offer the advantages of an increased rate of reaction and a stable enzyme system. Further, the high operating temperature and the acid pH restrict the growth of contaminating organisms. The three cellulases and the -glucosidase purified from *Thermoascus aurantiacus* have a temperature optimum of 70°C[16].

The mode of action of some cellulases has been determined by using a series of -1,4 oligosaccharides,[8 20 21]. The central bonds of the reduced cellulodextrins were the preferred sites of cleavage and kinetic data indicated that for these endocellulases the specificity region of the enzyme is five glucose units in length. The substrate specificity of celluloses is variable. Purified cellulases from a number of sources exhibit activity towards xylan and it is believed that the xylanase activity is an inherent feature of these enzymes[20]. One of the endocellulases and the -glucosidase from *T. aurantiacus* were capable of hydrolyzing the mixed -1,3; -1,6 polysaccharides such as CM-pachyman, yeast glucan and laminarin. This suggests that for some cellulases the 4- -glucosyl residue in the glycosyl portion of the linkage hydrolysed is not an absolute requirement.

The mechanism of action of cellulases has received scant attention. Kinetic studies[22] and chemical modification of the active site[23] of an endocellulase from *Aspergillus niger* have shown that carboxyl and tryptophan residues are essential for activity. In view of the functional similarity between lysozyme and cellulase (both cleave -1,4-glucosidic bonds) it is tempting to speculate that both enzymes have a similar mechanism with a carboxyl group acting as a general acid, protonating the leaving groups.

Saccharification Of Cellulose

In Japan several tonnes of cellulase are produced per annum and the majority of this is used in digestive tablets. The greatest interest in cellulases, however, lies with the production of glucose from cellulose. Cellulase preparations from *T. reesei* have been used to convert newsprint cellulose to glucose in batch and continuous systems with greater than 50% conversions and a final glucose syrup of up to 30%[24]. When the glucose is converted through to ethanol, production costs are in the region of 60¢ per litre, which is higher than the cost of ethanol from starch substrates such as barley (40-50¢/litre).

The fact remains, however, that cellulose itself is a cheap and renewable raw material. The high cost of glucose production results from problems encountered in the processing of the cellulose. These include delignification, pulping and the rate of cellulose degradation.

In the early 1960s, several countries (including New Zealand) instituted schemes for collecting at autopsy, the very large number of human pituitary glands required for treatment. In this country, the glands were sent to Prof. Alfred Wilhelmi in USA, a world leader in the extraction and purification of pituitary hormones, who in a sustained act of generosity returned hormones for therapeutic use to this country, at no cost. When Prof. Wilhelmi retired, the Medical Research Council of NZ formed a National Hormone Committee, to establish a pituitary extraction laboratory and to be its governing body. It was decided to site the laboratory in the Department of Biochemistry at the University of Auckland. The National Hormone Laboratory (NHL) thus came into being in 1977, with

EXTRACTION AND PURIFICATION FLOWCHART

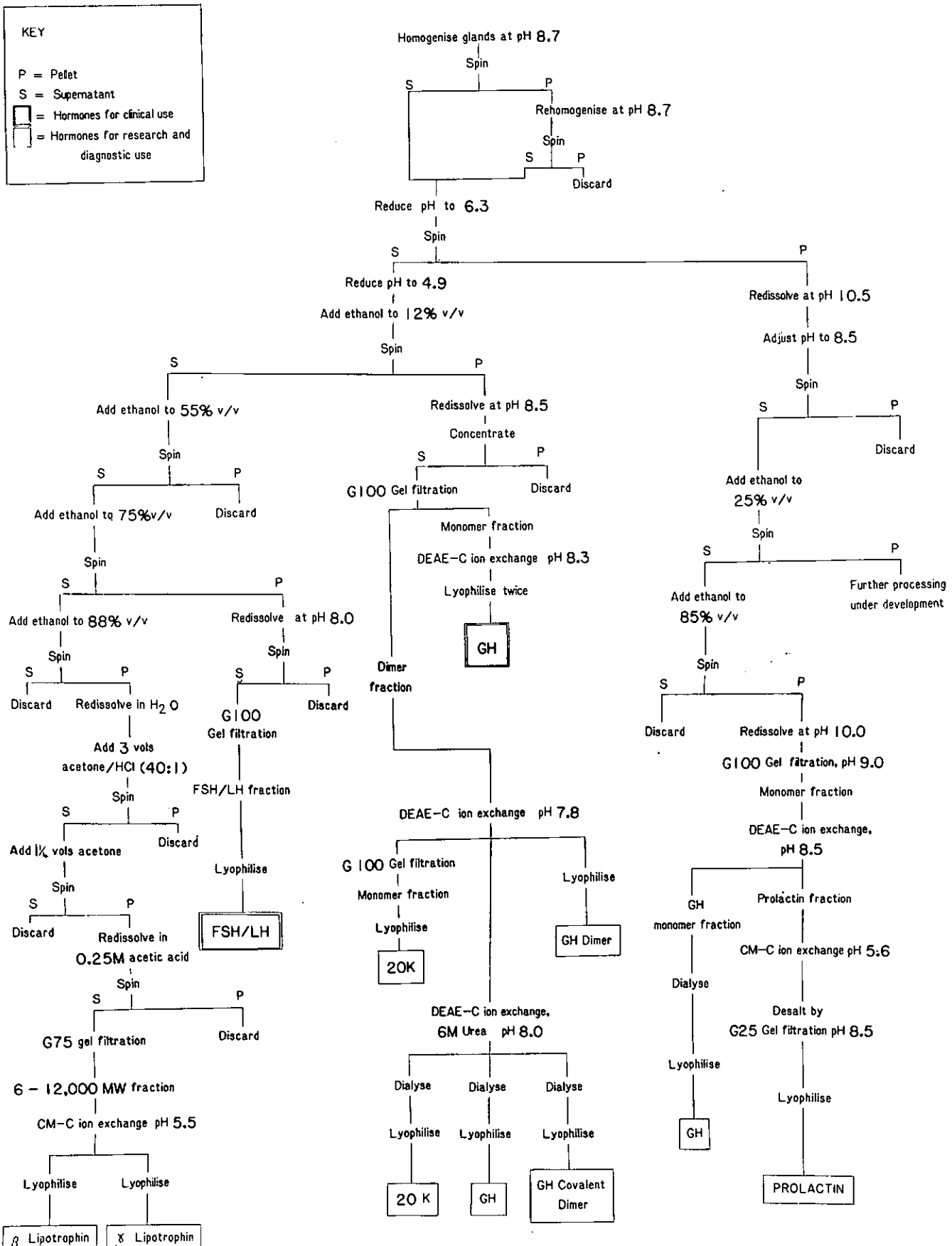

Fig 1: A flowchart of the main processes used for the extraction and purification of human pituitary hormones at the National Hormone Laboratory.

establishment and maintenance costs met by the Department of Health.

Hormone extraction began early in 1978 with the primary aim of purifying hGH for replacement therapy in children. Secondary objectives were the purification of human pituitary gonadotrophins for treatment and other pituitary hormones for diagnostic and investigational use. The essential requirements of an extraction process can be defined as achieving the maximum possible yield of products for clinical use, since the number of patients that can be treated is obviously proportional to the product of the number of glands collected and the extraction yield. However, quality cannot be compromised in achieving high yields. The extraction scheme employed in the NHL (Fig. 1) has been developed from a method used by Dr P.J. Lowry at St. Bartholomew's Hospital in London for the purification of hGH. The methods employed are fairly conventional protein purification techniques, such as fractional precipitation, gel filtration and ion-exchange chromatography. Two standards of working are used in the laboratory. When fractions ultimately destined for clinical use are being processed, special precautions are taken to avoid bacterial growth and contamination with toxic substances at every stage. Non-clinical fractions are processed under what would normally be regarded as clean biochemical working conditions. The extraction scheme has been refined to a stage where the yield and purity of the hGH produced are at least equal to those of any product made overseas. Although the yields and purity of other hormones compare favourably with those from other laboratories, there is clearly room for further improvement, and methods for the purification of side-fractions are being developed.

By international standards, the NHL operates on a relatively small scale. For a variety of reasons (including cost-effectiveness), it was decided that the NHL should be involved in as many aspects of the pituitary programme as possible. The NHL now organizes the collection of pituitaries throughout New Zealand, in collaboration with the NZ Society of Pathologists. It has recently taken over ampouling and testing of its clinical products, and hGH is distributed to patients throughout the country while pituitary gonadotrophins are sent to the two specialist centres in Auckland and Christchurch where they are used in the treatment of certain types of infertility. Such involvement with all stages of the manufacture and distribution of these products, including contact with the patients who benefit from them, gives particular satisfaction and motivation to the NHL staff. Considerations of patients' privacy preclude illustration of the dramatic effects of hGH on the growth of an individual patient, but the graph shown in Fig. 2 amply demonstrates the acceleration of growth rate evoked by hGH replacement in 10 severely undersized children (the examples were randomly chosen).

In addition to its production role, the NHL is actively involved in fundamental research, in its own right, and in collaboration with other groups in New Zealand, and overseas. Most of the research is concerned with the metabolism and metabolic effects of hGH (about which little is understood), and appraisal of the biological significance of a recently discovered hGH variant[2], which has been found to have an internal sequence deletion of 15 residues[3,4]. In addition to its endocrinological interest, this variant is the first protein discovered where the mutation arises from expansion of an intron (intervening DNA sequence) in the gene. It is possible that both hGH and this variant are products of the same gene. Methods are being developed for the measurement of this variant in blood samples because of its apparent universal occurrence in man. It will not surprise the reader to learn that such "fundamental" research has already yielded practical benefits: a radioreceptor assay has been

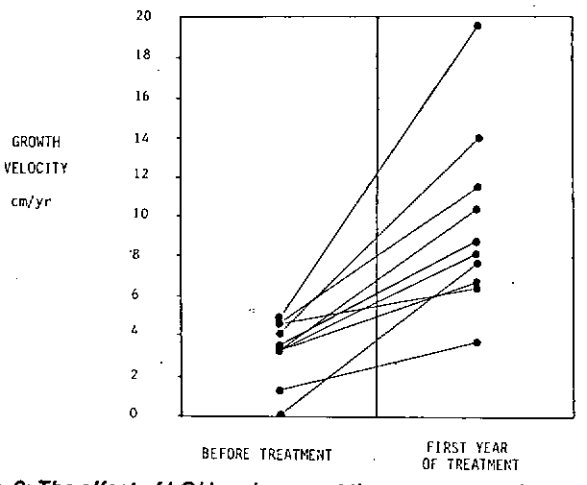

Fig. 2: The effect of hGH replacement therapy on growth rates in 10 growth hormone-deficient children.

developed from our studies on hGH receptors and this is now used routinely for determining the biological potency of clinical preparations, with far greater precision and far less cost than was achievable with **in vivo** bioassay techniques.

The world demand for hGH for clinical use far exceeds supply. Since snythesis is not a feasible proposition with current chemical technology, recent attempts to alleviate the supply problem have centred on recombinant DNA techniques. A semi-synthetic hGH gene has been inserted into the bacterium, *E. coli*[5], and hGH from this bacterial source is presently undergoing clinical trials. However, it is doubtful if the price of hGH will fall greatly in the foreseeable future, since the cost of producing the hormone in this way is much greater than from human pituitary glands.

In this connection, it is worth noting that the NHL is able to supply all our domestic requirements for hGH at one tenth of the world price (and this takes no account of the other hormones produced). Although genetic engineering techniques undoubtedly offer great promise in the manufacture of therapeutic substances, perhaps the above example illustrates that they may not always provide the ideal solution.

The decision to place a specialist pharmaceutical production unit within an academic institution was undoubtedly somewhat unorthodox in view of the large "service" component. However, we view this as a community venture which has provided unique opportunities and endeavours. From the standpoint of the National Hormone Laboratory, the experiment has been a success.

Cellulose: The Fuel of the Future

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Abstract

Energy production from Biomass can be regarded as the cornerstone of a sustainable energy source in the future. Cellulose is the most abundant and easily grown of our renewable energy sources. However, the degradation of cellulose is difficult due to the enert nature of the lignocellulose complex. The nature and structure of cellulose and its enzymatic degradation is described. Cellulases capable of rapidly degrading crystalline cellulose are required. A description of the production of cellulases and their mode of action and properties is presented.

Introduction

Cellulose, a linear (1—4) polyglucan, is produced over most of the earth's surface by photosynthesis. It has been estimated[1] that more than 150 billion tonnes of organic materials are photosynthesized annually and of this 50% is cellulose, 25% lignin, and 25% hemicelluloses, xyloses and pentoses. There is no question that cellulosic substrate could be used for the production of liquid fuels and essential chemicals, thus mitigating the effects of anticipated increases in the shortage of fossil source raw materials.

Economics will determine whether cellulosic substrate should be collected and transported in the first place and the degree to which it can be managed and manipulated prior to utilization. Moveover, two economic regimes must be inspected in each case; the economics of energy production, consumption and convervation, and the traditional dollar economics. The most abundant source of cellulosic substrate available includes municipal solid waste and agricultural and industrial refuse. More recently, however, a number of countries have formulated plans for energy farming and indeed an active and successful programme of energy farming for sugar is being practised in Brazil. In most countries for cellulose energy farming to be economical it would have to be combined with some other high value crop.

Nature And Structure Of Cellulose

Native cellulose generally contains more than 10,000 -anhydroglucose residues linked to form a linear molecule some 5 long. These chains form an aggregate of partly crystalline microfibrils which further aggregate to the cellulose fibre. Many models have been proposed for the structure of cellulose. In one model the cellulose chains are folded back and forth in the plane parallel to the basic structural unit of the cellulose fibre[2]. Chain folding takes place through the occurrence of three or four glycosidic bonds which represent weak points in the platellites and these can be equated with the "amorphous" areas in the cellulose structure. Other workers[3,4] believe that the strength and elastic properties of cellulose preclude a chain folding model. They argue that amorphous regions are caused by the beginning of new molecules, variation in crossover lengths and a staggered arrangements of chains. Helical arrangements have also been proposed.

It is apparent, however, on the basis of its crystallinity alone that a high degree of order exists in the native cellulose fibre. A consequence of this is that not even water molecules, let alone enzymes, can enter the structure. Hence enzymic degradation of native cellulose is difficult and restricted largely to amorphous areas, loose chain ends and exposed surfaces. In crystalline areas the hydrogen bonds, as well as glucosidic bonds, must be ruptured for hydrolysis to proceed. A further complication restricting enzymic attack on native cellulose is the presence of the high molecular-weight polymer lignin. This aromatic material wraps itself around the cellulose and protects it from attack. Indeed the majority of cellulose occurs in nature as a lignocellulose complex. Therefore, before rapid enzymic consumption of cellulose can be carried out, a pretreatment step is necessary that effectively both decrystallizes the glucose polymers and depolymerizes lignin.

Alkali treatment and ball-milling to a 200-400 mesh particle material are the two important pretreatments that have been found useful for the rapid hydrolysis of cellulose. Alkali-treatment loosens the lignin-cellulose complex by hydrolyzing the ester bonds between the uronic acid of hemicellulose and lignin. Ball-milling reduces the size (thus increasing the surface area) and also decreases the crystallinity of the material. The advantage of grinding by ball-milling over alkali delignification is that it increases both the surface area and bulk density; the resulting 20-30% cellulose suspensions give rise to high concentrations of glucose in the digest.

Energetics Of Cellulose Utilization

The photosynthetic equation can be represented as:

$$6\,CO_2 + 6\,H_2O \xrightarrow{\text{photosynthesis}} 6O_2 + C_6H_{12}O_6 \qquad G_o = 2{,}870\ kJ\ mol^{-1}$$

$$\xrightarrow{\text{combustion}}$$

Although the equation shown is for glucose the energetics for a glucose equivalent in a cellulose molecule are similar. When glucose is converted to ethanol by fermentation, only 7½% of the potential energy of the glucose is used:

$$C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2\,CO_2 \qquad G_o = -221\ kJ\ mol^{-1}$$

In the yeast cell some of this energy is conserved in the formation of 2 ATP molecules.

Hence in the combustion of ethanol with O_2

$$2C_2H_5OH + 6\,O_2 \longrightarrow 6H_2O + 4CO_2 \qquad G_o = -2648\ kJ\ mol^{-1}$$

compared to a G_o of - 2870 KJ mol $^{-1}$ kcal for the combustion of glucose.

As well as having only a small amount of the energy lost when cellulose is converted to ethanol, the ethanol produced in a yeast fermentation can exceed 90% of the theoretical yield. Consequently ethanol production from cellulose represents an efficient conversion in terms of both energy conservation and yield to a clean liquid fuel. The efficacy of replacing gasoline with ethanol for internal combustion engines has been well demonstrated in Brazil.

Enzymatic Degradation Of Cellulose

The literature dealing with the degradation of cellulose is confusing. The term cellulase has been used both for highly purified enzymes and mixtures of enzymes which degrade cellulose. These mixtures are sometimes called "cellulase complexes" and the components exhibit synergistic effects. Many microorganisms can grow only on degraded, swollen or soluble forms of cellulose, whereas truly cellulolytic organisms can grow on native, crystalline forms, such as cotton. Based on these

Max Shepherd has been appointed to the Foundation Chair in Experimental Oral Biology at the Dental School, University of Otago effective from August 1981. His primary research interests are with the mechanism of enzyme action and in the biochemical events controlling dimorphism in the yeast Candida albicans. He obtained his B.Sc. (Hons) in Chemistry at Canterbury University in 1965 and his Ph.D. at the University of Calgary in 1969. He has been a member of the Biochemistry Department at Otago University since 1969. His recreational interests include squash, tramping, fishing and bridge.

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Abstracts of Papers

Analytical

Analytical Options For The Automatic Control Of Industrial Bisulphite Liquors

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The suitability of amperometry, redox and colorimetric titrations, gas analysis and specific ion electrode techniques for an industrial application in the wool processing industry are discussed. Complex liquors containing coloured or oxidisable materials are involved, and constituents such as wool grease, non-ionic detergents, dyestuffs, polyethylene oxide chain compounds (used as processing oils) and inorganic particulates may interfere.

Currently available industrial monitoring and control equipment are reviewed, and the likely effects of processing conditions (such as liquor pH and temperature as well as potential interferences) on their reliability over extended operating periods are considered.

Preliminary Investigations Of South Island Lignites

R.M. Carr, M. Lovett and G. Collie
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(Poster Session)

Hydroliquefaction

More than 40 experiments have been carried out in small stainless steel autoclaves. The coal-solvent slurry was exposed to hydrogen gas at pressures in the range of 1100-5000 psi for 2-3½ hr at temperatures above 400°C.

Conversions ranged from 49% to 65% but the data is uncertain.

New autoclaves have been constructed and further experiments are planned.

Water Loss and Gain

The effect of humidity (100% R.H. - 0% R.H.) on powdered lignite samples is being studied by monitoring weight changes with electrobalances.

Ash Composition

Three methods have been used to separate ash. Gravity separation was ineffective. Hydrogen peroxide oxidation yields more ash than high-temperature oxidation due to the retention of volatiles at the lower temperature. Mineralogical content varies with ashing temperature and appears to be different from that reported in earlier investigations.

Measuring The Plastics Closure Efficiency Of Wine Containers By A Chemical Method

Arthur C. Kennett and Tony Eaton
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Once a well produced wine has been manufactured quality assurance has to be maintained by good storage in bottles with closures which effectively seal the contents against gas transmission.

Table wines and sparkling wines are usually preserved in the bottle against the ingress of oxygen by the presence of small amounts of sulphur dioxide while, in addition, the sparkling or carbonated wines require to retain the carbon dioxide produced or used in their manufacture.

Traditional cork closures are rapidly being replaced, mainly for economic reasons, with a wide range of plastics and composites. However, their technical efficiency has yet to be fully evaluated.

Results on seal efficiency for nine different closures including the conventional bark cork closure used to seal actual wine bottles and standard testing equipment are discussed in the paper.

Atomic Emission Spectroscopy: Multi Element Analysis Using A Rapid Scanning Spectrometer Coupled With An Inductively Coupled Plasma Source

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Labtest Equipment Co., Auckland

A computer controlled ICP — AES system will be described along with its performance for multi-element analysis using a high speed scanning monochromator. Consideration will be given to individual components of the system and examples of precision and detection limits will be illustrated.

Problems Of The Analysis Of Coal

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Coal is a heterogeneous mixture of coal substance, moisture and minerals.

Because of its heterogeneity, careful and accurate preparation and sampling is necessary before meaningful analysis can be attempted and the trend in ordinary chemical analysis to the use of microanalytical techniques does not apply.

The properties of the coal substance cannot be identified until the proportions of moisture and minerals are known. Water is relatively easy but there is no generally applicable method for determining the amount or quantitative composition of minerals mixed with coal. Usually they must be deduced from the amount and composition of the ash.

Modern chemistry has no acceptable method of describing the chemical structure of a complex mixture of metal organic three dimensional giant molecules that constitute coal. We are reduced to the use of concepts dating before modern chemistry. Ultimate analysis (percent carbon, hydrogen, nitrogen) uses methods from the era of Justus von Liebig and Johan Kjeldahl, and there is no generally applicable method for determining oxygen.

Proximate analysis (moisture, ash, volatile matter) is carried out under controlled but arbitrary conditions. Volatile matter in particular has little fundamental meaning since the amount can be altered arbitrarily by small changes in the test conditions.

The most accurate analytical determination in coal is the calorific value or specific energy — a physical measurement.

New Zealand coals cannot always be analysed by methods developed for European coals and some non-standard modifications appear inevitable until users of similar coals throughout the world can formulate standards.

A summary of recent work on the chemical properties and composition of New Zealand coals using novel methods for identifying the amount and composition of the associated minerals will be given.

HPLC Separation Of Nucleotides And Nucleosides In Fish And Their Use In Assessing Quality

John Ryder
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Since the declaration of the 200-mile Exclusive Economic Zone in 1978, the New Zealand fishing industry has developed dramatically, both in local and overseas markets. With this increased activity, there has been a corresponding demand for improvement in quality, especially for the export market.

Methods for assessment of quality therefore become of great importance.

One such method is the monitoring of levels of nucleotides and nucleosides in the fish muscle. Upon death, autolysis of the fish muscle takes place immediately, with resulting enzymic catabolism of ATP to yield inosine monophosphate (IMP). Bacterial enzymes become progressively more active in later stages, and together with the autolytic changes, produce inosine and hypoxanthine. It has been proposed that a quantitation of these compounds, the so-called K value, will give an indication of the age or "freshness" of the fish muscle, depending on the levels of each compound present.

The major problems associated with the present analysis have been the length of time required to assay the nucleotides/nucleosides and the lack of separation of two of the compounds.

This paper reports a procedure, using HPLC, which overcomes both these problems, given an analysis time one tenth the previous method and with baseline separation of all relevant peaks, in conjunction with good reproducibility and increased sensitivity.

Examples of uses of this procedure and of K values in assessing quality of fish will be discussed.

Quality Assurance In Chemistry

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British Standard BS 4778, in 1971, defined Quality Assurance as "all activities and functions concerned with the attainment of quality" (of a product or service). However, the meaning and the applicability of this term is still misunderstood or misinterpreted.

The importance of Quality Assurance and the extent to which it plays a vital role in the Chemical Laboratory, Chemical Development and Production, and Chemical Research will be discussed.

Selenium In Natural Waters

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With the recent approval of selenium topdressing as selenate to deficient pasture in New Zealand, the detailed measurement of selenium in river water is of some importance, particularly where water for human consumption is derived from treated catchments. Selenium was measured by a semi-automated fluorescence method, with the sensitivity enhanced by purging with nitrogen to lessen the fluorescence quenching, and interfering oxidative reactions of dissolved oxygen. Total selenium was determined after acid digestion, and selenite and selenate after anion-exchange chromatography. Selenate is theoretically the stable inorganic form in aerated river water, but it was below the detection limit (0.1 ng/L) in most samples.

Development Of A Capillary Discharge Lamp For Vacuum Ultra-Violet Atomic Absorption Spectroscopy

M.D. Lowe and M.M. Sutton
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There is an urgent requirement for more rapid, reliable and sensitive methods of analysis for the elements iodine, sulphur and phosphorus, which are of considerable importance in agriculture. This study has been aimed at the development of an atomic absorption spectrometer system for the determination of these elements, whose principal resonance lines are in the vacuum ultra-violet (vuv) region between 175 and 190nm.

A simple and inexpensive capillary discharge lamp (CDL) has been constructed, and the possibility of using it as a source of resonance radiation for atomic absorption measurements in the vuv region has been investigated. The spectra of iodine, sulphur and phosphorus have been produced by maintaining a current limited alternating current discharge in a helium-element vapour mixture flowing through a quartz capillary discharge tube. At wavelengths below 200nm resonance lines are strongly influenced by absorption due to molecular oxygen in the flame gases and the air. The optical path of the spectrometer system was therefore purged with argon.

The performance of the CDL has been evaluated by making atomic absorption measurements using both unshielded and argon shielded air-acetylene and nitrous oxide-acetylene flames. Analytical sensitivities and detection limits have been obtained and compared with data reported by other workers, who used an electrodeless discharge lamp and a modified hollow cathode lamp as sources of resonance radiation.

Initial studies have shown that the CDL is stable, projects a narrow intense light beam through the flame, and produces spectral lines with widths suitable for atomic absorption measurements. The CDL therefore possesses characteristics which should make it highly suitable and convenient for the measurement of non-metallic element concentrations by atomic absorption spectroscopy.

Comparative Analytical Studies On Some Ampicillin Capsules Marketed In Nigeria

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Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ife, Ile-Ife, Nigeria, (Poster Session)

Ampicillin, a semi-synthetic penicillin, is a broad spectrum antibiotic widely used in Nigeria in form of many brands and generics. The need to monitor the quality of these various ampicillin products arose from the fact that the drug (in capsule form) has been implicated in the inequivalence problems of drug preparations. Some samples of ampicillin were analysed for their ampicillin content in addition to chromatographic and spectrophotometric tests of identity. Bioavailability studies were carried out on three brands of the capsules (including Penbrittin Capsules) found to be chemically equivalent. The same products were subjected to dissolution rate tests. The method employed for the bioavailability studies was a randomnised cross-over design involving nine healthy male volunteers. One capsule of each brand was administered orally to the subjects. Samples of urine were collected at various time intervals and analysed spectrophotometrically by the method of Smith *et al* (1967). The cumulative dose excreted was subjected to statistical analysis with the innovator drug, Penbrittin Capsules, as the reference product. It was found that while the Ampicillin Capsules by Helm was bioequivalent to Penbrittin the other product, designated Ampicillin-X, showed statistically significant difference in its bioavailability relative to Penbrittin. The difference observed may have clinical implications because a patient on the Ampicillin-X may not derive the desirable cure from his ailments. It is, therefore, being suggested that the substitution of one brand of ampicillin capsules by another should be based on the results of a quality assurance test.

Analysis Of Cadmium In Whole Blood, Serum, Urine And Hair By Flameless Atomic Absorption Spectroscopy

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Toxicological studies require the analysis of heavy metals in many widely differing samples. In this paper a simple and sensitive method is described for the determination of cadmium in whole blood, serum, urine and hair. All the samples were digested in HNO_3 at 70° except hair, which was digested in a 1:1 mixture of HNO_3 and H_2SO_4. Although a low temperature necessitated longer digestion, the operation was conveniently carried out overnight. Ammonium dihydrogen orthophosphate was used as a matrix modifier. Detection limits (2) of 30 ng l^{-1} for cadmium are reported. The use of standard addition calibration was essential because of matrix interference.

A Multiresidue Method For Pesticide Residues On Kiwifruit And Berryfruits

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A simple method has been developed which can determine residues of over 15 common insecticides and fungicides to less

than 0.1 mg/kg on kiwifruit or berryfruits. Samples are extracted twice with methanol. Carbamates are determined directly by reversed phase HPLC with 254 nm UV detection following a hexane partition. Fungicides and organophosphates are partitioned into toluene, cleaned up on an activated carbon/cellulose/Florisil microcolumn and analysed by temperature programmed GC with a 5% OV-225 column and linearised electron capture detection. Recoveries, detection limits and blank chromatograms will be discussed.

Microdetermination Of Chlorophenols By High Performance Liquid Chromatography

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(Poster Session)

A wide variety of phenols may be introduced into the aquatic environment. Waste effluents of coking plants, brown coal distillery plants and the pulp and paper, and disinfectant industries are primary inputs of phenols into the environment. Phenols are components of many plastics and are raw materials for dyes and drugs. The toxic effects of phenols on man and aquatic life has been well documented.

While numerous analytical procedures exist for the "total phenols" content in water it is obviously apparent that there exists a great need for a method whereby analysis of individual phenols in water at low concentrations ($< 10^{-3}$ μg dm^{-3} or 1 ppb) is achieved. Methods involving analysis by: derivatisation for GLC, HPLC employing a post column reaction detector and fluorescence detection and normal phase HPLC have all been reported.

The aim of the present work is to develop a method, without necessity of derivatisation, for the direct analysis of a wide variety of chlorophenols in water by reverse phase HPLC.

Initially isocratic elution using acetonitrile-water eluent composition was attempted, whereupon 2 mixtures of 9 phenols were successfully separated in 17 mins. employing low pH conditions and UV detection at 230 nm. As more than 9 chlorophenols could not be separated isocratically, gradient elution was attempted, and various mixtures of 14 chlorophenols were separated over a period of 20-35 mins. at 215 to 235 nm using UV detection.

A schematic summary of the systematic development of reverse-phase HPLC with isocratic and gradient elution for the identification and analysis of aqueous chlorophenol mixtures is presented.

Biochemistry

The Properties Of Marine Wax Esters

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Chemical nature

The fatty acid and fatty alcohol composition of oils from a variety of deep sea fish caught within the New Zealand EEZ area were investigated. Although these are mainly associated with the wax esters, fatty acids related to other minor lipid constituents will be included. These findings will be compared with those of sperm and Jojoba seed oils.

Physical nature

Extensive comparative testings of the sulphurized derivatives of sperm oil and Jojoba seed oil have been carried out abroad. These are used as suitable engine oil additives for effective lubrication under high pressure-temperature conditions. The locally produced deep-sea fish oils could be used for the same purpose.

Biological nature

Marine wax esters offer important functions towards the existence of all marine life. The majority of energy sources utilized metabolically by marine animals are reserved in the wax ester form. However, these situations cannot be similarily used by land animals (including mankind). Results from some published metabolic investigations with wax esters together with other pertinent information will be discussed.

The Reaction Of Adriamycin With Haemoglobin

Diana Bates and Christine Winterbourn
Department of Clinical Biochemistry, Christchurch Hospital
(Poster Session)

The quinone drug Adriamycin is widely used for treatment of cancer. However it is also cardiotoxic, and this toxicity may be related to lipid peroxidation following the production of free radicals.

A similar mechanism may be involved in the oxidative haemoglobin breakdown and lysis of red blood cells caused by Adriamycin. We have studied the mechanism of the reaction of Adriamycin with oxyhaemoglobin, which results in the formation of methaemoglobin and the Adriamycin semiquinone free radical. The effects of the enzymes superoxide dismutase and catalase on the reaction rate suggest that superoxide and hydrogen peroxide are produced by subsequent reactions of the drug free radical. These species all contribute to the haemoglobin breakdown induced by Adriamycin, and also have the potential to initiate lipid peroxidation.

Hydroxyl Radical Production From Paraquat: A Possible Mechanism For Its Toxicity

Christine Winterbourn
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(Poster Session)

Paraquat is a highly toxic herbicide, which even ingested in small quantities, accumulates primarily in the lungs causing extensive tissue damage and usually death. Both the herbicidal action and toxicity of paraquat involve its metabolic reduction to the paraquat radical. While it is generally thought that subsequent reactions of this radical are the cause of toxicity, most investigators have focused on its reaction with oxygen to produce the superoxide radical as being the crucial step. However, in this, and other examples of oxygen toxicity it has not been possible to find reactions initiated by superoxide that would be sufficiently damaging.

In this presentation an alternative toxicity mechanism which is not dependent on superoxide production is proposed. We have observed a hitherto undescribed reaction between the paraquat radical and hydrogen peroxide which produces the hydroxyl radical. This radical is highly reactive and would be capable of causing extensive tissue damage. The reaction is very fast and efficient. It has a requirement for oxygen, and in air can be inhibited by superoxide dismutase, both of which are features of the *in vivo* toxicity mechanism. Intracellular conditions should be suitable for this reaction to occur, and it could be the basis of paraquat-induced tissue damage.

Evidence For An Increase In Viscosity Of Water Associated With Ion Pumping

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(Poster Session)

The Ca^{2+}-adenosine triphosphatase (ATPase) of sarcoplasmic reticulum binds two Ca^{2+} ions at specific sites on its cytoplasmic surface, and only then can be phosphorylated by ATP to form a phosphoenzyme, during the lifetime of which the two Ca^{2+} ions dissociate from their binding sites and appear on the other side of the membrane. Transmembrane Ca^{2+} concentration gradients of 10^3 can be generated and maintained by this pump. We have proposed a molecular mechanism for this and similar processes, in which the dissociation of the ions from their binding sites, the establishment of a gradient in chemical potential and the

direction in which diffusion down that gradient is allowed are all determined by an increase in the structure, hydrogen-bond energy and viscosity of interfacial water contained within oligomers of the phosphoenzymes. Previously we have shown that formation of the phosphoenzyme is accompanied by anomalous light scatter that one would expect to result from fluctuations in density, and by an increase in the polarisation of fluorescence of fluorescein, reflecting an increase in the viscosity of its microenvironment. In this report we describe experiments in which the effect of formation of the phosphoenzyme upon the visocisty of water was investigated using the spin probe, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPOL). Sarcoplasmic reticulum vesicles were prepared by standard methods and finally suspended on a protein concentration of 100 mg ml^{-1} in KCl (0.1M) buffered with tris-(hydroxymethyl) aminomethane (Tris) maleate (5 mM, pH 7). The electron spin resonance (ESR) spectrum of TEMPOL in a concentrated suspension of vesicles was sharp with three hyperfine energy-level splittings. With activation of the ATPase the highfield resonance fell and then returned to its initial value as the ATP was consumed. This is consistent with immobilisation of some TEMPOL which had been freely spinning in aqueous solution, when the highly viscous interfacial water of the phospheonzyme was formed. Calculations suggest that the amount of water involved is approximately 50 nm^3 per polypeptide chain.

A Linear 1 → 3 Galactan From *Pinus radiata* Callus Culture

D.R. Fenemor and I.G. Andrew*
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(Poster Session)

From a hot water extract of *Pinus radiata* callus cell walls, a neutral polysaccharide fraction was obtained, after α-amylase treatment, by passage through DEAE-cellulose.

Methylation analysis of this fraction revealed that it was mainly a linear 1 → 3 galactan, only slightly contaminated by residual starch.

Results from structural studies of the galactan and gel filtration data will be presented.

A Thermostable Protease From An Extremely Thermophilic Bacterium

D.A. Cowan* and R.M. Daniel
Department of Biological Sciences, University of Waikato

An extracellular protease from cultures of the caldoactive bacterium *Thermus* T-351, has been isolated, purified, and characterised. It is a metal-chelator-sensitive lytic protease with a molecular weight of about 20,000. This protease is more thermostable than any reported in the literature, and has a number of other interesting properties.

Collagen Fibre Diameters And Proteoglycans: An Interrelationship In Mammalian Tendons

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Proteoglycans are known to influence collagen fibril formation *in vitro* (1) and this has led to a study of the type of proteoglycan present in mammalian tendon in which collagen fibre diameter is known to vary with age and location (2). The proteoglycan carbohydrate side chains (glycosaminoglycans, GAG) were isolated and quantitated from the tail tendons of rats ranging in age from 1 day to 13 months and from the flexor digitorum profundus (FDP) tendons of rabbits of similar ages. In rat tail tendons from animals 1-5 days old there was a high concentration of GAG (>2% of dry weight) which decreased with age (≈0.25% after 8 weeks). In the young animals, chondroitin sulfate and dermatan sulfate each represented about 30% of the total GAG but, with increasing age, dermatan sulfate became predominant and chondroitin sulfate a minor component. Similar results were obtained with the rabbit FDP tendons except in the pressure bearing region where the tendon curves around the talus (3). In this location the total GAG content remained high throughout the age range and chondroitin sulfate remained a major component. The distribution of collagen fibril diameters has previously been determined in rat tail tendons over the same age range and also in regions of the FDP tendon of mature rabbits (2). These results support the thesis that the type of proteoglycan present plays a part in directing the organization of collagen fibres but, conversely, it is possible that collagen fibrils are involved in directing the type and amount of proteoglycan synthesized.

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Differentiation In Human Amniotic Fluid Cell Cultures: Studies On The Aromatization Of C_{19}-Steroids

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The major class of cell grown from diagnostic amniocentesis has been termed AF (amniotic fluid) and such cultures can be distinguished morphologically from the less frequent F (fibroblast) type cells.

Recently reported differences in these cell types include:

(a) Production of a glycoprotein specific for epithelial basement membrane by AF but not F cultures.
(b) Extracellular material with ultrastructural characteristics of Type I (connective tissue) collagen fibres in F but not AF cells.
(c) Biochemical evidence for Type I collagen in F cultures and for a basement membrane collagen in AF cultures.
(d) Production of human chorionic gonadotrophin by AF but not F type cells.

Such evidence indicates that F type cultures resemble typical fibroblast cultures from dermis and other connective tissues, whereas, the characteristics of AF cells could be attributed to a trophoblastic origin.

Because these cell lines did not appear to secrete estrogens in culture, it was decided to investigate their capacity to aromatize C_{19}-steroids and to explore the inducibility of the aromatase complex.

Cell preparations were incubated with 1 μCi[4-^{14}C]androstenedione in the presence of a NADPH-generating system. Steroids were extracted (recovery ≈ 90%) and chromotographed on small columns of magnesium oxide to separate substrate from products. The latter were then partially resolved by partition chromatography on short celite columns. Further characterization was achieved by thin-layer chromatography.

The results support the view that AF and F type cells from diagnostic amniocentesis have different origins, and evoke a number of questions concerning the control of estrogen production in AF cells.

20K hGH : New Hormone Or Evolutionary Oddity?

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20K is the name given to a variant of human growth hormone (hGH) which has a 15 residue internal sequence deletion. This variant (which is apparently universally present in humans) has almost certainly arisen from the expansion of a known intron in the pre-hGH gene. It is not known if 20K and hGH are translation

products of a common gene transcript. *In vivo* and *in vitro* tissue binding studies have been performed, in an effort to determine if this variant has a spectrum of receptor binding properties different from those of hGH, a prerequisite for classifying it as a hormone in its own right. The comparative binding of hGH and 20K to lactogenic and somatotrophic receptors in human, sheep and rat liver, and in sheep adrenals has been examined. In all cases, hGH and 20K have been found to bind to the same receptors though the affinity of 20K for the receptors is 5-10 times less than that of hGH. *In vivo* tissue binding studies in the rat are in agreement with the *in vitro* results, and incidentally confirm glomerular filtration as an important clearance mechanism for these hormones. We have not yet found any evidence to suggest that 20K has a specific physiological role to classify it as a new hormone, though further studies are in progress.

The Amphiphilic Helix-An Important Feature Of Lipid Associating Proteins

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Amphiphilic regions of proteins appear to be important in the binding of lipid to protein. These regions are predominantly α-helical with polar and non-polar residues distributed on opposite faces. Amphiphilic regions of apolipoproteins are probably responsible for determining interactions with lipid and with metabolic enzymes.

Model peptide studies based on the amphiphilic regions of apolipoprotein C-I, have demonstrated that the presence of the amino-terminal amphiphilic region is sufficient to allow weak but significant incorporation of peptide into Very Low Density Lipoprotein particles.

A series of peptides based on an amphiphilic region of apolipoprotein A-I have been synthesised and examined in model lipid binding studies in order to determine specific requirements for lipid binding.

Apolipoprotein A-I is responsible for activating the plasma enzyme lecithin-cholesterol acyl transferase (LCAT). The region of this protein thought to be responsible for activating the enzyme can be shown using Chou-Fasman parameters to be an amphiphilic helix with an unusually high number of ion pairs. These model studies have suggested new approaches to examining the interaction of serum enzymes with lipoproteins.

It is now possible to readily predict amphiphilic regions of proteins using axial projection or an unfolded lateral projection of its sequence.

An Intracellular Protease And Its Inhibitor From *Candida Albicans*

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Candida albicans can grow either as ovoid yeast cells or as a filamentous mycelium. Germ-tubes are the initial stage of the yeast-mycelium transition and this developmental change can be induced by bubbling air through a suspension of yeast cells for 24 hours and then incubating the cells with N-acetylglucosamine at 37°C. Similar developmental changes in *Saccharomyces cerevisiae* and other organisms have a critical requirement for intracellular protease. However, the intracellular proteolytic system of *C. albicans* has not been characterized and its role in the yeast-mycelium transition cannot be investigated until this work has been undertaken.

We have found an intracellular protease which degrades Azocoll and a macromolecular inhibitor of this enzyme in cell-free extracts of *C. albicans*. The protease can be detected using Azocoll but the assay is unreliable for quantitative measurement of enzyme activity. The standard techniques of enzyme purification, for example: salt fractionation, solvent fractionation, ion-exchange chromatography, have not been successful for purification of the protease.

These two basic problems: (1) establishing a reliable, reproducible assay for measuring protease and inhibitor activity and (2) purification of the protease and the inhibitor must be solved before their molecular and enzymatic properties can be studied. The strategies adopted to solve these problems and the proposed studies for characterising the protease and the inhibitor will be presented.

The Resolution Of 18 Amino Acids And Other Bacterial Cell Wall Components On An Amino Acid Analyser

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A method has been developed to resolve the 18 "protein hydrolysate" amino acids and the following bacterial cell wall components:— Glucosamine, galactosamine, muramic acid, diaminopimelic acid and glucosamine-6-phosphate. A technicon TSM amino acid analyser was used in the normal configuration for protein hydrolysate analysis except for a minor change to the buffer inlet tubing. Cell wall hydrolysate samples could therefore be run on an analyser dedicated to protein hydrolysate analysis with almost no disturbance to the machine. About 15 minutes was required to switch from one system to the other. Separation of the above compounds was completed in 160 minutes.

Glutathione S-Transferases And Insecticide Resistance In The Housefly

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The glutathione S-transferases are known to be involved in the metabolism of a wide variety of pesticides. In the housefly, it has become apparent that some strains, resistant to organophosphate pesticides, owe their resistance to very high levels of glutathione S-transferase activity. In the present study, the technique of affinity chromatography has been used to isolate these enzymes from five strains of housefly. It is apparent that the emergence of resistance involves not just an increase in the total amount of enzyme activity, but also a change in the identity of the principal enzymically active proteins. A provisional hypothesis is that this change involves not a structural mutation, but a change in the relative expression of existing genes.

The Production Of Insulin-Like Immunoreactive Material By Mouse Brain Cells In Culture

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Biochemical investigation of the central nervous system has often been frustrated by the lack of suitably robust biological preparations e.g. studies on the biosynthesis of hypothalamic peptide releasing hormones in the mid-1970s. However the search for transmissible agents in chronic neurological disorders, such as multiple sclerosis, has stimulated the introduction of improved methods for the establishment and maintenance of neurons in culture. We now report preliminary experience with a modification of one such procedure.

Cells derived from fetal mouse brain can be kept in defined media for several weeks and they produce insulin-like immunoreactive material. The significance of the latter finding and the influence of various factors upon its production *in vitro* will be discussed.

(This work was initiated by a grant from The Auckland Medical Research Foundation and is now supported by The Medical Research Council of New Zealand.)

Preparation And Properties Of Hexitol-Lysines

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Until recently, Schiff base condensations between reducing hexoses and the ε-amino of protein bound lysyl groups have not been reported. One major reason is the lability of such bonds to many of the conditions used for protein analyses. Such

conjugates are usually synthesized non-enzymatically in vivo and evidence of their occurrence in haemoglobin, collagen and crystallins has been documented. Of considerable interest has been the changes in these derivatives in pathological states such as diabetes and cataract formation.

One method of increasing the stability of such aldimine condensations is to reduce the bond and so form the hexitol-lysyl derivative. This paper reports a simple method for the chemical synthesis of some of these compounds which may then be used as standards during the analysis of the proteins. Aspects examined include some of the conditions to optimize the synthesis, acid and alkaline hydrolysis behaviour, electrophoretic and chromatographic properties.

Kinetics Of The Reconstituted Artificial L-Lactate Oxidase From *Mycobacterium Smegmatis*

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The natural flavin, FMN, in L-lactate oxidase from *M. smegmatis* is replaced by the artificial flavin, iso-FMN (6,7-dimethyl FMN). The tight binding of this flavin to the apoprotein showed no slow secondary spectral changes. One of the problems in making reconstituted lactate oxidase with flavin analogues for mechanistic study is heterogeneity. By the different solubility and centrifugation properties, we were able to isolate the fully functional reconstituted enzyme.

This artificial enzyme catalyses the oxidation of L-lactate via the same pathway as that for the native enzyme. The only striking difference between the iso-FMN enzyme and native enzyme is the reactivity of the reduced enzyme-pyruvate complex with molecular oxygen and not the reductive half reaction. The low turnover number and partial uncoupling (50%) of the normal oxidative decarboxylation of L-lactate can be explained by the alteration of the kinetic parameters (rate constants).

Protein Cross-Linking As A Probe For Investigating Bacteriophage T4 Structure

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To investigate the topological relationships between the polypeptides of the bacteriophage T4 we have used protein-protein cross-linking, a technique which has been applied to many other complex macromolecules including the ribosome, nucleosomes, cell membranes and viruses. The general protocol is to react the macromolecular structure (in our case bacteriophage) with a compound which is reactive towards particular amino acid side chains. The polypeptides are then separated by SDS-polyacrylamide gel electrophoresis. Cross-linked species will migrate as bands having the combined molecular weights of the monomers. Following electrophoresis in the first dimension the gel strip is excised and exposed to conditions which will cleave the cross-links. The gel strip is then placed at the origin of a second slab gel and electrophoresed in a second dimension. Unchanged components form a diagonal in the two dimensional gel while cleaved components lie below the diagonal and may be identified by their position.

At this time we have completed a survey of seven commercially available, cleavable protein-protein cross-linking reagents, having investigated conditions suitable for their use as cross linkers and for the cleavage of the cross-links so formed. The reagents we have found most suitable for use with phage T4 are 2-iminothiolane; 4,4'-dimethyldithiobispropionimidate; 4,4'-dithrobissuccinimidyl propionate and succinimidyl 3-(2 pyridyldithio) propionate. The conditions of use and cleavage of each of these reagents and preliminary results will be discussed.

Membrane Proteins In Fertilization

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Fertilization is the process in which sperm and egg fuse to form a single diploid cell. This cell is the fertilized egg and, given appropriate conditions, it develops into a mature adult of the species. Fusion initiates activation of the egg. The fusion process itself occurs between a defined area of the sperm plasma membrane, in mammals, the equatorial segment, and the egg plasma membrane. Mammalian sperm only fuse with the egg after the acrosome reaction, despite the prior exposure of the equatorial segment. There is, therefore, evidence that specific membrane constituents render the equatorial segment capable of fusing with the egg. Formal evidence for the involvement of proteins or glyco-proteins is, however, lacking. Nevertheless, it is now clear that in general only proteins have the necessary structural complexity to be able to recognize ligands.

Two assays are currently under development to enable the identification of sperm plasma membrane responsible for fusion with, and possibly activation of, the egg. The first employs fluorescence microscopy and an image intensifying detection system to monitor the transfer of fluorescent label from membrane fragments and reconstituted membrane proteins to the egg plasma membrane. The second assay under physiological conditions tests membrane proteins for their ability to induce a characteristic electrical response from the egg.

The Cell Wall Structure Of *Selenomonas ruminantium* Peptidoglycan And Lipopolysaccharide Composition

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The cell walls of gram negative bacteria contain a peptidoglycan layer composed of glucosamine, muramic acid, alanine, glutamic acid and diaminopimelic acid. In most species this layer has lipoprotein molecules covalently bound to it. An outer membrane of lipopolysaccharide and phospholipid surrounds the peptidoglycan layer. Our studies have shown that lipoprotein is either not covalently bound to the peptidoglycan of *S. ruminantium*, or is present in extremely small amounts compared to other species.

Lipopolysaccharides from *S. ruminantium* were very heterogeneous. Fractions were obtained with qualitative and quantitative differences in carbohydrate composition. The main fatty acid present in all lipopolysaccharides examined was 3-hydroxytridecanoic acid.

In Vitro Mucin Utilisation By Anaerobic Bacteria

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Mucins from the gastrointestinal tract are thought to be available as fermentable substrate for many of the colon anaerobic bacteria. To detect mucin utilisation most investigators have looked for gross utilisation of a single mucin by pure bacterial cultures. Only very low numbers of mucin degrading bacteria have been detected by this method.

Because of the complexity of mucins possessing a large number of different glycosidic linkages it is proposed that their degradation may be by a sequential process involving several bacteria which each cleave only a limited number of linkage types. We have utilised g.l.c. sugar analysis to show that pure cultures of *Bacteroides fragilis* can use a small proportion of the mucin molecule. This limited cleavage would probably not be detected by other methods but is consistent with the sequential model of degradation. Gastric mucin was degraded to a greater extent than colon mucin although both molecules have a similar composition. Factors that may limit the mucin degradation will be discussed.

Proteinase Activity And The Growth Of Cultured Human Cells

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(Poster Session)

A neutral serine proteinase has been purified from the plasma membrane fractions of human lymphocytes and granulocytes. A

rabbit antiserum to the enzyme inhibits its proteolytic activity. The antiserum and γ-globulin purified from it can retard or completely inhibit the growth of normal human skin fibroblasts in culture. Non-immune rabbit sera or γ-globulin had no effect on control fibroblast cultures. Monovalent antibody was equally successful in inhibiting fibroblast growth. The growth of other human cell types was also inhibited by the antiserum.

The Factor Responsible For The Antibacterial Activity Of Some Mammalian Cationic Proteins Identified Crystallographically As The Polyamine Spermine

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We have reported the isolation and partial characterisation of a strongly cationic antibacterial substance from several mammalian tissues and bovine seminal plasma. This substance occurs in a bound form in various protein fractions, and is responsible for the antibacterial activity of these. The molecular weight of the free antibacterial substance was estimated by gel filtration chromatography to be between 2 000 and 3 000. It was thought to be a peptide, but our continuing study has shown that this was incorrect.

In the course of preparation of larger quantities for further characterisation, we found that it crystallised as a phosphate. We were able to identify it by crystallography as the polyamine spermine. A commercial preparation of spermine (Sigma Chemical Co.) was found to have the same antibacterial properties and the same unusual behaviour on polyacrylamide gel electrophoresis (being found on the outer surface of the gel on staining). It was also eluted at the same volume on gel filtration chromatography, much earlier than it should have been according to its calculated molecular weight of 202.

Spermine has been known for some time to occur in a wide range of tissues and secretions, but its biological role has not been known. Our discovery that spermine is the active component of several cationic antibacterial proteins suggests the possibility that its role may be in defence against infection, in the form of protein complexes.

The Structure Of Azurin, A Blue Copper Protein

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Azurin, the blue copper electron transfer protein, has been extracted from *Alcaligenes denitrificans*. purified and crystallised[1]. The crystals, which diffract to 2A resolution, have been the subject of an X-ray crystallographic structure analysis.

An electron density map, phased by 4 heavy atom derivatives, has been calculated at 3Å resolution. The copper atom lies in a hydrophobic pocket, bound to two His residues, one Cys and one Met, in a distorted tetrahedral geometry. The copper site appears to be homologous with those in other blue copper proteins[2,3]. Details of the polypeptide chain folding and of possible electron transfer pathways to and from the copper atom will be presented.

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Acetyl-CoA Carboxylase In Plant Tissues

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Acetyl-CoA carboxylase catalyses the reaction:

$$\text{acetyl-CoA} + CO_2 + \text{ATP} \xrightarrow{Mg^{2+}} \text{malonyl-CoA} + \text{ADP} + P_i$$

which is the first committed step in the ulitisation of acetyl-CoA for fatty acid synthesis. The enzyme plays an important role in the control of this pathway in mammalian tissues, yeast and *E. Coli*, but the significance of this enzyme in the control of fatty acid biosynthesis in plants is unknown.

We have partially purified acetyl-CoA carboxylases from both maize and barley leaves and some of the kinetic properties of these enzymes have been characterised. Enzymes from both sources show very similar properties with optimum pH for activity at 8.4. The Michelis constant (K_m) for acetyl-CoA is 0.1 mM and for $NaHCO_3$, 2.0 mM. Investigation into the requirement for ATP and Mg^{2+} indicates that the Mg-ATP complex is the substrate for the enzyme, with free ATP inhibiting and Mg^{2+} activating. The requirement for divalent cation was specific for Mg^{2+}, with Mn^{2+} only giving 25% of the activity observed with Mg^{2+}. Monovalent cations, especially K^+, were found to activate the enzyme, depending on the $NaHCO_3$ concentration. Unlike the enzyme from mammalian sources, plant acetyl-CoA carboxylase was not activated by citrate, but this metabolite inhibited the enzyme by complexing Mg^{2+}. Intermediates in the pathway for fatty acid biosynthesis which inhibit acetyl-CoA carboxylase, include malonyl-CoA, palmitoyl-CoA, ADP and CoA.

Binding Of Antitumour Drugs To Natural DNA : NMR Studies At 300 MHz.

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The complexes formed between a number of clinical and experimental antitumour drugs and short pieces of duplex random-sequence DNA have been studied by proton magnetic resonance at 300MHz. Under appropriate conditions, the only resonances to appear in the low field (10-14 ppm) are those from the thymidine N-3 and guanine N-1 imino protons which form -N-H...N-hydrogen bonds during basepairing. These are seen as two well-resolved envelopes centred at 13.70 and 12.65 ppm, asigned respectively to the A-T and G-C base pairs.

Titration of DNA with drugs causes characteristic changes in these envelopes. Drugs which bind by intercalation between the base pairs cause an increasing upfield shift of both envelopes. Compounds which bind on the outside of the DNA cause smaller but definite downfield shifts of the envelopes. These changes are interpreted in terms of drug-induced shielding of the imino proton resonances, and results for 70 drugs have been classified.

For compounds whose mode of binding is known by other methods, complete agreement with the NMR results are found, indicating this to be a new and complementary technique for determination of the mode of binding of small ligands to DNA. In addition, qualitative lineshape analysis of the imino proton envelopes allows drugs to be classified as exchanging between binding sites at a rate either slow or fast (on the NMR timescale). The kinetics of a series of derivatives of m-AMSA are briefly discussed.

The Use Of Perfluoroalkanoic Acids As Volatile Ion Pairing Reagents In Preparative HPLC. The Synthesis, Purification And Biological Testing Of The Proposed Anorexigenic Peptide, Pyr-His-Gly

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The condition anorexia nervosa, characterised by self-imposed starvation, leads to drastic loss of body weight. Recently a urinary peptide has been isolated from patients suffering from anorexia nervosa. This peptide (both natural and synthetic) was reported to cause food aversion and prolonged body weight reduction. In addition to the potential anorexigenic properties reported for this material, this peptide is of interest due to its structural similarity to thyroliberin (TRH) and the association of anorexia nervosa with disturbances of hypothalamic-pituitary function.

This report describes the successful use of the ether soluble, ion pairing reagents perfluoropropionic and perfluorobutyric

acid in the preparative and analytical reversed phase HPLC of underivatised peptides. The preparative separation of a 1 g sample of Pyr-His-Gly, the proposed anorexigenic peptide, is described on C_{18}-silica which was packed in a flexible-walled cartridge and subjected to radial compression. The mobile phase consisted of an aqueous solution of perfluorobutyric acid (5mM) and a flow rate of 100 ml/min was used. The purified peptide was simply isolated by neutralizing and freeze-drying the corresponding peak and then extracting the excess ion pairing reagent with ether. The product was then shown to be homogeneous by analytical HPLC and amino acid analysis. The tripeptide failed to show any effect on food intake, water intake or body weight in female rats. Similarly no effect was noted on the reproductive cycles of the rat.

When One Is Two: The Extracellular Polysaccaride Gum From *Rhizobium* Strain CB744

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The structure of the extracellular polysaccharide gum from the nitrogen-fixing *Rhizobium* sp. strain CB744 (a member of the slow growing group) has been determined by biochemical and chemical methods. It s properties have been studied by various physical methods. While it acts as a single polysaccharide, in fact it consists of two strongly interacting polysaccharides; a β -1,4-**D**-glucan and an unusual β -galacto-α-mannan. The later is a 1,4-α-**D**-mannan in which each 6-hydroxyl is substituted by a β-galactosyl residue, with 71% of the galactose units present as 4-**O**-methyl-galactose. The existence of two separate components was confirmed by affinity chromatography. The strong interaction between the two different polysaccharides may help explain the recognition of the plant host and the symbiotic bacteria.

Antibiotic Substances From New Zealand Plants: Polygodial, An Anti-Candida Agent From *Pseudowintera colorata.*

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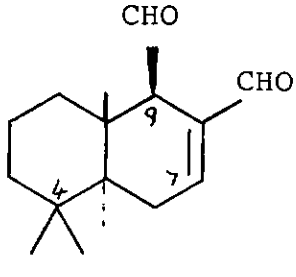
(Poster Session)

A bicyclic sesquiterpene dialdehyde "Polygodial" has been isolated from leaves of the New Zealand tree *Pseudowintera colorata* and shown to possess strong antibiotic activity against the yeast *Candida albicans.* Polygodial was also found in *Drimys winteri* but was absent from *P. axillaris* and *P. traversii.*



Polygodial
(9 -drim-7-en-11, 12-dial)

The Role Of Colloidal Species In The Formation Of Macroscopic Protein Films

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(Poster Session)

Protein adsorption at solid/liquid interfaces is a physical phenomenon of relevance in many biological and tehnological systems. Although protein adsorption in model systems generally proceeds to monolayer coverage, the formation of macroscopic films can be induced by colloidal organic or inorganic bridging species. This effect may be important in processes ranging from the soiling of milk lines to the formation of dental plaque and may also provide a means of recovery of dissolved proteins.

The Preparation And Properties Of A Nitrogen Analogue Of S-Adenosylmethionine

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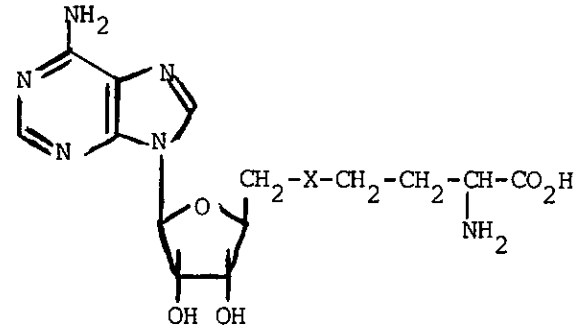
(Poster Session)

S-Adenosylmethionine (SAM) (I; X=S$^{\oplus}$-CH_3) is the ubiquitous methyl donor in metabolism. Although a number of analogues of SAM have previously been described, and tested for biological activity, these have mostly involved modification of the aminoacid chain length or changes to the methyl group itself. Analogues in which the S$^{\oplus}$-CH_3 group is replaced by N-H or N-benzyl are also known, but the nearest analogue (I; X=N-CH_3) has, rather surprisingly, not previously been prepared. We describe the preparation, stereochemistry, and biological activity of this compound (I; X=N-CH_3) and of some closely related adenosine derivatives.



The Separation Of Lipophilic Protein Mixtures By Reversed Phase HPLC, With Reference To The Examination Of Apolipoprotein Profiles Of Hyperlipidemic Patients

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The high efficiency separation of proteins by reversed phase high performance liquid chromatography was made possible by the use of mobile phases which contained ion-pairing reagents. Now that the initial development phase has been completed, it is possible to apply this chromatographic technique to the rapid and highly efficient separation of complex mixtures of proteins present in biological samples. A number of crude apolipoprotein samples isolated from human very low density lipoprotein (VLDL) and high density lipoproteins (HDL) were analysed with this technique. A μ Bondapak-alkylphenyl column was used, and the mobile phase consisted of a 1% solution of the polar, ion-pairing reagent triethylammonium phosphate. A shallow gradient of acetonitrile (37 to 42%) was used to elute the apolipoproteins. The order of elution of apolipoproteins was as follows: C_x, C-I, C-III_2, C-III_1, C-III_0, C-II, A-I and A-II. This order is consistent with the known polarity of the proteins, ie., the most non-polar of the VLDL apoproteins, apolipoprotein C-II, was the last to be eluted, while apolipoprotein C-I, with the lowest non-polar surface area eluted first. The recovery of the individual apolipoproteins was 80 to 95% and the individual peaks were characterized by amino acid analysis, UV absorption spectra and chromatography of pure protein standards.

It would be expected that in addition to apolipoproteins, other proteins with significant hydrophobic regions will be readily separated by this reversed phase HPLC system, and therefore the application of the technique to membranous and other lipid-associating proteins should be a fruitful area of future study.

Structure And Function Of The Hemocyanin From The Paua *Haliotis Iris*

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The hemocyanin from the gastropod *Haliotis iris* is present in the hemolymph as a large molecule with molecular weight about 9×10^6. Electron microscopy shows molecules with a cylindrical structure having eight fold symmetry. It can be dissociated into subunits with mass 330,000 daltons. Ion exchange chromatography yields three fractions, which on SDS gel electrophoresis give bands corresponding to masses 200,000, 320,000 and 430,000 dalton respectively. Proteolytic digestion, carried out with trypsin on whole hemocyanin molecules yielded an aggregate some 20-30 times the size of the original native molecule, formed by end-to-end polymerization, and a fragment (the so-called "collar" fraction) which dissociates, according to SDS gel electrophoresis, into 50,000 dalton fragments.

Electron micrographs of the protein subunits reassociated in the presence of Ca^{2+} show a cylindrical structure typical of the native protein, and the absorption spectrum of the reassociated protein is the same as that of the native hemocyanin.

Oxygen binding experiments with the native hemocyanin, the 1/10, 1/20 molecules and "collar" fraction show a positive Bohr effect and positive shift with increasing temperature.

From amino acid analyses of native, 1/10, 1/20 particles and the "collar" fraction, it was concluded that there were no gross differences in composition between the dissociation products.

Changes In Organic Acids And Sugars During Ripening And Post-Harvest Storage Of Sweet Cherries

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Three commercially grown varieties of sweet cherries were studied for their ripening behaviour with emphasis on the gross changes in organic acids and sugars in the edible portion of the fruit. The parameters were studied at weekly intervals, from approximately one week after fruit set to the point of complete ripening as determined by the orchadist. The changes in organic acids and sugars were also studied over 5-7 weeks after harvest stored at 1°C under two different conditions. Gas chromatography of trimethyl-silyl derivatives of the constituents under study showed that malic acid, fructose and glucose occurred in appreciable amounts.

The path of ^{14}C as $^{14}CO_2$ fed to the leaves will also be discussed in relation to these constituents.

The Mechanism Of Hydroxylation At C-22 During Formation Of Ecdysone In The Ovaries Of The Desert Locust

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Ecdysteroids (insect moulting hormones) occur in relatively large amounts in the newly laid eggs of insects compared to their concentration in insect haemolymph. In biosynthetic studies on *Schistocerca americana gregaria* (Desert Locust), radiolabelled ecdysteroid precursors can be incorporated into ovarian ecdysone to levels of 1%. Two stereospecifically labelled cholesterols, obtained from incubating **3R**, **2R** and **3R,2S** 2-^{14}C/2-^{3}H mevalonic acids with rat liver preparations, were injected into separate batches of maturing adult female locusts and the radiolabelled ecdysteroid fraction isolated from each set of egg pods. The purified ecdysones were treated to give the 2,3-acetonide and 22-oxo-2,3-acetonide derivatives and the $^{3}H/^{14}C$ ratios measured to account for loss of tritium at C-22. The cholesterol containing the C-22 **pro-S** tritium retained this label in the biosynthesis of ecdysone. It was removed on the formation of the 22-oxo derivative. In contrast, the cholesterol containing C-22 **pro-R** tritium lost its label on hydroxylation. These results indicate that C-22 hydroxylation in ecdysone biosynthesis is direct and is accomplished with retention of configuration, in agreement with most other biological hydroxylations.

Kinetics Of Protein Synthesis By *Rhizobium* Bacteroids

Brian D. Shaw
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(Poster Session)

While the separation of rhizobial polypeptides by molecular weight on polyacrylamide gels (as the sodium-dodecyl-sulphate aggregates) resolves thirty to forty bands, the combined separation effected by isolectric point and molecular weight on two-dimensional gels resolves 10 times as many.

Autoradiographed bands from a one-dimensional separation can be quantitated from densitometer tracings by using simple approximations, or by more complex computational procedures which fit a series of Gaussian curves to the tracing. With two-dimensional gels, estimates of the radioactivity of spots must either be made by a direct method such as excision and counting, or by manual or computational densitometry.

Rhizobium bacteria from lupin root nodules will continue to reduce acetylene (a measure of nitrogen fixation) if isolated under inert gas, and incabated in a 300 milliosmolar solution at a dioxygen partial pressure of 0.2%. Added ^{35}S-methionine is incorporated into protein, and its quantitative distribution can be analysed as described.

The use of such methods to analyse the effects of compounds including dioxygen on the protein synthesis of these agronomically-important bacteria will be illustrated.

Inhibition Of Cell-Free Protein Synthesis By Low-Molecular-Weight Nuclear RNA Of Lactating Rat Mammary Glands

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(Poster Session)

Poly(A)-containing RNA isolated from the nuclei of several tissues of the guinea pig inhibits mRNA-directed protein synthesis in the wheat-germ cell-free system. Lactating mammary gland nuclei is a particularly good source of this inhibitory activity.

Similar inhibitory activity has now been found associated with the poly(A)-containing nuclear RNA of lactating rat mammary glands. In order to further characterise the inhibitor, and to determine whether or not the inhibitor activity can be attributed to the U series of low-molecular-weight nuclear RNAs, we have fractionated total RNA, poly(A)-containing and poly(A)-minus preparations from rat mammary glands by urea-acrylamide gel electrophoresis. Bands were identified using ethidium bromide fluorescence, and with a photographic template, they were then excised and the RNA isolated and quantitated. The ability of each fraction to inhibit protein synthesis directed by various messenger RNAs was measured. Inhibitory activity was found with many RNA species, some of which inhibited initiation of protein synthesis, whereas others inhibited elongation. Neither poly(U)- nor poly(A)-directed protein synthesis was affected by any of the nuclear species.

Barley Leaf RNase Activity In Response To The Pathogen *Puccinia Hordei*

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(Poster Session)

During the infection of barley leaves by a pathogenic fungus such as leaf rust (*Puccinia hordei*) the overall response of the host is a reflection of the metabolic changes occuring at and around the infection site. These metabolic changes are in turn governed largely by the metabolism, in the affected cells, of RNA.

A key factor in RNA turnover is the RNase which exists as two polymorphic forms (isozymes) in the uninfected leaf. In response to the pathogen the activity of the host's original RNases changes and a new RNase is produced. This enzyme is produced not by

the pathogen but the host. The characteristics of these RNases will be described and the time course of the activity changes will be discussed in relation to their possible role in disease resistance.

Secondary Structure Of The Major Bovine Caseins In Solution

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The caseins of bovine milk are hydrophobic proteins and associate reversibly with one another to form aggregates. These can be dispersed by dissociating solvent systems, or, conversely, can form much larger conglomerates after addition of calcium chloride to their solutions. In order to gain a greater understanding of the protein-protein interactions that occur at a molecular level and that lead to these association reactions, an attempt has been made to determine the secondary structure of the major casein fractions.

The circular dichroism spectra below 250nm indicated that none of the caseins had more than 35% periodic structure (α-helix or β-sheet) at pH 7. Low concentrations of calcium chloride did not affect the spectrum but 5M guanidine hydrochloride diminished the periodicity of the proteins. Spectra in the 255-350nm region for β-casein were indistinguishable at pH 7, in guanidine hydrochloride or in calcium chloride solution. The α_{s1}-casein spectrum, however, was altered markedly in transferring from guanidine hydrochloride to pH 7 buffer and again on the addition of calcium chloride to the protein solution. It seems likely that the environment of the tripeptide Trp 164, Tyr 165, Tyr 166 might be responsible for these effects.

Structure prediction on the basis of the primary sequence data also suggested that there was little periodic structure, but did indicate that in each protein three or four regions that were likely to be α-helix and a similar number likely to be β-sheet. Interestingly, the major sites of chymosin attack in α_{s1}-, β- and κ-casein are all in regions of predicted β-sheet structure surrounded by aperiodic structure. These regions in α_{s1}- and β-casein are also the major segments tht interact with the fluorescent hydrophobic probe, anilinonaphthalene sulphonic acid.

Glycogen As A Metabolic Monitor

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The hugely polydisperse glycogen of liver (1) has been shown to be stored in at least two compartments (2), namely in the cytosol and in the lysosomes. Further the two locations hold glycogens of different molecular size (2) and overall structure (3,4). This means that size and/or structural analysis of the total glycogen extracted from liver can give information on the metabolic state of both compartments in the liver of the whole animal. Further, since degradation within the lysosome is independent of the "normal" degradative processes in the cytosol and since the lysosome is a target for anti-inflammatory drugs, glycogen may be used as a metabolic monitor of the effect of these drugs on the whole cell metabolism *in vivo*. Little information is currently available on the effects (if any) of hormones or lysosomes and preliminary experiments along these lines will be reported.

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Glycerol 3-Phosphate Dehydrogenase In *Mycobacterium Smegmatis*

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Mycobacteria metabolize glycerol via two pathways. It can be oxidized by an NAD-glycerol dehydrogenase to dihydroxyacetone and then phosphorylated or phosphorylated to glycerol 3-phosphate which is oxidized by a glycerol 3-phosphate dehydrogenase to dehydroxyacetone phosphate. Reddy (1969)[1] used L-[3-^{14}C] glycerol to show that *in vivo* different strains of mycobacteria use either one or both of these pathways. The glycerol 3-phosphate dehydrogenase has been variously reported to be either NAD(P)+ -linked or acceptor-linked. Our object was to isolate and characterize the acceptor-linked enzyme and to investigate the growth conditions under which the different pathways are expressed.

Initial work indicated that the enzyme was probably membrane-bound which suggested that it might be usefully isolated from a purified membrane fraction rather than from whole cells. Protoplasts were prepared from lysozyme-treated cells and lysed by osmotic shock. The membranes were washed free of cytoplasm and then 60-70% of the glycerol 3-phosphate dehydrogenase activity was solubilized by extracting for 15 minutes in 0.5% Triton X-100. The enzyme was further purified using conventional techniques. Kinetic and functional data will be presented.

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Phospholipid Stimulation Of Solubilized Acyl-CoA:Diacylglycerol Acyltransferase

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The acyl-CoA:diacylglycerol acyltransferase (DGAT) from rat liver microsomes can be solubilized in 100mM cholate and partially purified by sucrose density gradient centrifugation. When the centrifugation is carried out in the presence of 50mM cholate delipidation of the DGAT occurs and full activity can only be obtained by adding back phospholipids. A cholate dilution procedure was used with the final cholate concentration between 2.5 and 7.5 mM. With unfractionated microsomal phospholipids a 10-fold stimulation of DGAT activity was obtained at a final phospholipid concentration of 200 uM. With single phospholipids, the acidic phospholipids, phosphatidylserine and phosphatidylinositol were the most effective, but mixtures of phosphatidylcholine and phosphatidylserine were more effective than phosphatidylserine alone.

Estimation Of Protein Synthesis In Lambs

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The techniques for measuring the *in vivo* rates of protein synthesis in the whole body and in individual tissues have been successfully pioneered, in rats, by Waterlow and Stephen (1967) and Garlick *et al.* (1973). We have also used the technique of a continuous 6 hour infusion of [^{3}H]-tyrosine to measure blood tyrosine flux and whole body protein synthesis in growing 8, 12, 16 and 20 week old lambs fed grass or milk. Since the growth rates of the animals were also measured, protein degradation was estimated as the difference between the rates of synthesis and growth.

In the 8 week old lambs fed grass, the percentage daily body weight gain, fractional whole body protein synthetic rate and fractional degradation rate were 0.81 ±0.19, 5.7 ±1.8 and 4.8 ±1.9 respectively which did not change significantly between 8 and 20 weeks. In contrast, in the 8 week old lambs fed milk, the percentage daily body weight gain (1.36 ± 0.3), fractional whole body protein synthetic rate (9.2 ± 0.8) and fractional degradation rate (7.8 ± 0.9) decrease with increasing age. The mean whole body protein synthesis rate of 19.7 ± 5.5g/kg$^{0.75}$ for all the lambs, however, was similar to the rate of 17.0 ± 4.2g/kg$^{0.75}$ for the animals such as the rat, pig and man (Waterlow *et al.*, 1978; Reeds & Lobley, 1980) illustrating that there is little interspecies variation in protein synthesis.

The rates of protein synthesis in individual tissues were also determined by measuring the specific activities of intracellular and bound tyrosine on determination of the infusions. Very high rates of protein synthesis were found in the rumen and small intestines compared to muscle. The implications of these results will be discussed.

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Enzymes From Extremely Thermophilic Bacteria

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Bacteria with growth optima above 70° C can be isolated from a wide range of environments, and exhibit a diversity of metabolic capabilities comparable with mesophilic bacteria. New Zealand hot springs appear to be a particularly good source of such bacteria. The enzymes of extreme thermophiles are generally stable at the growth temperature of the organism, but in most other respects are similar to enzymes from mesophilic bacteria. A variety of hypotheses have been advanced to account for this stability.

Enzymes are an increasingly important class of industrial catalyst. Because of their thermostability, the use of enzymes from extremely thermophilic bacteria has potential economic advantages over the use of those derived from mesophiles.

Design Of Transition State Analogues For GABA Transaminase

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Compounds which inhibit the mitochondrial enzyme 4 amino butyrate-2 oxo-glutarate amino transferase (GABA transaminase) increase brain levels of the inhibitory neurotransmitter GABA and may exert an anti-convulsion action in the mammalian central nervous system. A number of irreversible "catalytic inhibitors" of GABA transaminase have been synthesised and several of these are potent anti-convulsants, but their action is generally not specific to GABA transaminase.

An alternative approach which may provide both potent and highly specific inhibitors, involves the use of the transition state structure of the enzyme catalytic reaction as a template for the design of transition state analogues. We have designed, synthesised, and tested several of these inhibitors and found that they not only inhibit the enzyme *in vitro*, but also prevent chemically induced seizures in mice for up to two days following oral administration.

Chemical Education

Games And Simulations In Chemistry Teaching

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The first part of this paper will be devoted to an analysis of what is meant by gaming and simulation and what useful part the techniques can play in chemical education. It will be followed by a discussion of examples of materials produced in various countries, together with a consideration of the steps involved in constructing a good simulation.

The paper will conclude with some observations on the necessity of curriculum reform if the use of these techniques is to become widespread.

After presentation of the paper there will be a workshop session in which those interested can study some sample simulations and games and possibly enact some in small groups.

Training: Toward A Life-Long Habit

Graham R. Little
Centre for Continuing Education, University of Auckland

It's a changing world! Oh groan, groan, comes the cry, another writer of cliches and truisms. In defence I would point to the reality of truisms as that so true it is universally ignored. I repeat, it IS a changing world. That means that if you graduated, like me, ten years ago, you are likely to be so far out of date it is frightening.

The speed of technological change is quickening, new facts, new techniques, new theory, simply keeping abreast of one's own field is difficult enough: however, the prospect of achieving some real generality, the broad base of knowledge and understanding so many of the 'old school' seemed to achieve, is akin to dipping one's soul in liquid nitrogen. But generalists we must become. More and more solving the real problems in the community demands skills extending far beyond 'chemistry'. If nothing else it demands an appreciation of politics, of pressure groups, and of diplomacy; finally, even as chemists, we need to become managers.

This is the backdrop, the concern with the training needs of professional scientists, chemists in particular, ten, twenty, and more years beyond graduation. The needs are varied and most often individual, but must be met via institutional means. The University of Auckland Centre for Continuing Education has taken an initiative and sought to develop a series of courses suitable for chemists.

During the early part of 1981 the writer contracted with the Auckland Centre to explore the training needs of chemists, and to formulate a training programme able to be implemented by the Centre. The paper reviews the results, the process used, and the response from firms, professional associations (OCCA, Corrosion Association, NZIC, etc.), and individuals. Finally the paper attempts to identify some of the criteria and questions which need to be considered in establishing the NZIC's role in continuing education as we move towards a new century.

Diploma In Surface Coatings Technology

Neil Edmonds
Auckland Technical Institute

The Oil and Colour Chemists Association have joined in with the Auckland Technical Institute to run an extensive post-graduate course on the manufacture and application of synthetic resins and surface coatings. Based on an established Australian course, it is designed to extend the knowledge and skill of personnel employed in the resin, coatings and printing ink industries as well as suppliers to these industries and the end user of these products.

With the initial 2-year course nearing completion the time is appropriate to review progress.

Some Statistics Relating To Selection And Difficulty Of Chemistry In Secondary Schools

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The proportion of students selecting to study chemistry in the final 2 years of secondary school is declining and this decline has accelerated since the introduction of theory-oriented prescriptions in the late 1960's. Analysis of the 1980 statistics shows that the proportion of students studying chemistry varies with sex, ethnic origin, type of school and locality. The difficulty which students encounter with chemistry, physics and biology in the School Certificate Science Examination is compared.

Chemical Hazards

Safety At The Workplace

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An attempt will be made to rationalise the various approaches developing worldwide to achieve safety at the work place with particular reference to hazardous chemicals. These approaches will be compared with the New Zealand experience and the various legislative steps introduced to implement safety in this country.

Contamination Of Water Supplies By Hazardous Chemicals

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There are few towns in NZ where water supplies originate from a catchment which is generally accessible to the public. However with the normal pattern of growth observed in NZ over the last few decades many towns are finding their local stream or spring is no longer adequate and are having to look to rivers for their future sources. Human activity in these river valleys may vary from a small farm and farmhouse, to intensive farming, large towns with industry, and with railways and state highways passing through the valley.

The water supply authority faced with this growth realises there are certain changes in philosophy between operating a "protected" water supply scheme and a water supply from the lower reaches of a large river in a busy catchment. One of the more important differences is that the water supply authority is no longer in control of its water source or its quality and that water treatment is required to produce high quality drinking water, at all times, from a predicted range of water qualities.

Unpredictable water quality changes of concern to water supply authorities often involve substances causing taste or health problems. These substances may result from agricultural or industrial use, or from disposal by intention, spillage or accident.

This paper will discuss the problems of administration and communication concerning hazardous chemicals with the host of organisations involved: it will discuss the problems of identifying these substances in the raw water intake by chemical and biological methods; it will discuss the water treatment options and costs available to a water supply authority.

The paper will conclude with some case histories.

Hazardous Chemicals Information Retrieval And Use

Arthur C. Kennett and Sonya Donoghue
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This paper discusses the information available to Chemists and other persons who are involved in dealing with hazardous chemicals.

The problems of obtaining the right information rapidly and using it to deal successfully with chemical problems are debated. Incidents with hazardous chemicals are illustrated by selected case histories of incidents in the Auckland area commencing with the Parnell Civil Defence Emergency of 1973.

It is postulated in the paper that manufacturers of a chemical product should know its chemical constitution. If they are unaware of the chemical products' dangers or fail to recognise the need for making the information freely available they should not be allowed to distribute the product.

The difficulties encountered with trade names (whether incorrectly or correctly spelt) and their chemical identification to enable the Chemist to give reliable advice to a client e.g. neutralisation and safe disposal of the chemical, are given.

In conclusion, the paper deals with the types of information which are most helpful to the Emergency Services if the Chemist is not immediately available at the scene of the incident.

The Disposal Of Toxic Wastes By Incineration

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One method for the disposal of toxic wastes is incineration. For complete destruction various combustion parameters must be assured, such as sufficiently high temperatures and adequate residence time in the combustion zone of the incinerator. Monitoring of the combustion process is essential to ensure satisfactory operation, and testing of the exhaust gases may be required to confirm complete destruction of the waste.

Two examples of equipment used in New Zealand for the destruction of wastes will be described. Monitoring and testing procedures will be discussed, including a technique being developed by us for measuring residence times in furnaces. The possible use of existing New Zealand incinerator facilities for toxic waste destruction will also be examined.

The Work Of The National Hazardous Chemicals Information Centre

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National Poisons And Hazardous Chemicals Information Centre, Dunedin

Following the recommendations of the Commission of Enquiry into the Parnell Civil Defence Emergency in February 1973 when drums of an organophosphorus cotton defoliant were damaged in transit, the National Hazardous Chemical Information Centre (NHCIC) was established in Dunedin in conjunction with the existing National Poisons Information Centre. A list of chemicals was obtained by circularising all manufacturers in New Zealand for details of chemicals used or stored. Data sheets were prepared listing chemical name, synonyms, a description of the chemical's appearance, the immediate health hazard that it presents to persons exposed to it, whether protective equipment is required to handle it and suggested management procedures in the event of fires or spillages involving the compound.

The files reached an operational state in August, 1979, and NHCIC "went public". Since then numerous enquiries have been dealt with.

The operation of NHCIC together with some examples of enquiries directed to the centre will be discussed.

The Role Of *In Vitro* Tests In Detection And Regulation Of Carcinogens

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The identification of carcinogens is a necessary prerequisite to the control of their distribution and use. While animal tests give the most meaningful information, they involve considerable time and expense. The high dose levels of material required and apparent species specificity of many carcinogenic agents make it difficult to be certain of the relevance of such data to the human situation. Attention has turned in more recent years to more simple *in vitro* test systems, such as the bacterial mutagenicity assay known as the 'Ames test'. Validation studies suggest that such tests can give comparable results to rodent models in predicting human carcinogenicity.

When new compounds are under consideration, no single test is conclusive, but a carefully selected battery of such tests can give persuasive evidence, at least sufficient to indicate priorities for animal testing. Promising approaches involve a combination of *in vivo* and *in vitro* tests. These provide information on metabolism and absorption of a compound with far greater sensitivity than either test alone, and in combination with a cost benefit analysis, could provide sufficient data upon which to base regulatory controls.

The quality of many chemicals varies between batches, and it would be unrealistic to perform animal tests on each batch. In this situation *in vitro* tests can usefully supplement chemical analysis to detect suspicious impurities, and thereby protect the user from possible carcinogenic hazard.

The Fire Service And Chemical Hazards

A.W. Bruce
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The paper, supported by colour slides, will portray the observations of a senior commanding officer in the NZ Fire Service following a recent study tour on behalf of the NZ Fire Service Commission to the United States, Canada and the United Kingdom.

The specific brief on those visits was to investigate and report on fire protection, firefighting, techniques and training, specifically affecting the petrochemical industry and in particular liquified petroleum gas and natural gas.

This was achieved by visiting numerous refineries and chemical plants, involving production, distribution, storage, transportation, together with associated fire equipment, fire-fighting, training and safety procedures.

The paper will endeavour to make a realistic appraisal of the associated problems with particular emphasis on liquified petroleum gas as a fuel of vital concern to the future of New Zealand.

In association with the Ministry of Energy and the petroleum and gas industries, the Fire Service has an important part to play in dealing with any projected problems.

Development Of The Control Of Toxic Substances In New Zealand

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The history of the legislation controlling toxic substances in New Zealand is briefly reviewed. There is an international move away from the control of chemicals after their toxic hazards have become apparent during a period of use and misuse towards an "anticipatory" approach involving a premarket evaluation of new chemicals. The particular problems this approach poses for New Zealand are discussed in relation to the new Toxic Substances Act 1979 and its regulations.

Chromatography

Analytical And Semi-Preparative Purification Of Peptides And Proteins Using HPLC With Radially Compressed Reverse Phase Columns

M.T.W. Hearn, B. Grego and C.A. Bishop*
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The analysis and purification of peptides and proteins using reversed phase high performance liquid chromatography (HPLC) is now an established technique. A recent addition to the wide range of columns available for this purpose has been the introduction of polyethylene cartridges containing spherical, silica based reversed phase packing material, which achieve maximal efficiency when radially compressed. Using these cartridges it has been possible to achieve separations of mg quantities of both whole proteins and the trypic digest products of proteins without loss of resolution. Post chromatographic recoveries of loaded proteins have been assessed by the analysis of radio-actively labelled samples and found to be in the range of 95 to 100%. The proteins used for this study include the following: human thyroglobulin 19 S, guinea pig thyroglobulin 19 S (^{125}I labelled), ovine thyrotrophin (^{125}I labelled and unlabelled), β, γ endorphins, human growth hormone, bovine insulin, angiotensin I and chick lysozyme.

The Analysis Of Carotenoids Of Green Plant Extracts By HPLC

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Though there have been several reports of analyses for carotenoids by HPLC utilising silica columns and straight phase separations only one or two brief reports have been made using reverse phase techniques with a C18 column.

Initial separations did not have the resolution of normal phase techniques and poor peak shapes. Now a separation has been achieved equal to any achieved on straight silica utilising a multi-solvent system initially developed at the Roche Marine Pharmacology Laboratory.

This system has been adapted to allow rapid measurements of lutein and β carotene in protein extracted from lucerne and ryegrass/clover in order to study the effects of various factors and modifications of extraction techniques on the level of carotenoids in the protein extracts.

Though ideally a gradient system is required to resolve a system of such wide polarity an isocratic system can be utilised using two solvent mixtures and a three-way valve.

The effect of light and oxygen on carotenoid yields has been shown to be critical both in the analytical extraction and in the production of protein extracts from the green crop.

Determination Of Mothproofing Agents By HPLC

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Wool Research Organisation of NZ, Lincoln

Larvae of a small number of species of moths and beetles are able to digest keratin, making them pests of clothing, blankets, upholstery fabric and carpets. A number of commercial moth-proofing agents are available to protect wool, and provided the fibres have been adequately treated little damage occurs. By measuring the residual amount of mothproofing agent present in the wool the type of product used and the level of treatment can be determined.

The active constituent of one of the mothproofing agents is a mixture of isomers of a chloro-2-chloromethyl sulphonamide diphenyl ether. Using a reverse phase radial compression column and a methanol/water solvent, rapid separation of the mothproofing agent is obtained. The normal phase separation mechanisms, which can occur with the lower C18 coating of radial compression columns, are suppressed by adding a quaternary ion pairing reagent to the solvent.

An alternative separation method involves the use of a CN bonded phase column and non-polar solvents. In this situation the CN bonded phase packing performs similarly to a normal phase silica column but it eliminates the possibility of deactivating the column and reduces the time for the column to equilibrate with the solvent.

Another mothproofing agent has the synthetic pyrethroid, permethrin, as its active constituent. The **cis:trans** isomers can be readily separated using the CN bonded phase technique.

Inverse Gas Chromatography Of Wool

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Inverse Gas Chromatography (IGC) is a method for examining the physical chemistry of surfaces. Unlike conventional gas chromatography, which usually involves the investigation of complex injected material on well characterised substrates, with IGC it is the column packing which is investigated by the injection and elution of (usually simple) interactive probe molecules.

A column was packed with wool and the C_1 to C_5 linear alcohols were injected as probe molecules. Changes in retention volume with water content and chemical pretreatment of the wool, and with temperature, provided information which lead to a better understanding of the wool surface.

Capillary Column Gas-Liquid Chromatography Of Wax Esters And Triglycerides

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Forest Research Institute, Rotorua

Columns packed with high-temperature-stable phases (Dexsil;Silar) have been previously used for gas liquid chromatography (glc) of waxes and triglycerides. Whereas these columns provide satisfactory resolution of simple homologous series, resolution of complex mixtures cannot readily be achieved. High elution temperatures are also required (e.g. C_{46} alkyl ester elutes at ca 330°C on Dexsil phase). Use of short (ca 5 m) silicone gum wall-coated open tubular capillary columns offer increased resolution over that of packed columns, and also show a useful temperature advantage (e.g. C_{46} alkyl ester elutes at ca 270°C), which can be important when attempting gas chromatography-mass spectrometry (gc-ms) studies owing to limitations on interface temperatures. Recent availabilty of persilylated wall-coated capillary columns which display both high resolution and thermal stability enable temperature programming to 350°C. Examples of glc and gc-ms analyses of some natural waxes and triglycerides will be illustrated, together with a discussion of capillary column injection techniques for these low-volatility compounds.

An Approach To Total Optimisation Of Gas Chromatographic Systems

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The "window analysis" approach to optimisation of mixed solvent selection in GLC, initiated by us in 1975, has since been extended to take in other important parameters such as temperature, carrier gas composition and, in HPLC, solvent composition, pH and substrate composition. The technique is now extended to the more general problem of solvent choice and loading and, finally, of analysis time. This permits identification of the maximum performance of any system and, thus, in principle, of the conditions for absolute optimisation of a given separation.

Examples, for both packed and open columns, are given and the agreement between calculated and experimental performance is shown to be excellent. The method thus allows, for the first time, unequivocal choice between solvents, or their mixtures, and between packed and open tube columns.

Vintage Amygdalin? — Its Pharmaceutical Assessment

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Chemistry Division, DSIR, Petone

Amygdalin (D-mandelonitrolo-β-D-glucosido-6-β-D-glucoside) has been promoted as a drug for use in cancer chemotherapy. It is presented as a solution for injection, a lyophilised powder for injection, or as tablets. Formulations have been examined by Chemistry Division as part of the MEDIQUAL programme of drug quality surveillance.

In aqueous solutions above pH7, amygdalin undergoes rapid epimerisation at the benzyl carbon atom to form a mixture of amygdalin and neoamygdalin. Hydrolysis of the nitrile group to form an amide or carboxylic acid occurs more slowly. In acid conditions, amygdalin degrades to benzaldehyde, free sugars and free cyanide.

An HPLC assay has been developed which rapidly separates amygdalin and neoamygdalin by using a 25cm Zorbax CN column and 2 percent aqueous acetic acid. The method was used to assess commercial amygdalin preparations.

The determination of optical rotation, regularly used by manufacturers to control neoamygdalin content, is insensitive to epimerisation and can be misleading.

Preparative-Analytical HPLC For The Separation Of Plant Waxes

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Forest Research Institute, Private Bag, Rotorua

Complex natural extracts such as plant waxes need to be separated into constituents classes before individual compound analysis is possible. Preparative-TLC is one standard method but is difficult to use quantitatively and for that reason has been replaced with an HPLC alternative.

Several methods and equipment combinations were evaluated based around separations on silica packings. Sample sizes varied from 1 to 50mg. Though simple and cheap, systems incorporating Sep-paks suffered from solvent leaks, contamination and physical deterioration, restricting their use. Alternative metal equivalents were more satisfactory. Although a mass detector was used principally, UV detection could also be used with the solvent systems finally chosen.

The routine method developed provided a quick and flexible separation of neutral plant wax constituents in micro- to milligram amounts prior to further GC or GC-MS analysis.

Separation Of Unsaturated Fatty Esters

Cecil B. Johnson
Applied Biochemistry Division, DSIR, Palmerston North

A method was developed for the separation of fatty esters according to the number of double bonds that they contain. This method is based on the reverse phase column chromatographic separation of mercuric acetate derivatives of the unsaturated esters.

Three modes of operation were developed:

1. The esters (in methanol) were injected into a methanol-water mixture on the top of the column. Saturateds were eluted with methanol and unsaturateds (as a group) with ethylene glycol dimethyl ether.
2. The esters were injected into a methanol-water-acetic acid mixture and the unsaturateds (followed by the saturateds) eluted, in turn, by a series of solvents containing increasing proportions of methanol.
3. Saturated esters were removed by mode 1, after which the column was washed with a methanol-water mixture followed by the solvents in mode 2.

This method has been used in the analysis of tallow and for the preparative isolation of polyunsaturated fatty esters from natural sources. The esters may also be separated on the basis of their chain lengths on the column.

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The Crystal Structure Of Trans-$[Co(en)_2(C_2O_4)(OH_2)](CF_3SO_3).2H_2O$ And The Reactions Of The Trans-$[Co(en)_2(C_2O_4/H)\ (OH/H)]^{0,+,2+}$ Species

D.A. Buckingham, C.R. Clark, G.M. Miskelly* and J. Simpson
Department of Chemistry, University of Otago

A crystal structure of **trans**-$[Co(en)_2(C_2O_4)(OH_2)]$ $(CF_3SO_3).2H_2O$ has been completed (monoclinic, $P2_1/c$, a=9.537, b=14.708, c=13.55.7 Å; β =102.9°) in order to confirm our previous stereochemical assignment[1]. The rates of reaction for the **trans** isomer are significantly slower than for the **cis** isomer, a difference noted also in the cyclisation of **cis** and **trans**-$[Co(en)_2(NH_2CH_2COO)(OH_2)]^{2+}$,[2,3]. However, the cyclisation of **trans**-$[Co(en)_2(C_2O_4)(OH_2)]^+$ proceeds via the **cis**-isomer, in contrast to the cyclisation of **trans**-$[Co(en)_2(NH_2CH_2COO)(OH_2)]^{2+}$ which, for ^{18}O-tracer experiments, has been shown to occur via direct substitution at the Co(III) centre.

The pK values and reaction rates for the **cis**- and **trans**-$[Co(en)_2(C_2O_4/H)(OH/H)]^{0,+,2+}$ will be compared and the differences discussed in terms of the stereochemical relationships of the H_2O (or OH-) and $C_2O_4^{2-}$ (or $HC_2O_4^-$) ligands in the two isomers.

References

1. G.M. Miskelly, Abstracts PII-15, COMO-10 Conference, Queenstown, New Zealand, May 1981.
2. C.J. Boreham, Ph.D. Thesis, The Australian National University, July 1978.
3. C.J. Boreham and D.A. Buckingham, *Inorg. Chem.* accepted for publication.

The Interaction Of Dioxygen With The Oxalyldihydrazide Complexes of Copper(II)

K.J. Oliver and T.N.M. Waters
Department of Chemistry, University of Auckland

The oxalyldihydrazide complex of copper(II) has long been used as an analytical reagent because of its very high extinction coefficient (ε 29 000 at 515 nm). It has been found that dioxygen is an essential constituent in the development of the colour leading to reports that the compound responsible was an oxygen carrier. Suggestions that the system was a model for some biological processes involving copper quickly follow.

The roles of dioxygen, base, and carbonyl-containing compounds in the chemistry of the reaction have been investigated and two stages leading to compounds showing intense blue and intense purple colours have been identified. X-ray structural, e.s.r. and other physical and chemical data will be presented.

The Addition Reactions Of Group IV B Hydrides (C, Si, Ge, Sn) To Cobalt Carbonyls

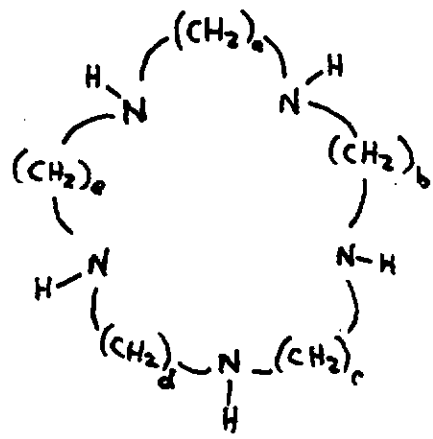
S.P. Foster (Student Paper Competition)
Department of Chemistry, Waikato University

Group IV B Metal-Cobalt Carbonyls have been limited to a maximum of 5 metal skeletal atoms. Recent work on the reactions of Group IV B hydrides (Si, Ge, Sn) with cobalt carbonyls have shown ways of extending this metal skeleton.

Metal Complexes Of Pentaazamacrocyclic Ligands

P. Osvath, N.F. Curtis, D.C. Weatherburn
Chemistry Department, Victoria University of Wellington

A wide variety of transition metal complexes with pentaazamacrocyclic ligands of ring size ranging from 15 to 20 have been prepared.



Spectral characteristics, stability and chemical properties of the complexes have been investigated, and will be presented.

Effect Of Aluminium Content On The Catalytic Activity Of ZSM-5

L.P. Aldridge*, D.M. Bibby, and N.B. Milestone
Chemistry Division, DSIR, Petone

ZSM-5 is the zeolite catalyst which will be used by Mobil to convert methanol to gasoline. The zeolite is synthesised in a hydrothermal system containing silica, alumina, a base and a "templating ion" such as a primary amine or tetra ethyl ammonium hydroxide or tetra propyl ammonium hydroxide.

It has been shown that the catalytic site in the zeolite is related to the hydrogen "cations" required to balance the negative charge resulting from the substitution of aluminium for silicon in the zeolite framework. The more alumina present in the zeolite the more potential catalytic sites are available.

We have investigated the catalytic activity of a series of ZSM-5 zeolites with varying aluminium composition. These zeolites had Al_2O_3 contents ranging from 0 to 4% and were prepared using 3 different "templating ions": (1) butyl amine, (2) tetra ethyl ammonium hydroxide (TEA) and (3) tetra propyl ammonium hydroxide (TPA). The zeolites were used to convert methanol to gasoline, the composition of which were monitored over a 24 hour period.

We have found that when the Al_2O_3 content of ZSM-5 falls below 0.05% the zeolite will not convert methanol to gasoline. As the Al_2O_3 content of ZSM-5 increases the composition of the gasoline produced changes markedly.

We have also found that ZSM-5 zeolites with the same Al_2O_3 content which were prepared using the three different "templating ions" have different catalytic properties in that the product compositions of the gasoline produced by these zeolites are different. We speculate that the distribution of the aluminium atoms within the zeolites and hence the distribution of catalytic sites is responsible for this effect.

Adsorption Of Alcohols From Aqueous Solutions Onto ZSM-5 Zeolites And Silicalite

N.B. Milestone, D.M. Bibby
Chemistry Division, DSIR, Petone

The synthesis of high silica zeolites has led to zeolites with some unusual properties. Among these are those of exceptional thermal stability and hydrophobocity. The hydrophobic properties are dependent on the aluminium content of the zeolite and give these zeolites the ability to absorb small organic molecules from dilute aqueous solutions.

Adsorption studies of water-soluble alcohols have been made using the zeolite ZSM-5 synthesized with a range of aluminium contents and the pure silica analogue silicalite. The amount of alcohol adsorbed depends on both the aluminium content of the zeolite and the alcohol being absorbed. Ethanol is more strongly adsorbed on ZSM-5 than silicalite but this order is reversed for the less water soluble butanol.

Electrochemical Synthesis Of Metal Thiocyanates And Related Complexes

Michael J. Taylor and Brian C. Dobson
Department of Chemistry, University of Auckland

Anodic oxidation of metals in the presence of suitable ligands can provide a simple preparative route to various complexes. The technique lends itself to convenient control of reaction conditions and allows such factors as the oxidation state of the metal and the stoichiometry of the product to be regulated.

In this work we are interested in the development and application of non-aqueous electrolyte systems. The preparation of thiocyanate complexes of various metals using a system based on thiocyanic acid in acetonitrile will be described, and other systems will be outlined. Structural methods, including IR, Raman and NMR spectroscopy, are employed to characterise the products.

A Scale Of Electronegativity, Based On Diatomic Molecular Force Constants

Derek W. Smith
University of Waikato

IN 1982 electronegativity, as a semi-quantitative concept, will celebrate its Golden Jubilee. Despite the advances in experimental and theoretical chemistry over 50 years, electronegativity remains alive and well: the concept is still fundamental to an orderly study of chemistry. However, the most popular scales in use up to the present time suffer from serious defects. Electronegativity is defined as a property of an atom in a molecule; only the Pauling scale (and then only partially) is based on molecular data.

This paper will describe how a scale of electronegativities can be established using the force constants of diatomic molecules. The resulting scale is in good agreement with existing ones, and is consistent with experimental molecular properties and chemical periodicity.

Mössbauer Spectroscopy Of Iron In Float Glass

I.W.M .Brown
Chemistry Division, DSIR, Petone

The use of Mössbauer spectroscopy to study the site symmetry and structural behaviour of iron in glass is now well established and has been applied to a number of glass-forming systems particularly in the field of silicate chemistry. While many studies have encompassed systematic studies of simple two or three component glass-forming systems there has been limited work on complex multicomponent glasses and glasses of major commercial significance. Further, there has been little or no use made of Mössbauer spectroscopy in the examination of crystallisation and devitrification phenomena in glass.

This paper examines devitrification behaviour in a commercial Float glass using iron (as Fe_2O_3) as a tracer for the devitrification

process. Mössbauer spectroscopy and X-ray diffraction techniques are used to study the inter-relation between glassy and crystalline phases formed under a range of firing conditions.

Kinetic Studies Of The Decomposition In Acid Solution Of Copper Complexes

Paul G. Graham, Peter Osvath and David C. Weatherburn*
Chemistry Department, Victoria University of Wellington

Over the past few years we have been studying the decomposition in acid solution of a series of copper(II) complexes with polyamine ligands. The ligands have included simple diamines, triamines, tetraamines and triaza-and pentaazamacrocycles. A wide range of kinetic behaviour has been observed, some reactions are independent of the acid concentration, other reactions display a first or second order dependence upon $[H^+]$ and other systems show more complex kinetic behaviour. Nevertheless all the data can be accommodated within a single mechanistic scheme and this scheme will be presented and discussed.

The Mechanism Of The Secondary Alkylation Of Coordinated Dinitrogen

Joseph Chatt, Wasif Hussain, G. Jeffery Leigh, Heinrick Neukomm, Christopher J. Pickett and Douglas A. Rankin*
ARC Unit of Nitrogen Fixation and School of Molecular Sciences, University of Sussex, Brighton, England BN19RO, and Wool Research Organisation of NZ, Lincoln

The monoalkylation of co-ordinated dinitrogen in complexes **trans** - $(M(N_2)_2(dxpe)_2)$ (M = Mo, W; dxpe = bis 1,2 (disubstituted phosphino) ethane) occurs via a reaction pathway whose rate determining step involves loss of one of the co-ordinated dinitrogen molecules. Some of the resulting monoalkylated diazenido (1-) products are known to undergo further alkylation. The mechanism of this secondary alkylation has been studied using cyclic voltametry and the results are reported in this paper. The reaction has a S_N2 mechanism. The rate of loss of co-ordinated dinitrogen in the monoalkylation reaction and the rate of secondary alkylation are sensitive to the metal and coligands. Factors which affect these reactions rates will be discussed.

Sulphur Compounds Formed In The Cement Kiln

R.A. Kennerley
Chemistry Division, DSIR, Petone

Sulphur present in the coal and the raw material react in the kiln to form mainly alkali and calcium sulphates and SO_2. Some alkali sulphate is volatilized and trapped by the precipitators but SO_2 escapes into the atmosphere. Alkali sulphates which remain and are incorporated in the cement dissolve in the concrete mixing water at a faster rate than sulphate present in the cement as added gypsum. Alkalies which are released rapidly during mixing influence the rate of setting and strength development of concrete. Standard specifications limit the total sulphate in cement; hence where appreciable alkali sulphate is present, the amount of gypsum which can be added to optimize setting and hardening is restricted.

Sulphur compounds also deposit in the kiln and create operating problems for cement manufacturers. The nature of the various compounds which form, including $(Ca_2SiO_4)_2.CaSO_4$ (Sulphospurrite) and $3CaO.3Al_2O_3.CaSO_4$ will be described and examples where the formation of these compounds have created difficulties in New Zealand will be cited.

The importance of relatively low ($\leq$ 2.5%) sulphur coals to the New Zealand cement industry will be stressed, and the world-wide interest in minimizing the sulphate and alkali contents of portland cement will be mentioned.

Novel And Unusual Compounds In The Vapours Above Molten Salts

H. Bloom
Chemistry Department, University of Tasmania

Vapour pressure measurements for molten salts and their mixtures gave results in the 1950's which could not be explained on the basis of simple unassociated vapour species. In explanation, many complex compounds were postulated, such as Li_3Cl_3 above molten lithium chloride and $CsPbCl_3$ above mixtures of lead chloride and caesium chloride.

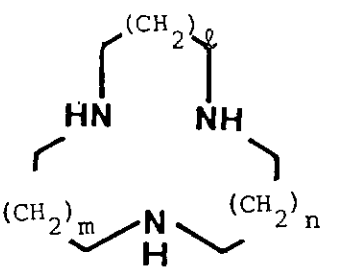
More recently, mass spectrometry of vapours emerging from Knudsen cells and Raman spectroscopic measurements on salt vapours have established a large number of unusual compounds. Some examples are, PbClBr, $NdAl_4Cl_{15}$, $UAlCl_8$, $KPbBr_3$, $Cu_4Br_2Cl_2$, $LiCu_2Cl_3$,$NaAg_2Br_3$ and many other gaseous species including trimers, tetramers and pentamers of the halides of Groups 1A and 1B.

In this lecture the experimental techniques, results and attempts to explain these unusual compounds, will be presented.

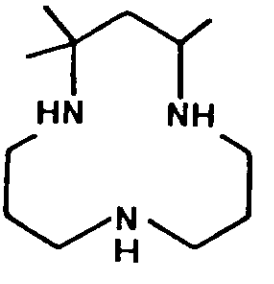
Preparation And Kinetic Studies Of Triazamacrocyclic Copper(II) And Nickel(II) Complexes

P.G. Graham* and D.C. Weatherburn
Department of Chemistry, Victoria University of Wellington

Copper(II) and nickel(II) complexes of triazamacrocyclic ligands have been prepared with a variety of ring sizes and with differing arrangements of methyl substituents.



ℓ,m,n = 0-4



$2,2,4\text{-}Me_3[12]ane\text{-}1,5,9\text{-}N_3$

The nickel(II) complexes are either five-coordinate in aqueous solution, as for example, 2,2,4-Me_3[12]ane-1,5,9-N_3-nickel(II), or six-coordinate, such as [12]ane-1,5,9-N_3-nickel(II). The properties of a number of these complexes will be discussed.

The acid-decomposition reactions of the copper(II) and nickel(II) complexes have been studied, and show large differences in the rates and activation parameters for each metal ion. Complexes of ligands with larger ring sizes (12-membered and higher) tend to react at a rate independent of acid concentration with increasing acid concentrations. The effect of methyl substituents on the decomposition reaction has also been studied, and the results of these kinetic investigations will be reported.

Formation And Thermal Stability Of Low Al Content ZSM-5 Zeolites Prepared From Amine Containing Systems

D.M. Bibby, N.B. Milestone, L.P. Aldridge
Chemistry Division, DSIR, Petone

ZSM-5 zeolites are synthesised in hydrothermal systems typically containing organic bases such as tetraalkyl ammonium hydroxide or primary amines at temperatures around 150°C.

ZSM-5 can be synthesised with Al content ranging from about 3 percent (Si/Al ratio c20) down to the pure silica end-member known as silicalite. The whole range of Al containing ZSM-5 zeolites can be synthesised using tetra-alkyl ammonium hydroxide containing systems, but we have been unable to prepare ZSM-5 with Si/Al ratios greater than about 200 when using amine containing systems. In amine containing systems where the starting composition has a Si/Al ratio greater than 200 the excess silica form separate phases such as cristobalite (SiO_2) or kenyaite ($NaSi_{11}O_{20.5}(OH)_4.3H_2O$).

ZSM-5 zeolites with Si/Al ratios between 100 and 200 prepared from amine containing systems have been found to be relatively thermally unstable. The ratio of decomposition decreases as the Si/Al ratio falls, and at Si/Al ratios below 100, which corresponds to more than 1 Al atom per unit cell, ZSM-5 appears

to be stable indefinitely. In comparison ZSM-5 zeolites with Si/Al ratios in the range 100 to 200 which have been prepared from tetraalkyl ammonium hydroxide containing systems are stable to temperatures in excess of 1000°C.

Thermal Decomposition Of Some Ni II Thiourea Chloride Complexes

Martin P.B. Attard and John O. Hill*
Department of Inorganic and Analytical Chemistry, La Trobe University, Bundoora, Victoria 3083, Australia

Programmed Probe Analysis (PPA)[1] has been employed to identify the decomposition products and to derive the thermal decomposition mechanism of a series of nickel II thiourea chloride complexes of stoichiometry $Ni(tu)_4Cl_2$. Two types of two-step mechanisms are evident: for (tu) = thiourea, methylthiourea, HCl is lost in both decomposition steps whereas for (tu) = dimethylthiourea, dibutylthiourea, ethylthiourea, allylthiourea and naphthylthiourea, HCl is lost only during the second stage of decomposition. The proposed thermal decomposition mechanisms are rationalised in terms of the inductive effect of the R group(s) of the thiourea ligand.

1. J.F. Smith, *Int. J. Mass Spectrom. Ion Physics, 26,* 149 (1978).

Metal Ion : Amino Acid Ester : 1,2-Diamine = 1:1:1 Complexes. Stability Constants And Rates Of Alkaline Hydrolysis Of The Ester Function

K.I.G. Wilson and P.J. Morris*
Chemistry Department, University of Waikato

Stability constants have been measured, using SCOGS, for various $ME(1,2\text{-amine})^{2+}$ complexes, where M = Cu^{2+}, Ni^{2+}; E = 2,3-diaminopropanoic acid methyl ester, 4,5-diaminopentanoic acid methyl ester, histidine methyl ester; 1,2-diamine = 1,2-diaminoethane, 1,2-diaminocyclohexane. There is generally no enhancement of the stability of the 1:1:1 complexes (often seen in other mixed ligand systems) as compared to the 1:2 complexes. Consequently 1:1:1 mole ratio solutions contain about 66% $ME(1,2\text{-diamine})^{2+}$ and 17% each of ME_2^{2+} and $M(1,2\text{-diamine})_2^{2+}$.

Kinetic studies on the alkaline hydrolysis of the ester function at various M^{2+}:E:1,2-diamine mole ratios, will be reported. The kinetic complications associated with disproportionation in these labile systems will be discussed. The effects of systematic changes in the structure of the ester and the 1,2-diamine, on the rate constant for ester hydrolysis will be examined.

The Chemistry, Structures, Spectroscopy And Bonding In Mixed-Valence Complexes

R.J.H. Clark
Department of Chemistry, University College, London

A survey of synthetic routes to linear-chain mixed-valence complexes of platinum and palladium, for example, of the types $[M^{II}(LL)_2][M^{IV}(LL)_2X_2]Y_4$, where LL = a bidentate aliphatic amine, X = Cl, Br or I, and Y = Cl,Br,I,BF_4^-,ClO_4^- etc., will be presented. Such complexes are of considerable interest as one-dimensional materials since their electrical conductance is highly pressure dependent (by as much as 10^9 on their being compressed to 140 kbar) and intermediate between that of insulators such as K_2PtCl_4 ($\sigma_{11} < 10^{-8}\,\Omega^{-1}cm^{-1}$) and "metals" such as KCP, $K_2Pt(CN)_4Br_{0.30}.3H_2O$ $\sigma_{11} \approx 10^2\,\Omega^{-1}cm^{-1}$). The key structural features of the complexes will be discussed with reference to recent X-ray analyses on $Cs_2[Pt^{II}(NO_2)(NH_3)X_2][Pt^{IV}(NO_2)(NH_3)X_4]$, X = Cl or Br, and $[Pt^{II}(NH_3)_4][Pt^{IV}(NH_3)_4Br_2](HSO_4)_4$.

The electronic, Raman and resonance Raman spectra of these complexes will be presented, together with polarization data. The resonance Raman spectra yield long overtone progressions in the X-Pt^{IV}-X symmetric chain stretching mode, providing information on harmonic frequencies and anharmonicities as well as on the nature of the geometric change in the molecules on excitation from the ground to the intervalence state. This change will be related to the one-dimensionality of the electrical conductance of the complexes. The nature of the bonding in, and spectroscopy of, these class II mixed-valence complexes will be contrasted with that in class III (i.e. delocalised) complexes, such as ruthenium red, $[Ru_3O_2(NH_3)_{14}]^{6+}$.

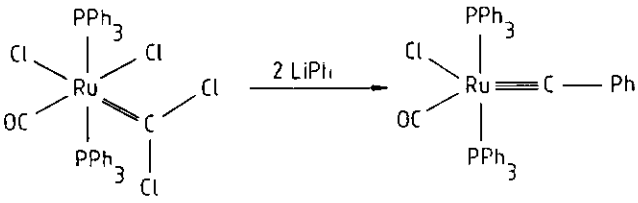
Metal-Carbon Multiple Bonds

A.H. Wright (Student Paper Competition)
Department of Chemistry, University of Auckland

Much of the recent research in organometallic chemistry has concentrated on multiple metal-carbon bonds. While of obvious intrinsic interest, unsaturated metal-carbon bonds are also frequently proposed as intermediates in industrial catalytic processes.

Research in our laboratory has recently concentrated on a particularly reactive type of carbene complex (containing a metal-carbon double bond). These dihalocarbene complexes, such as $RuCl_2(CCl_2)(CO)(PPh_3)_2$, react with many nucleophiles to give products derived from reactions of the co-ordinated ligand.

In particular, reactions with carbon nucleophiles have led to a series of carbyne complexes (containing metal-carbon triple bonds).



Further studies of these species is currently under way.

The Energy Of d Electrons From Ferrous Ions In Minerals

L.P. Aldridge*, G.M. Bancroft and J.S. Tse
Chemistry Division, DSIR, Petone, and Chemistry Department, University of Western Ontario, London, Ontario, Canada

We have calculated the energies of d electrons in ferrous cations using the extended Huckel Method (with self consistent charge) from the positions of the Fe^{2+} cation and the nearest neighbour oxygen anions. The energies of the d electrons in Fe^{2+} were calculated within gillespite, (which has 4 oxygen atoms surrounding the Fe^{2+} in a square planar environment) garnet (where the 8 oxygen atoms are situated at the corners of a cube) and the olivine M1 and M2 sites (with 6 oxygens arranged in a distorted octahedron). These energies were compared to the observed energies derived from adsorption or Mossbauer spectra and there was a linear relationship between the observed and calculated energies. (The correlation coefficient was 0.98)

$E_{calc} = 1.57\ E_{obs} - 537\ (cm^{-1})$

The Mechanisms Of Induced Aquation And Base Hydrolysis Of Cobalt(III) Complexes. Do Intermediates Of Reduced Coordination Number Exist?

D.A. Buckingham
Department of Chemistry, University of Otago

The mechanisms of substitution in inorganic complexes and the question of common intermediates of reduced coordination number and their lifetimes has interested inorganic chemists for the past 40 years. Today we still do not have a complete understanding of these processes.

For acido-cobalt(III) species induced aquation (Hg^{2+},NO+, Cl_2, HOCl, OH-) are particularly facile, and intermediates of reduced coordination number are believed to exist in some cases (D mechanism). The I_d proposal, with the intermediate reacting with the environment provided by its solvation shell, has received

increasing support and it now appears that in aqueous solution the lifetime of this intermediate is so short that it cannot control the speed, or direct the position of entry, of the incoming group. These matters are decided by events occurring before the group being replaced has left the metal.

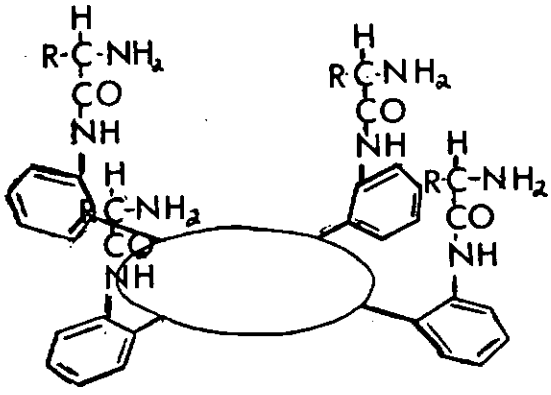
We now have good evidence for entry of groups from the intermediate solvation sheath, and evidence that the intermediate, if it exists at all, has a lifetime so short that metal-ligand bonds do not have sufficient time to readjust to a new environment.

This chemistry will be discussed in terms of the induced aquation and base hydrolysis reactions of **cis**-$[Co(en)_2(glyO)X]^+$ (X = Cl, Br), t-$[Co(tren)NH_3X]^{2+}$ (X = Cl, NCS^- $MeSO_3^-$ Me_2SO, N_3^-), **anti**-p, **syn**-p and t-$[Co(Metren)NH_3X]^{2+}$ (X = Cl, Br, N_3) and the parent $[Co(NH_3)_5X]^{2+}$ (X = Cl, N_3) complexes.

Picket Fence Porphyrins Containing Appended Metal Binding Groups

D.A. Buckingham, C.R. Clark and W.S. Webley*
Chemistry Department, University of Otago

Differences in the lability of the two metal ion binding sites in amino acid appended (glycine, alanine, phenylalanine) picket fence porphyrins (fig.) allow the formation of heterobinuclear complexes, $[M_1(N_4)\text{-}(P)M_2]^{n+}$, with inter-metal distances of ca 5 Å. The Cu-Fe porphyrin holds promise as a model for the active site of cytochrome oxidase and e.s.r., electrochemistry and catalytic activity (dioxygen reduction) parameters for this system are reported. Metal insertion into the free base forms of the porphyrin ligands occurs via rapid complexation of M^{2+} by the



N_4 site and its subsequent rate limiting intramolecular transfer into the porphyrin nucleus. These metallation reactions are the fastest yet observed for porphyrins and appear to have some relevance to the enzymic metal insertions of protoporphyrin IX.

A strategy to create a molecular binding site above the plane of iron, manganese and ruthenium porphyrins is currently being developed with a view toward exploring the hydroxylase activity of these species toward appropriate occluded substrates. Synthetic approaches to these systems are also discussed.

Mass Spectrometry

Mass-Spectrometry Of Sporidesmin Metabolites

P.T. Holland and R. Fairclough
Ruakura Agricultural Research Centre, MAF, Hamilton

Microsomal degradation of the facial eczema toxin sporidesmin has been studied by chromatographic and mass-spectrometric techniques. A number of lipophillic metabolites have been characterised by high resolution mass-spectrometry and detailed analysis of their fragmentation patterns. Loss of the disulphide bridge is accompanied by hydroxylations.

Investigation Of Long-Chain Alkyl Esters And Macrocyclic Lactones By EI And CI Mass Spectrometry

R.A. Franich
Forest Research Institute, Rotorua

The structures of some natural long-chain alkyl esters and simple macrocyclic lactones have been investigated using direct inlet- and gas chromatography- mass spectrometry in both electron impact and chemical ionisation modes.

The Attachment Of Low Energy Electrons To Dicyanogen In The Gas Phase

Bruce J. McIntosh* and Peter W. Harland (Student Paper Competition)
Chemistry Department, University of Canterbury

The study of negative ion formation by low energy electron impact under single collision conditions in the gas phase leads directly to the evaluation of thermochemical parameters for free-radicals, ions and molecules which would be difficult to obtain by any other means. The major problems associated with negative ion studies are the manipulation of near thermal energy electron beams, low capture cross-sections, difficulties resulting from a finite electron energy distribution and the necessity to measure the translational energy of the ions formed. A mass spectrometer with a specially designed ion source will be described and data for dicyanogen, C_2N_2, and related small molecules, will be presented.

Flowing Afterglow Studies Of Ion-Molecule Reactions Of Phosphine

C.G. Freeman, P.W. Harland, M.J. McEwan and L.M. Parker
Department of Chemistry, University of Canterbury

A series of gas phase ion-molecule reactions involving phosphine (PH_3) have been studied in a flowing afterglow system incorporating a quadrupole mass filter. Reactions involving both phosphine as a neutral reactant

$$X^+ + PH_3 \longrightarrow \text{Products}$$

and positive ions generated from phosphine

$$PH_n^+ + R \longrightarrow \text{Products}$$

(n = 0, 3 or 4)

have been investigated. Rate coefficients are reported and compared with collision rates calculated from classical models.

Ion-molecule processes involving phosphine are of importance in the chemistry of interstellar dust clouds and are possibly relevant to the chemistry of the atmosphere of the planet Jupiter.

Defensive Secretion Of NZ Tenebrionid Beetles — The Identification Of 4-Methylhex-1-en-3-one In *Amarygmus tristis*.

C. Gnanasunderam, R. Hutchin and H. Young
Entomology and Horticulture and Processing Divisions, Mt Albert Research Centre, DSIR, Auckland

In addition to the usual quinones and hydrocarbons found in the defensive secretions of this type of beetle two branch chain ketones have been identified in the secretion from *Amarygmus tristis*. The structures of the ketones have been shown by GC/MS to be 4-methylhex-1-en-3-one and its saturated analogue. This is the first report of the isolation of the unsaturated ketone from natural sources.

Organic

Aspects Of The Chemistry Of Phosphoranes

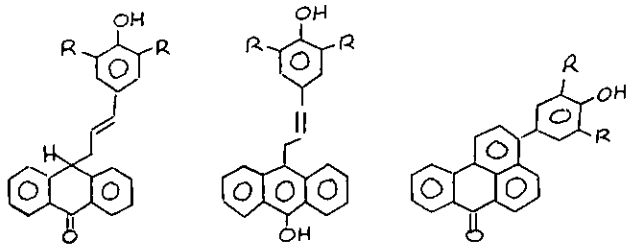
J.I.G. Cadogan FRS
BP Research Centre, Sunbury-on-Thames, England
(Review Lecture)

Synthesis and reactions of novel phosphoranes (5-coordinate organophosphorus compounds) will be discussed with respect to their hitherto undetected presence as intermediates in organophosphorus reactions. Phosphoranes are also precursors in the formation of reactive intermediates and non-phosphorus heterocycles.

Anthraquinone Pulping — A Mechanism For Benzanthrone Formation

Jacqueline A. Hemmingson
Chemistry Division, DSIR, Petone
(Poster Session)

Vinyl-substituted quinone methides such as 4-allylidene-2, 6-dimethylcyclohexa-2, 5-dienone react with anthrone in basic solution to give an isomeric mixture of (I) and (II). Both isomers, when refluxed in base, cyclise and dehydrogenate to give the benzanthrone (III). Each isomer, when the double bond of the cinnamyl substituent is hydrogenated is oxidised by $K_3Fe(CN)_6$ to a methylene cyclohexadienone. These quinone methides, when refluxed in base, also give benzanthrone (III). A mechanism for benzanthrone formation involving this type of quinone methide as an intermediate is discussed.

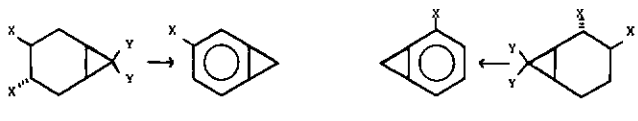


I II III

Aromatization Routes To Cycloproparenes

Brian Halton*, and Clifford J. Randall
Department of Chemistry, Victoria University of Wellington

The dehydrohalogenation of 7,7-dihalobicyclo[4,1,0]hept-3-enes has provided a viable route to a number of cycloproparenes[1]. Recent studies have shown that the Δ^3-olefinic linkage of such compounds can be replaced by halogen substituents at the 3- and 4-positions[2]. Thus compounds (1a) and (1b) undergo base-induced tris-elimination to the cycloproparenes (2a) and (2b) respectively. The mechanistic details of these reactions based on studies employing the 'mixed' tetrahalo compound (1c) will be discussed. In addition, the results of studies aimed at the synthesis of 2-halocyclopropabenzenes (4), ideal precursors for a single dehydrocyclopropabenzene product, will be presented.



(1) (2) (4) (3)

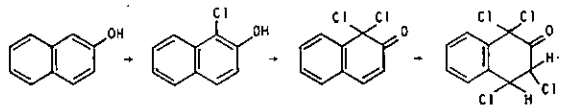
a) X = Y = Br b) X = Y = Cl c) X = Br; Y = Cl

1. B. Halton, *Ind. Eng. Chem. Prod Res. Dev., 19,* 349 and references cited therein (1980).
2. P.J. Garratt, and W. Koller, *Tetrahedron Lett., 4177* (1976); W.E. Billups, W.T. Chamberlain, and M.Y. Asim, *ibid., 571,* (1977).

Stereochemistry Of The Formation Of 1,1,3,4-Tetrachloro-2-Ketotetralin By Chlorination of 2-Naphthol

Judith M. Brittain, David J. Calvert, Peter B.D. de la Mare, Paul A. Newman, and Hitomi Suzuki*
*Chemistry Department, University of Auckland, Private Bag, Auckland, New Zealand, and *Chemistry Department, Ehime University, Matsuyama 790, Japan.*

The reaction of 2-naphthol with molecular chlorine in acetic acid gives in successive stages 1-chloro-2-naphthol, 1,1-dichloro-2-keto-1,2-dihydronaphthalene, and 1,1,3,4-tetrachloro-2-ketotetralin.

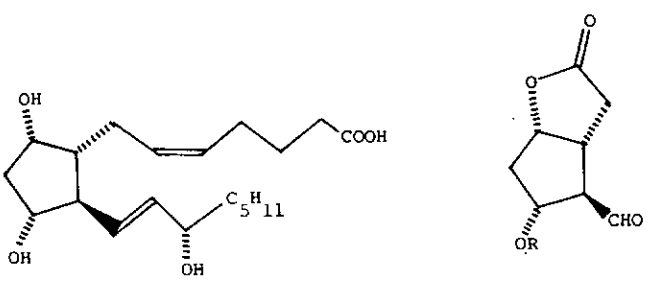


The alicyclic ring in the ketotetralin would be expected to be flexible and the value of the vicinal ($^3J_{H,H}$) coupling (4.8 Hz) does not distinguish satisfactorily between a *cis*- and a *trans*-configuration for this compound. When the high-resolution ^{13}C spectrum is considered also, however, the results are more satisfactorily explained by attribution of the compound as the *cis*-isomer. The argument supporting this assignment will be presented and the mechanistic significance will be discussed briefly.

The Use Of Carbohydrates As Chiral Precursors To Prostaglandins

Stephen R. Haines (Student Paper Competition)
Department of Chemistry, Victoria University of Wellington

Prostaglandins are a class of naturally occurring compounds containing several chiral centres. The structural features typical of the family are displayed by prostaglandin $F_{2\alpha}$ (1). The implication of these compounds as local hormones in important physiological processes, coupled with an extremely high potency, has made prostaglandins the subject of intense research activity in recent times. Much of the work has involved new synthetic strategies to provide intermediates, e.g. lactone (2), capable of conversion into a variety of prostaglandins. Novel approaches to such intermediates using carbohydrates as chiral starting materials will be discussed.



(1) (2)

Stereospecificity In Dehydration Reactions

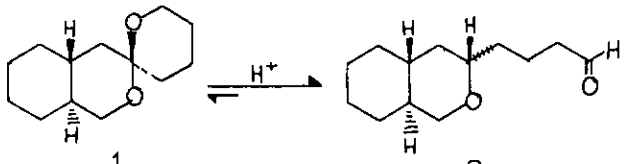
J.M. Coxon and J.R. Gibson
Chemistry Department, University of Canterbury

The results of our recent studies on the dehydration of decalols will be reported. Dehydration in these systems can occur with or without rearrangement and the stereospecificity of the reactions has been probed by deuterium labelling experiments. A detailed mechanism for the reactions is proposed.

Stereoelectronic Control In Hydride Transfer To Cyclic Oxenium Ions

Daryl Rowan* and Pierre Deslongchamps
*Applied Biochemistry Division, DSIR, Palmerston North, and Departement de Chimie, Universite de Sherbrooke, Sherbrooke, Quebec, Canada

In the course of a study of stereoelectronic effects (anomeric and exo-anomeric effects) in acetal systems such as (1), we observed an acid catalysed interconversion of the acetal function to the corresponding aldehyde-ether (2). The stereospecific formation of (2) either by intra or intermolecular hydride transfer (pH=4, $NaBH_3CN$, reduction to the ether-alcohol) can be explained by stereoelectronic control of the hydride transfer. This must occur anti to the "developing" oxygen lone pair and an axial hydride transfer allows a preferable chair conformation of the initial reaction product. These results provide further evidence for stereoelectronic control of hydrolysis reactions (P. Deslongchamps. *Tet., 31*, 2463, (1975))



1 2

Aromatic Nitrations With A Difference

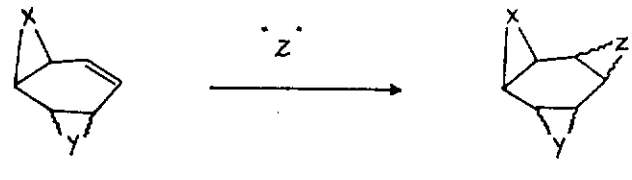
K.E. Richards
Department of Chemistry, University of Canterbury, Christchurch

This review lecture will cover recent developments in the formation and reactions of *ipso*-nitration products including recent work carried out at the University of Canterbury.

New Methods For the Synthesis Of *Bis-* And *Tris-*σ-Homobenzenes

Martin G. Banwell
Dept. of Organic Chemistry, University of Adelaide, Adelaide, South Australia

The *tris*-σ-homobenzenes of general structure (2,X,Y,Z = NR, O or CR^1R^2) their precursors the *bis*-σ-homobenzenes (1,X,Y = NR, O or CR^1R^2) and a variety of closely related compounds are of considerable synthetic, mechanistic, theoretical and biological importance. In view of their diverse chemical roles there is a continuing need for new and versatile synthetic approaches to these types of compounds.



(1) (2)

The present paper will describe strategies which appear to offer means by which a wide variety of substituents can be introduced at all positions within the molecular frameworks of (1) and (2) with a high degree of regio- and stereochemical control.

A New Way Of Forming Lignin — Carbohydrate Bonds

G.J. Leary
Chemistry Division, DSIR, Petone

Model lignin p-hydroxybenzyl alcohols in dilute aqueous solution are smoothly etherified by sugars to give benzyl sugar ethers. The mechanism of formation and the structures of these model lignin-carbohydrate compounds suggest that analagous lignin-carbohydrate benzyl ether bonds will form in lignified plants. Evidence for the occurrence of these in wood will be discussed.

Diazonium Coupling To Guaiacol And Veratrole And The NMR Of The Azophenols Produced

J.W. Ronaldson
Ruakura Agricultural Research Centre

The coupling in acid of nitrobenzenediazonium salts to guaiacol is straight forward. Only 2,4-dinitrobenzenediazonium salts in acid couple to veratrole. The nmr of each product showed the expected spectrum except that for 4'-hyroxy-3'-methoxy-2,4-dinitroazobenzene. For this compound in acetone-d6 there were the expected 16 lines but in chloroform-d there were 26 or more lines. This anomalous spectrum is not explained by isomerisation to a quinone hydrazone structure.

In those cases where there was no coupling to veratrole, on raising the pH to c. 4.5 a Gomberg reaction occurred, producing the corresponding biphenyls.

The Structure Of Agar From *Gracilaria secundata*

Donald J. Brasch*, Chaw T. Chuah and Laurence D. Melton
Departments of Chemistry and Human Nutrition, University of Otago

Aqueous extraction of the New Zealand red seaweed, *Gracilaria secundata* gave a low sulphated agar polysaccharide which gelled strongly. The structure of the agar has been investigated by methylation analysis, controlled degradations of the permethylated agar, enzyme analysis, ^{13}C—nmr spectroscopy. It is concluded that the agar is mainly composed of the familiar structure of (1-3) and (1-4) linked galactose residues, but an important variation occurred in that there are some adjacent 3,6-anhydro-L-galactose residues. The distribution of the 6-O-methyl groups was thought to be in 'blocks'.

The Galactan Sulphate From The Edible Red Seaweed, *Porphyra columbina*

Donald J. Brasch, Hi Mui Chang, Chaw T. Chuah and Laurence D. Melton*
Departments of Chemistry and Human Nutrition, University of Otago

A galactan sulphate has been isolated from the edible New Zealand seaweed *Porphyra columbina* and its structure established by a combination of methylation, methanolysis, treatment with alkali followed by methylation, and ^{13}C-nmr. The polysaccharide belongs to the porphyran class, and consists of 3-linked β-D-galactose residues and 4-linked α-L-galactose residues. The 3,6-anhydro-L-galactose and L-galactose-6-sulphate total approximately half of the sugar units, the other half being made up of D-galactose and 6-O-methyl-D-galactose. Some evidence is presented which suggests the galactan sulphate does not have a completely alternating structure.

New Metabolites From *Plocamium cartilagineum*

J.W. Blunt*, M.H.G. Munro, Yeow Kok Kon
Department of Chemistry, University of Canterbury, Christchurch

Eight new monoterpenoid metabolites have been isolated from the marine organism *P. cartilagineum*, and their structures determined through the combined use of ^1H and ^{13}C nmr spectroscopy, MS and GCMS techniques. A feature of this work has been the manner in which this combination of techniques has been applied to the solution of structures where the amount of each metabolite available for examination was only about 10mg.

Organophosphorus Analogues Of The Antitumour Agent N-[4'-(9-acridinylamino)-3'-methoxyphenyl] methanesulphonamide (m-AMSA)

G.W. Rewcastle*, B.C. Baguley and B.F. Cain**
Cancer Research Laboratory, Auckland University School of Medicine.

Replacement of the methanesulphonamide group in m-AMSA with a number of phosphoramide and related substituents gave a series of compounds whose antitumour activity and DNA binding characteristics varied over a wide range.

The compounds were synthesised by mild acid-catalysed coupling of 9-chloroacridine with the requisite substituted aniline derivatives, with these latter species being prepared by reduction of the analogous nitro or benzylurethane precursors. Several different methods were used for the initial incorporation of phosphorus from the inorganic substrates, PCl_5, PCl_3, and $POCl_3$.

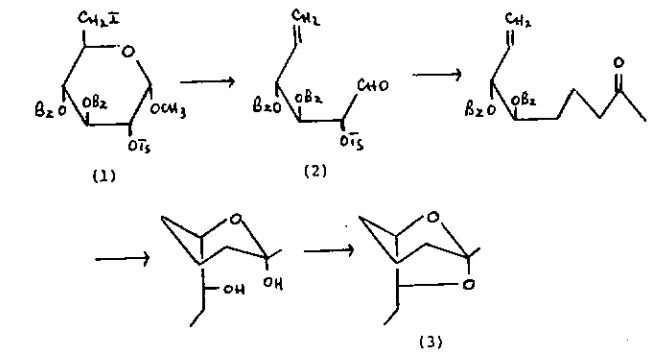
For increased biological activity a marked preference was noted for oxygen containing substituents on the phosphorus atom, with the most active congener displaying activity comparable to that of the clinically utilized agent m-AMSA.

** Deceased January 1981.

Synthesis Of The Insect Pheromone (1R)-exo-Brevicomin From Glucose

R.J. Ferrier and P. Prasit
Chemistry Department, Victoria University of Wellington

The title compound (3), which is the sex attractant of the female Western pine beetle *(Dendroctonus brevicomis)*, has been synthesised from the readily available D-glucose derivative (1) by way of the unsaturated aldehyde (2) as outlined. This last compound also affords access to functionalised cyclopentanes e.g. (4) and (5) from which prostaglandin intermediates can be produced.



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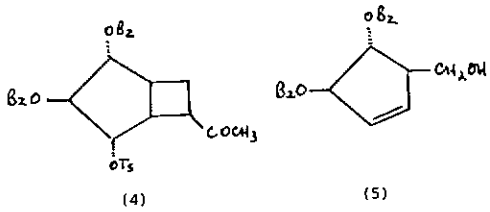


(4) (5)

Medicinal Plants Of Fiji And South Pacific Islands

Ajit Singh
University of the South Pacific, Suva, Fiji

Plants of Pacific Islands, particularly Fiji have been surveyed. Alkaloid and antimicrobial activities of several have been assayed and reported. The reported folklore medicinal uses and parallel identified alkaloid constituent is noted.

^{13}C-NMR Spectroscopic Analysis Of Seaweed Polysaccharides: Porphyran

Richard H. Furneaux*, Ian J. Miller, and Herbert Wong
Chemistry Division, DSIR, Petone

Porphyran, the water soluble galactan obtained from members of the *Porphyra* family of red seaweeds, has an agar-type structure (alternate 1,3-linked-D-and 1,4-linked-3,6-anhydro-L-galactopyranosyl units), modified by (i) partial 6-O-methylation of the D-galactose units, and (ii) partial replacement of the 3,6-anhydro-bridged units with their supposed biosynthetic precursor, i.e. L-galactose 6-sulphate. Working with the common New Zealand alga, *Porphyra columbina*, we have been able to demonstrate that its water extractable polysaccharide is a block polymer, comprising unsubstituted agar-type regions and sulphated oligosaccharide regions. The techniques of selective hydrolysis, dialysis and ^{13}C-NMR spectroscopy will be described.

Acid-Catalysed Cyclisation Of Some Methoxyphenylethyltrimethylcyclohexanols

B.W. Axon, B.R. Davis*, M.G. Hinds, S.J. Johnson and P.D. Woodgate
Department of Chemistry, University of Auckland, Auckland

The acid-catalysed reactions of two p-methoxyphenylethyl-trimethylcyclohexanols, precursors to tricyclic diterpenoids, were investigated. Treatment with boron trifluoride — ether, or tin(IV) chloride, or methanesulphonic acid — phosphorus(V) oxide produces both a bicyclic alkene and a tricyclic molecule, with the former being converted to the latter in protic media. Conditions were established for the formation of the naturally occurring **trans** A/B ring stereochemistry and the mechanism of cyclisation explored with the aid of deuteriated substrates. Polyphosphoric acid-catalysed cyclisation produces a more complex mixture. Extension of this work to the m-methoxy phenylethyltrimethylcyclohexanols and related systems will be discussed.

Total Synthesis Of Deoxyribonucleic Acid Fragments: The Preparation Of Four Decadeoxyribonucleotides

William A. Denny*# and Werner Leupin#
Cancer Chemotherapy Research Laboratory, University of Auckland School of Medicine (*) and Dept. of Chemistry, University of California at San Diego, La Jolla, California (#)

For self-complementary decadeoxyribonucleotides, d-ApTpApTpApTpApTpApT, d-ApTpApTpGpCpApTpApT, d-ApTpApTpCpGpApTpApT and ApApApApApTpTpTpTpT were chemically synthesized from commercially available 2'-deoxy-ribonucleosides by the phosphotriester method, using liquid-phase procedures. Efforts were concentrated on keeping the experimental techniques as simple as possible, concomitant with obtaining high-purity products at each step. The molecules were elaborated from the 5' end of an initial 3'-O-benzoylated monomer according to the scheme: monomer + monomer → dimer + dimer → tetramer + dimer → hexamer + tetramer → decamer. Yields in the coupling steps averaged 70%. Removal of the blocking groups involved a three-step procedure, followed by purification of the product by ion-exchange chromatography, with an overall efficiency of about 60-65%.

These procedures allowed the preparation of approximately 0.1 milliMole (300mg) of each decamer, in an overall yield from the 2'-deoxyribonucleosides of about 10%, and occupied four man-months. Such simplified procedures for the preparation of large amounts of defined-sequence DNA oligonucleotides of high purity make these molecules relatively accessible for a variety of different studies.

Incomplete Acetylation In The Methylation Analysis Of Polysaccharides

I.G. Andrew and D.R. Fenemor
Department of Chemistry, Biochemistry and Biophysics, Massey University

During a study on *Pinus radiata* cell wall structure, the

polysaccharide composition of fractions was explored by methylation analysis, with combined gas chromatography — mass spectrometry (GC/MS). In this technique, the partially methylated sugars, derived from hydrolysis of permethylated polysaccharides, were reduced to alditols and acetylated with acetic anhydride plus pyridine, yielding suitable volatile derivatives for GC/MS.

When pyridine was omitted from the acetylation mixture, a practice used by some workers, it was noted that additional peaks sometimes appeared on gas chromatograms. These were observed when glass columns were used but not with a steel column. Analysis of these peaks by mass spectrometry suggested that they were due to incompletely acetylated derivatives bearing free hydroxyl groups. When a mixture containing such peaks was reacetylated with pyridine as catalyst, these peaks were shown to be absent from subsequent chromatograms and there was a corresponding increase in size of certain of the peaks for fully acetylated derivatives.

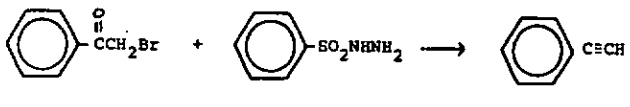
The nature of several of the underacetylated derivatives was investigated and it appeared that underacetylation was most frequent in those derivatives carrying one or two hydroxyl groups between two methoxyl groups. That steric hindrance could play a role in the observed phenomena was supported by the observation that complete acetylation of a branched-chain alditol, apiitol, is very slow, even in the presence of pyridine.

The assistance of Dr K. Couchman with mass spectrometry and Professor R. Hodges with interpretation of mass spectra is gratefully acknowledged.

Alkynes From α-Substituted Ketones And Aryl Sulphonyl Hydrazines

Michael W. Bryant, Robin A.J. Smith and Lisa Wong
Department of Chemistry, University of Otago
(Poster Session)

The reaction of $ArSO_2NHNH_2$ with certain -substituted ketones produces alkynes in reasonable yields e.g.:



The scope and limitations of this reaction have been examined and the results of varying the reactants and the reaction conditions will be reported. Significant acid catalysis is observed with a variety of catalysts (HBr, Nafion-H, $BF_3.Et_2O$, $SnCl_4$) under specific conditions.

Experiments with preformed hydrazones have shown they are not intermediates in the reaction. Possible mechanistic schemes will be presented.

Homolytic Reactions Of Polyhalogeno-Organic Species

Roger Bolton, E. Philip Mitchell, Jillian M. Seabrooke, and Gareth H. Williams
Chemistry Department, Bedford College (University of London), London NW1 4NS, England

The use of polyfluoroaliphatic and polyfluoroaromatic liquids (e.g. C_6F_6, $C_6F_5.CF_3$) as heat-transfer liquids is determined by their thermal stability. In the absence of heterolytic reagents, this is conditioned by their susceptibility to homolysis and to subsequent attack by the polyhalogeno-organic free radicals so generated (eqn. (1)):

$$R_f F \xrightarrow{\text{abstraction}} R_f^{\cdot}; \quad R_f^{\cdot} \xrightarrow{R_f^{\cdot}} \text{Polymers (1)}$$

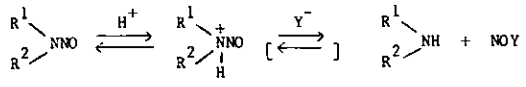
In assessing new possible heat-transfer fluids, two aspects have been considered. The first measures the relative ease of abstraction of halogen from saturated systems by the pentafluorophenyl radical; this reaction parallels the necessary initiation process, and the polarity of the attacking radical is likely to be similar to that of the R_f. intermediate. The second process deals with the attack of some polyfluoro-polyhalogeno-benzenes by phenyl radicals in an attempt to find compounds of greater stability towards this radical than hexafluorobenzene itself.

Surprisingly, the displacement of chlorine and bromine from aromatic carbon atoms occurs less easily than that of fluorine, although the converse is true in the aliphatic systems. The cause and significance of this is discussed.

Nitrosamines, Alkyl Nitrites And Thionitrites As Nitrosating Agents

D.L.H. Williams
University of Durham, Durham, England

There is much current interest in nitrosamines because of their well established carcinogenic properties and since they are readily formed from secondary (and tertiary) amines and sources of nitrous acid. Secondary amines occur widely in nature and sodium nitrite is used as a food preservative, so that there is very real danger to humans from in vivo nitrosamine formation. The work to be described here refers to the reactions of nitrosamines in acid solutions **i.e.** to the denitrosation (sometimes called transnitrosation) reaction. In many cases it is necessary to suppress the reverse reaction (**i.e.** of **N**-nitrosation) by the removal of the free nitrosating species as it is formed; sodium azide, hydrazine, hydroxylamine aniline derivatives, sulphamic acid, urea and thiols (species X) have been used, and their relative reactivities have been established by a kinetic analysis method. For many nitrosamines the rate determining step is the attack by the



$$NOY + X \longrightarrow \text{removed}$$

nucleophile Y- and the reactivity of Y- correlates surprisingly well with the Pearson nucleophilicity parameter for the following range of nucleophiles, Cl-, Br-, SCN-, I-, $SC(NH_2)_2$. It is possible to change the rate determining step to an earlier step (the proton transfer) in three ways (a) by structural changes in the nitrosamine, (b) by change of solvent and (c) by working at high [Y-]. These implications will be discussed both for denitrosation and for the reverse reactions **i.e. N**-nitrosation and the predictions tested experimentally.

Both alkyl nitrites and thionitrites undergo denitrosation reactions also. These have been examined kinetically in acid solution and the results will be discussed and compared with those found for nitrosamines.

Parton Symposium

Equilibrium Properties Of Fluid Mixtures: Theory And Experiment

M.L. McGlashan
Department of Chemistry, University College, London

We now, at last, have statistical-mechanical theories, containing no, or at most one, adjustable parameter, of fluid mixtures at least of the simplest kinds of molecules. These theories depend *faute de mieux* on the use of an equation of state for a pure fluid that is still grossly wrong around the critical point. The nature of the theories, and the failings of even the "best" modern equation of state, will be discussed. Computations of the equilibrium properties of fluid mixtures from these theories are still far from trivial; we need better methods of numerical analysis. Much too little is known experimentally about the *(p, T, x)* phase diagrams of fluid mixtures of simple molecules, especially of those that give three fluid phases; we need more measurements of such phase diagrams. (We no longer need measurements, which have become too easy, of the excess mixing functions made over only short ranges of temperature close to room temperature and at a pressure close to atmospheric.)

Lead-Acid Traction Batteries For Electric Road Vehicle Propulsion

D.A.J. Rand
CSIRO, Institute of Earth Resources, Division of Mineral Chemistry, P.O. Box 124, Port Melbourne, Victoria 3207, Australia

The successful introduction of electric vehicles into the road transport sector depends critically on the development of a high-performance, reliable and economical electrochemical power source. At present, the lead-acid battery is the only system commercially available for electric vehicles. Nevertheless, little information exists on the behaviour of lead-acid batteries operating under duty cycles normal to electric vehicle on-the-road service.

This paper defines areas in which both fundamental and applied work are required to optimize lead-acid battery performance, and reports the findings of research projects now in progress in CSIRO laboratories.

Kinetics Of The Heterogeneous Carbon Catalysed Ferricyanide — Sulphite Reaction In Aqueous Solution

J.M. Austin and J.A. Kennedy
Department of Chemistry, University of Canterbury, Christchurch

Although there are many indications in the literature of the heterogeneous catalysis of redox reactions, few studies have been reported of the heterogeneous kinetics of these reactions.

We here report investigations of the rate of the ferricyanide — sulphite reaction in 1M KNO_3 aqueous solutions at 298K both in the presence and absence of activated charcoal powder by following the optical density of the system with respect to the ferricyanide ion at a wavelength of 420nm. From these observations we deduce that the following rate law applies to the heterogeneously carbon catalysed reaction:

$$d[Fe(CN)_6^{3-}]dt = k_{Het}\,[Fe(CN)_6^{3-}]^{0\cdot3}[SO_3^{2-}]^{0\cdot6}$$

where $k_{Het} = 8 \pm 3$ ℓ mol^{-1} sec^{-1}.

Unlike Interaction Second Virial Coefficients

P.J. McElroy*, Hadian Hashim, and R. Battino#
Department of Chemical Engineering, University of Canterbury and #Wright State University

Measurements of the pressure change of mixing of gases, P, are used to estimate the excess second virial coefficients,

Since $\varepsilon = B_{12} - (B_{11} + B_{22})$

and $\varepsilon = RT\Delta P/[P^2(1 + \Delta P/P)\,2Y_1Y_2]$

the unlike interaction virial coefficient, B_{12}, may be estimated with an accuracy similar to that of B_{11} and B_{22}.

In the temperature range 298K to 398K the following systems have been studied

1) n-hexane - benzene
2) n-hexane - cyclohexane
3) benzene - cyclohexane
4) acetone - methyl acetate

Previous studies of system 3) have been complemented and confirmed and the systems 1), 2), and 3) represent measurements of all binary interactions of the three components. System 4) represents an extension of the study into polar systems.

The results have been compared with the behaviour predicted by the Tsonopoulos modification of the Pitzer and Curl correlation and by the Hayden and O'Connel correlation.

The acetone-methyl acetate measurements were made in cooperation with J.D. Olson (Union Carbine Corp) whose G^E values from PT'χ measurements differ by 33% depending on which of the B_{12} correlations is employed.

Molten Salt Solutions

H. Bloom
Chemistry Department, The University of Tasmania, Hobart, Tasmania

Molten salts are ionic liquids which conduct electricity well. They are also good conductors of heat and are excellent solvents. Many different classes of compounds dissolve in molten salts e.g. other ionic salts, most gases, many organic compounds and metals.

Molten salts are used on a large scale in industry as solvents in electrochemical processes, for example the extraction of aluminium. They have actual or potential uses as solvents for organic reactions, liquid-liquid solvent extraction and in fuel cells.

The mixing of two or more salts may modify their physical and chemical properties dramatically due to the formation of complex ions and complex vapour phase compounds. Some molten salt systems can be quite low melting while others are stable only at very high temperatures. Their range of electrochemical and thermal stability is in general much greater than any other liquids (except molten metals).

An understanding of the physical properties of molten salt solutions is important to the study of liquids as well as for practical industrial purposes.

The future may see molten salt systems as important solvents for preparation of many inorganic and organic chemicals, for disposal of waste products, the gasification of coal and oil, fuel cells, and the production of metal surfaces with very altered properties.

Electrochemical Aspects Of Corrosion In Geothermal Fluids

P.T. Wilson and K.A. Lichti
Industrial Processing Division, DSIR, Petone

Corrision in geothermal power systems is complicated by the presence of the gases, carbon dioxide, hydrogen sulphide and ammonia. The concentration of these gases in the steam from the Ohaki-Broadlands geothermal field is ten times that for steam from the Wairakei field. The effect of different concentrations of these gases will be compared by describing the corrosion phenomena observed at the Ohaki-Broadlands Br22 test site compared with that observed at Wairakei. These observations of corrosion in-situ will be compared with the thermodynamic predictions of Pourbaix diagrams calculated at the temperature and chemical composition of the geothermal fluid.

Steric Hindrance To Hydrogen-Bonding In Alcohols

R.H. Stokes
Department of Chemistry, University of New England (NSW)

The extent of hydrogen-bonding in alcohols is reflected in the enthalpy and free energy changes which occur on dilution with non-polar solvents. The lecture will be concerned specifically with recent work by Dr. H.T. French in Armidale, which clearly shows how in the isomeric butanols the extent of hydrogen-bonding is reduced by steric hindrance when the branched-chain members are compared with the normal isomer.

Some Implications Of Chromatographically Derived Solution Data

J.H. Purnell
Department of Chemistry, University College of Swansea, Singleton Park, Swansea SA2 8PP, Wales

The great majority of solution data that have entered the literature in the past decade have been derived from chromatographic studies. Results obtained for certain mixed solvents in GLC have proved difficult to encompass within the framework of conventional solution models and this has catalysed recent interest in the properties of such solvents. Recent work in HPLC has provided further examples of behaviour of the same kind. Classical thermodynamicists have, largely, rejected the findings and sought an explanation in experimental inadequacy or over-approximation. The number of systems so discussed is, however, a small, and selective, fraction of those for which information is available. The considerable volume of evidence, it is suggested, cannot be dismissed so readily and there appears to be a clear implication of a need to reconsider, in very great detail, the fundamentals of solution theory as it now exists.

Tricritical Phenomena In Fluid Mixtures

Robert L. Scott
Department of Chemistry, University of California, Los Angeles

A tricritical point is most easily visualized as a point at which three coexisting phases simultaneously become identical, i.e. where two menisci simultaneously vanish. However the lack of symmetry in typical fluid mixtures precludes a path by which one can take a closed system from three phases to one phase through a tricritical point. For such mixtures the phase rule forbids a tricritical point in systems of fewer than three components. Indeed most experimental studies have been carried out on four-component systems at atmospheric pressure.

However we have found that certain ternary hydrocarbon mixtures simulate binary mixtures, but permit the experimenter to vary at will the magnitude of the "binary" interaction. In these "quasi-binary" mixtures two components are sufficiently similar that their mole ratios are substantially the same in all three coexisting phases. Temperature, pressure, and composition measurements have been made on three-phase systems in the tricritical region of three quasi-binary systems: methane + (n-pentane + 2,3,-dimethylbutane), methane + (2,2,-dimethylbutane + 2,3,-dimethylbutane), and ethane + (heptadecane + octadecane). The binary system ethane + octadecane is close to a tricritical point, with a three-phase region only 0.16 K in breadth; it shows critical opalescence in all three phases. All the results are consistent with the theoretical prediction that the approach to the tricritical point is governed by critical exponents which are essentially "classical", i.e. those of a "mean-field" theory.

"Symmetrical" tricritical points are found in a few binary fluid mixtures: $^4He + ^3He$ and mixtures of sulfur with certain solvents. They are also possible in mixtures of d,ℓ-enantiomers and in three-component mixtures of such a pair with an optically inactive fluid. The possibility of finding such tricritical points heightens the interest in looking for non-ideal behaviour and liquid-liquid phase separation in mixtures of optical isomers.

Ionic Diffusion Coefficients And Their Use In Newer Formalisms For Electrolyte Solutions

R. Mills
Diffusion Research Unit, Research School of Physical Sciences, Australian National University, Canberra

A brief introduction defines ionic self-diffusion coefficients and describes the main experimental techniques for measuring them.

A review is made of work in this area in the decade 1955/1965. Highlights such as the testing of the Onsager Limiting Law for ionic diffusion are outlined and also the frustrations due to lack of a suitable theory for concentrated solutions.

In 1973 a revival of interest in ionic self-diffusion coefficients occurred because by this stage, formalisms for using these data in concentrated solutions had been developed. In this development, this Unit has acted as the prime source of experimental data and in consequence there has arisen active collaboration with a number of overseas groups. Such studies include the structure around ions in aqueous solution (with H.G. Hertz), the use of Linear Response Theory in electrolyte solutions (with H.G. Hertz and H.L. Friedman), the nickel chloride problem (with N.H. March) and normal mode analysis of electrolyte solutions (with P. Turq).

The current experimental programme at the National University and its relation to the projects listed, will be described.

The Dissociation Of Copper (II) Sulphate In Water-Methanol And Water-Dioxan Mixtures

R.A. Matheson* and J.C. Tunnicliff
Chemistry Department, University of Otago

Spectrophotometric (UV) and conductivity (Λ) measurements have been used to study the dissociation of the copper sulphate ion pair in water-methanol and water-dioxan mixtures at 25°C. In contrast to the situation obtaining in water, the choice of distance of closest approach was not found to be critical in the analysis of either UV or Λ data for our mixed solvents. However, with increasing % of organic component in the solvent mixtures, complications due to bisulphate formation and solvolysis of the Cu^{2+} ion became more troublesome. In particular, it was found necessary to add a small amount of perchloric acid to the copper sulphate solutions in order to achieve stable conductivities. A correction, based on pH measurements, was then applied to compensate for the effect of added H^+ and ClO_4^-, and of the bisulphate formation and solvolysis reactions, upon the measured conductivities. The following dissociation constants (K) were obtained. (Figures in parentheses standard deviations, not total uncertainties).

	10^4K/mol dm^{-3}	
	UV	Λ
20% Dioxane (w/w)	76 + 2	78.0 + 0.5
40% Dioxane	2.8 + 0.2	5.84 + 0.07
30% Methanol	75 + 3	81.2 + 0.9
50% Methanol	14.4 + 0.6	17.6 + 0.2
70% Methanol	1.9 + 0.2	3.68 + 0.03

Possible causes for the discrepancies between UV and Λ results for 40% dioxane and for 70% methanol will be discussed.

The Transference Numbers Of Aqueous Cadmium Chloride Solutions

K. Indaratna, A.J. McQuillan* and R.A. Matheson
Department of Chemistry, University of Otago

Transference number measurements are capable of revealing the nature and mobility of ionic species in solution and the variation of transference numbers with concentration reflects the equilibria existing between these species. It has been long established that aqueous cadmium chloride solutions are highly associated and at concentrations above 4 mol kg^{-1} have negative cadmium constituent transference numbers due to the presence of species such as $CdCl_4^{2-}$. In more dilute solutions, at 0.1 mol kg^{-1}, the $CdCl^+$ ion pair is the major cadmium constituent and only below 0.01 mol kg^{-1} does the free Cd^{2+} ion predominate.

The indirect moving boundary method has recently been used to measure the transference numbers of cadmium chloride solutions in the concentration range 0.004 - 0.1 mol kg^{-1}. The basis of the method and the conditions required to obtain precise results will be discussed. Combination of these recent results with earlier results for concentrated solutions reveals a complex variation of the transference numbers with concentration.

Deuterium Isotope Effects In Liquid-Liquid Phase Diagrams

David V. Fenby, Zorawar S. Kooner and Jagjit R. Khurma
Department of Chemistry, University of Otago, Dunedin

In 1934 Hall et al. examined the effect of increasing deuterium content on the lower critical solution temperature of water + nicotine and on the upper critical solution temperature of water + phenol. They reported that the former was lowered and the latter raised as the deuterium content of the water increased. Further, it was found that the deuterium isotope effects were considerable (e.g. for water + phenol, extrapolation to 100% D_2O indicated an increase of 11.8 K in the UCST). Since this work there have been many studies of deuterium isotope effects in liquid-liquid phase diagrams, these being motivated by diverse interests.

During the last decade there has been some interest in the explanation of these effects in terms of intermolecular interactions. In such discussions the question of the relative strengths of the hydrogen and deuterium bonds is of fundamental importance. Tsvetkov and Rabinovich have proposed an explanation based on a consideration of hydrogen bond and dispersion interactions. Our recent measurements on various water + n-hexane and water + phenol systems are in qualitative accord with the predictions of the Tsvetkov-Rabinovich model.

Recently there have been a few studies of the effects of deuterium exchange reactions on liquid-liquid phase diagrams. We have completed preliminary studies of various water + phenol systems involving OH/OD exchange reactions.

Solvent Structure And Ionic Equilibria In Biological Systems

Phillipa M. Wiggins
Department of Medicine, University of Auckland School of Medicine, Auckland

In vivo most biochemical reactions take place in interfacial regions where it is possible that the structure of the solvent is significantly perturbed. Examples of highly ordered networks of water molecules in macromolecular crystals show that organisation of water by biological surfaces does occur. Of particular interst are the membrane proteins which are cation pumps, linking hydrolysis of adenosine triphosphate (ATP) to transport of highly hydrated small cations (Ca^{2+}, H^+ or Na^+) against macroscopic gradients in electrochemical potential. Alternatively, under suitably imposed conditions, those same pumps can synthesise ATP in a reaction which normally has a positive ΔG; that reaction, too, involves highly hydrated ions.

It is suggested that when the protein complexes are phosphorylated they undergo a configurational change which imposes a high degree of order upon an interfacial aqueous phase. All contained solutes change in absolute activity, the increment in chemical potential increasing with degree of hydration. During the lifetime of that conformation ionic equilibria in the interfacial solution shift in favour of less highly hydrated species, and gradients of chemical potential are established across its interface with the bulk aqueous phase. Therefore the chemical work of either ATP synthesis or cation transport is achieved.

Electron Spin Resonance Solution Studies Of Ion Pair Phenomena In Some Alkali Metal-Aromatic Hydrocarbon Radical Ion Systems

B.M. Peake* and G.J. Hormann
Chemistry Department, University of Otago, Dunedin

The polarity of the solvent has long been known to markedly influence the nature of interactions between pairs of ions. Electron Spin Resonance (ESR) has been used to study such solvent effects in a number of paramagnetic alkali metal-aromatic hydrocarbon radical anion systems and has provided much information on equilibria between the ion pair and the free anions and on the structure of the ion pairs. These results will be briefly reviewed for a number of aromatic hydrocarbon systems including naphthalene, 1,2,3,6,7,8-hexahydropyrene and spirobifluorene.

Kinetic Parameters In Reversed Micelles

Charmian J. O'Connor* and Terence D. Lomax
Chemistry Department, University of Auckland

Alkylammonium carboxylate salts form aggregates in non-polar solvents which typically only involve 3-10 monomer units per aggregate. The addition of a reactive ester to such a system results in a non-linear dependence of the observed pseudo first order rate constant (for appearance of product) on detergent concentrations. Analyses of the rate data in these "reversed micellar" systems have borrowed extensively from the nomenclature and concepts used in aqueous detergent systems, giving results which appear to be reasonable — provided the assumptions used in the derivation are true.

However, such procedures do not allow for the fundamental physical differences between the two reacting systems. In benzene solution, not only are the forces which predominantly govern the aggregation of these salts different, but relative acidity and nucleophilicity of reagents may change depending on their local environment.

Explanations for the rapid formation of product in these systems need not invoke the involvement of the aggregate. Chemically sensible arguments can be put forward whereby aggregation has a negative effect on reaction rate and solvent effects can explain the observed catalysis.

Recent investigations have documented the decomposition reaction of *p*-nitrophenyl acetate in a variety of aklylammonium carboxylates and alkyldiamine bis(dodecanoates) and that of *p*-substituted phenylbenzoates in dodecylammonium propionate and tetramethylenediamine bis(dodecanoate) solutions in benzene. These studies provide some basis for a non-micellar answer to the observed catalysis.

Group Contribution Methods Of Estimation Of Liquid-Vapour Equilibria For Non-Electrolyte Solutions

N.F. Pasco, T. Steele and A.G. Williamson
Department of Chemical Engineering, University of Canterbury, Christchurch

In recent years a number of treatments have been produced which aim either to predict the behaviour of multicomponent mixtures in terms of binary mixture behaviour or which aim to predict the behaviour of mixtures in terms of group contributions. These treatments are discussed and compared. The similarities among the treatments are such that the differences between the various treatments are often less than the differences between any one of them and experimental data. Some directions in which any (or all) of the treatments might be improved as prediction methods are discussed.

Microcalorimetric Studies Of Wetting And Adsorption

A.G. Langdon* and D.H. Everett
Chemistry Department, University of Waikato, Department of Physical Chemistry, University of Bristol

The ampoule cell assembly of a Tian-Calvet microcalorimeter has been modified to incorporate a magnetic seal breaking mechanism for use in heat of wetting studies. The new device has been used to investigate the structure of C_6 to C_{18} hydrocarbon wetting films and the thermodynamics of adsorption from solution.

Mercury (II)-Assisted Aquation Rates Of Chloropentaamine Cobalt (III) Complexes — The Effect Of High Ionic Strength

D.A .House
Chemistry Department, University of Canterbury, Christchurch

The variation of rate constant with ionic strength for reactions between ions, is known as the primary salt effect. For reactions between ions of unlike sign, the salt effect is governed by the principle of ionic strength, i.e., by some form of the Debye-Huckel-Bronsted-Davies equation. However, reactions between ions of like sign, e.g.,

$$CoCl(N_5)^{2+} + Hg^{2+} = Co(N_5)(OH_2)^{3+} + HgCl^+$$

do not appear to obey such a relationship (A.R. Olsen and T.R. Simonson, *J. Chem. Phys., 17,* 1167 (1949). In such cases, the rate is determined by the concentration of the supporting electrolyte of opposite sign and specific salt effects are often apparent.

The second-order rate constants for the title reactions are generally determined by spectroscopic changes under pseudo-first-order conditions ($[Hg^{2+}] > 10[Co(III)]$), involving the use of high ionic strengths (0.2-3.0 **M**) adjusted by the addition of acid. There is no consensus as to the acid or the ionic strength to be used (although I = 1.0 **M**, $HClO_4$ is common), and intercomparison of the literature data is difficult.

To facilitate such comparisons, we have measured the Hg^{2+}-assisted aquation rate of racemic-**cis**-$[CoCl(en)_2(imidazole)^{2+}]$ in $HClO_4$, HNO_3, H_2SO_4 and CF_3CO_2H over the ionic strength range 0.1-3.0 **M** at 298.2 K.

Empirical relationships relating the rate constant (k_{-Hg}, $M^{-1}s^{-1}$) to the ionic strength (I) are:

$HClO_4$ (I = 0.1-3.0 **M**)	$\log k_{-Hg} = 0.26I + 1.56$
HNO_3 (I = 0.1-3.0 **M**)	$10^3 k_{-Hg} = 56.91 + 21$
CF_3CO_2H (I = 0.3-3.0 **M**)	$10^3 k_{-Hg} = 23.0$
H_2SO_4 (I = 0.5-2.0 **M**)	$10^3 k_{-Hg} = 63.6$

These data show that for the title reactions, there is probably no background electrolyte in the concentration range 0.1-3.0**M**

that is free from specific interactions with the reactants. Consequently, we suggest a "standard state" of I = 1.0 **M**, $HClO_4$ be used wherever possible.

The Physical Chemistry Of Solutions Of Copper (I) And Silver (I) In Acetonitrile-Water

A.J. Parker and P. Singh
Murdoch University, Murdoch, Western Australia

Copper (I) and silver (I) are selectively solvated by acetonitrile whereas Na^+ and Cu^{2+} are selectively solvated by water, in acetonitrile-water mixtures. This produces marked differences in the chemistry of these four ions on transfer from water to acetonitrile-water mixtures. This will be illustrated by discussion of nmr spectra, free energies of transfer, enthalpies of transfer, entropies of transfer, conductance changes, transfer partial molal volumes and electrode kinetics of the Cu^{2+}, Cu^+, Cu^0 system. The copper-acetonitrile chemistry is of special interest because of the applications to copper hydrometallurgy and the stabilisation of Cu^+ in the presence of acetonitrile.

Electron transfer with the Cu^{2+}, Cu^+, Cu^0 system is fast in acetonitrile-water at various electrodes. There is a very substantial loss of entropy when the equilibrium $Cu^{2+} + Cu^0 \rightleftarrows 2Cu^+$ is transferred from water to 30% vv acetonitrile-water. The mobility of Cu^+ and Ag^+, but not Na^+ is greatly decreased on addition of acetonitrile to water. After an initial increase for all three cations, the partial molal volumes of Ag^+ and Cu^+ decrease much more than does that of Na^+ as acetonitrile is added to water.

An Interpretation Of The Anomalous Properties Of Water

Robin J. Speedy
Chemistry Department, Victoria University of Wellington

Evidence is presented to suggest that the metastable superheated, stretched and supercooled states of liquid water are bounded by a line $p_s(T)$ at which $(\partial p/\partial V)_T = 0$. Since it is a condition of stability that $(\partial p/\partial V)_T \leq 0$, the line $p_s(T)$ bounds the region on the pT diagram where water can exist as a thermodynamically stable or metastable liquid and $p_s(T)$ is called the limit of stability.

The locus of $p_s(T)$ is estimated and it is shown that the anomalous expansivity, compressibility, heat capacity, viscosity etc. of water can be simply predicted as consequences of the anomalous form of $p_s(T)$.

The Dissolution Mechanism Of Ionic Solids

G.A. Wright
Chemistry Department, University of Auckland

The dissolution or crystallisation of ionic solids in contact with electrolyte solutions involves the transfer of ions and sometimes electrons across the double-layer at the interface. Experiments can be conducted on the kinetics of ionic dissolution under conditions such that the rate is not controlled by mass transport or nucleation, leaving the potential difference in the inner double-layer as the key variable effecting the ion transfer rate. Some interesting rate laws are known for the oxidative dissolution of cuprous oxide, the reductive dissolution of magnetite and the dissolution of boehmite in hydrochloric acid. In the latter case, Daroux has determined the rate law $v = k_1A(Cl^-)(H^+)^{1/2}/(1 + K_2(Cl^-))$, which is consistent with a mechanism involving cation transfer with coordination to two Cl^- to give $Al(OH)_2Cl_2^-$, and anion transfer with the assistance of one proton.

In the case of salts of low solubility such as barium sulphate the rate law is $v = kA(c_s - c)^n$, where c_s is the concentration at equilibrium saturation, and n = 2 (1,1 and 2,2 salts) or 3 (2,1 or 1,2 salts). This law was first established for $PbSO_4$ by Jones in 1963, and at least ten cases are now known. But surprisingly this simple rate law has not yet been adequately explained in terms of a self-consistent mechanism obeying the law of microscopic reversibility. This paper reports a new attempt at formulating the mechanism in terms of double-layer charging by adsorbed ions and potential dependent rate constants for ion transfer. The interfacial potential difference is predicted to change monotonically towards the equilibrium value, the sign of the change indicating which ion dominates in the adsorbed layer.

Mixture Potentials In Chemistry

M. Spiro
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Many redox couples such as

$$Cd^{2+} + 2e^- \rightleftharpoons Cd(s) \qquad (1)$$

rapidly set up reproducible electrode potentials. Their measurement leads to many useful thermodynamic quantities: to changes in Gibbs free energies, to stability and solubility constants, and to activity coefficients. H.N. Parton and his school have made signal contributions to this field.

It is the purpose of the present lecture to discuss what happens at a given electrode when two or more couples are simultaneously present (but are not at equilibrium with each other). An example is a cadmium rod dipped into a solution of Fe^{3+} ions : the reaction

$$2Fe^{3+} + Cd(s) \longrightarrow 2Fe^{2+} + Cd^{2+} \qquad (2)$$

takes place slowly and both the couples Cd^{2+}/Cd and Fe^{3+}/Fe^{2+} are present together. This situation must be treated in kinetic and not just thermodynamic terms, i.e. in terms of the current-voltage curves of the two couples. When both couples are present together, the two curves simply add up : this is the famous addivitiy principle of Wagner and Traud. The one point in the joint curve at which the net current is zero defines the mixture (or mixed) potential E_{mix} of the system.

These ideas can be applied in several branches of chemistry. One important area is the dissolution of metals, whether desirable (as in the leaching of metals from ores) or undesirable (as in corrosion). Another large field is the catalysis by metals of redox reactions. The electroless deposition of metals provides a practical example; so does the development of photographic plates.

More recently, several cases have been discovered of the breakdown of the additivity principle. This occurs when the electrode discriminates between the two couples. Such discrimination is essential for certain purposes, in particular for the successful operation of a photogalvanic cell for solar energy conversion.

The Effect Of Structure On Acid Strength In 5,5-Disubstituted Barbituric Acids, Including A Comparison With Dicarboxylic Acids — The Malonic Acids In Particular

D.R. Baird, R.H. McKeown*, R.J. Prankerd and Wong Ooi
Department of Pharmacy, University of Otago

First thermodynamic dissociation constants (25°C) are examined for the effect of C(5)-substituents on acid strength in a series of 27 5,5-disubstituted barbituric acids (I). Steric factors have been investigated as an additional effect on reactivity in these derivatives. A linear free energy relationship (l.f.e.r.) has been established by multiple correlation, with independent variables polar and steric substituent constants, and its significance for the series will be discussed. Acid strength in derivatives increases with increasing steric effects in C(5)-substituents, e.g. (1; $R^1=R^2=Me$, $pK_1=8.51$ and 1; $R^1=R^2=Et$, $pK_1=7.98$), contrary to what would be expected for electronic effects alone for these substituents. Moreover this steric acid-strengthening trend is less with the isopropyl group (I; $R^1=Me$, $R^2=Pr^i$, $pK_1=8.45$) than the ethyl group (1; $R^1=Me$, $R^2=Et$, $pK_1=8.28$). This steric effect is considered to be more important in the undissociated molecule where solvation due to weaker dipole-dipole interactions would be more susceptible to disruption than in the anion, where stronger ion-dipole interactions are relatively less affected. This difference in steric effects on solvation in the initial and final states is believed to be responsible for a decrease in the standard free-energy change for dissociation and the acid-strengthening effect observed. This is essentially the converse of the explanation for steric acid-weakening in aliphatic carboxylic acids, where hindrance to solvation in the anion of the acid, which is thus less stable with

respect to the acid (RCO_2H) has long been accepted; this results in an increase in the standard free-energy change for dissociation. A suitable substituent might, therefore, have a steric effect on both initial and final states thus cancelling itself out so far as overall reactivity is concerned.

Attention is drawn to this last possibility since the C(5)-isopropyl group clearly does not increase reactivity in derivatives in proportion to the steric effect anticipated for the group from steric constants. The close parallel for alkyl substituent effects in the corresponding malonic acids, from which barbituric acids are derived, suggests that the cause of these effects is of similar origin. An intramolecular hydrogen-bonded anion hypothesis has most often been advanced to account for substituent effects in malonic acids and it is difficult to imagine how this might apply in the barbituric acid derivatives.

Ionic Surface Mobility in Electrolyte Solutions

S.I. Smedley*, D. McFarlane*, M. Ryan* and J. Talon**
*Department of Chemistry, Victoria University of Wellington and **Physical and Engineering Laboratory, DSIR, Petone

The interpretation of conductance measurements on ionic solids close to the melting point, has led to a proposal that surface ions have a much greater mobility than ions in even the bulk liquid state. This finding has encouraged us to search for a similar phenomenon in the surface of electrolyte solutions. In order to detect such a phenomenon we have used two techniques to enable us to separate surface and bulk conductivities. The first involves the measurement of conductance with two parallel wires of different depths of immersion in the fluid, the second involves the study of detergent films of a known dimension. From these dimensions, and the known bulk conductivity we calculated the expected conductance, which was always less than the measured conductance.

Our experiments have been conducted with 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} mol dm^{-3} KC1 solutions containing various concentrations of a non ionic surfactant. The films were pulled from solution into a saturated, temperature controlled atmosphere, and conductance measurements were made at a film thickness of 625 nm, as determined from the interference bands. Measured conductances were proportional to KCl concentration, but independent of surfactant concentration. The surface conductances G_s were calculated by subtracting the expected conductance G_c from the measured value, the ratio of G_s/G_c varied with concentration as follows:—

Concentration of KC1/mol dm^{-3}	0.1	0.01	0.001	0.0001
G_s/G_c	1.6±0.1	1.66±0.03	1.80±0.05	2.0±0.2

The surface conductances are well in excess of those that would be expected from the surface adsorbtion phenomena, and suggest to us the possibility that surface ions have much higher mobility than ions in the bulk phase.

1981 NZIC JUBILEE CONFERENCE PROGRAMME

Plenary Lectures

August 24: Prof. B.S. Hartley, FRS, Imperial College, London.
"Biochemistry and Biotechnology"

August 25: Dr P.B. Weisz, Mobil Research USA
"Shape-Selective Catalysis — New Horizon for Chemistry and Industry".

August 26: Prof. J.I.G. Cadogan FRS, BP (U.K.)
"The Energy Problem: Some Chemical Initiatives".

August 27: Prof. A.J. Parker, Mineral Research Unit, Murdoch Univ. W.A.
"Solvents as Solutions to Minerals and Energy Problems".

SYMPOSIA

Industrial

AUGUST 25

SESSION 1: (11:00-12:30)

Theme Address & Natural Product Industries
Chairman: Sir Geoffrey Badger

1. Chemistry in Industry — A Review — A.W. Mackney
2. 50 Years of Chemical Developments in the Rubber Industry — S.M. Betty, Empire Rubber Mills Ltd
3. 50 Years of Chemical Developments in the Tanning Industry — Dr. B.R. Mann

SESSION 2: (2:00-3:30)

Fuels and Energy
Chairman: Dr C.J. Maiden

4. Liquid Fuels in New Zealand — A.L. Titchener Auck. University
5. "Synthetic Fuels Via Methanol" — Dr. P.B. Weisz, Mobil Oil, USA
6. Production of methanol — Dr. P. Marsden, I.C.I. UK.

AUGUST 26

SESSION 3: (11:00-12:30)

Mineral Based Industries
Chairman: Professor A.J. Parker

7. 50 Years of Chemical Developments in the Steel Industry — Dr. D.F. Christian
8. 50 Years of Chemical Developments in the Cement Industry — W.T. Paulin
9. Chemical Developments in the Salt Industry — Dominion Salt Ltd Speaker

Chemistry in New Zealand

AUGUST 27

SESSION 4: (11:00-12:30)

Natural Product Industries
Chairman: Dr. J. Rogers

10. 50 Years of Chemical Developments in the Pulp & Paper Industry — R.B. Hall
11. 50 Years of Chemical Developments in the Fertiliser Industry — W.E. Russell
12. 50 Years of Chemical Developments in the Sugar Industry — P.A. Simpson

SESSION 5: (2:00-3:30)

Polymer Industries
Chairman: Dr. P.K. Foster

13. 50 Years of Chemical Developments in the Glass Industry — J.J. Wearne
14. 50 Years of Chemical Developments in the Ceramic Industry — W. Vose
15. 50 Years of Chemical Developments in the Polymer Industry — A.C. Kennett, Chem. Div., DSIR Auckland.

AUGUST 28

SESSION 6: (9:00-10:30)

Management in the Chemical Industry
Chairman: Dr. J. Watt

16. Instrumentation in Chemical Industry — M. Collins
17. Operational Research in the Chemical Industry — Dr. T. Ball
18. Management in Technically Based Industries — J.P. Goulter

Agricultural

August 25: 11-12.30 Symposium: **Fertilisers for the Future**
Chairman: N.A. Cullen, MAF, Ruakura
Speakers: D.J. Higgins, Director, N.Z. Fertiliser Manufacturers' Research Association "Phosphates"
K.W. Steele, MAF, Ruakura "Biological nitrogen"
I.G. Menzies, Petrochemical Corporation of N.Z. "Synthetic nitrogen"

August 25: 2-3.30 Symposium: **Biochemical Understanding and Agricultural Improvement (I)**
Chairman: Dr D.E. Wright, Head Office, MAF
Speakers: G.B. Russell, Applied Biochemistry Division, DSIR "Can Chemistry help in the development of insect resistant plants?"
R.E. Mitchell, Division of Horticulture & Processing, DSIR "The chemical and biological intricacies of plant pathogenic bacteria"
C.E. Pankhurst, Applied Biochemistry Division, DSIR "Biochemical aspects of host specificity with *Rhizobium* — legume symbiosis"

August 25: 2-3.30 Symposium: **Biochemical Understanding and Agricultural Improvement (II)**
Chairman: Dr R.W. Bailey, Applied Biochemistry Division, DSIR
Speakers: R.T. Gallagher, Research Division, MAF "The rogue neurotoxins of ryegrass staggers"
H. Young, Division of Horticulture & Processing, DSIR "The volatile aroma compounds of N.Z. subtropical fruits"
J.S. Ayers, Dept. of Chemistry, Biochemistry and Biophysics, Massey University "The recovery of protein from whey by cellulosic ion exchangers"

August 27: 2-3.30 Symposium: **Living with Pesticides**
Chairman: Professor E.G. McQueen, Dept of Pharmocology, University of Otago
Speakers: Dr Cleve A.I. Goring, Dow Chemical Company "Problems in pesticide development"
B.B. Watts, Agricultural Chemicals Board "Regulation of pesticides"
Commission for the Environment (speaker to be named) "Pesticides in the environment".

Pharmaceutical

August 24: 2-3.30 Symposium: **Bioavailability and Pharmacokinetics of Drugs**
Chairman: Dr P. Carroll, Wellcome Aust. Pty

Speakers:
1. Dr M. Kingsford (Chem. Div. DSIR Wellington) Topic "The Adventures of Frusemide Formulations"
2. Dr R. Briant, Pharmacology Dept, Auckland School of Medicine
Topic "Relevance of Pharmacokinetics to Clinical Practice".
3. Mr D.G. Ferry (Toxicology Research Unit, Otago Medical School).
Topic "Analysis of Drugs in Biological Fluid".

August 24: 4-5.30 Symposium: **Drug Design**
Chairman: Prof. E.G. McQueen, Dept. of Pharmacology, Otago Medical School
Speakers:
1. Dr W. Denny "Cancer Chemotheraphy: Medicinal Chemistry's Greatest Challenge".
2. Dr K. Taylor (Pharmacology Dept. Auck. School of Medicine)
Topic "Drugs from the Sea — Marine Natural Products of Pharmacological Interest".
3. Dr P.R. Andrews, Victorian College of Pharmacy Ltd Melbourne.
Topic "Theoretical Approaches to Drug Design".

Education And Technology

August 26: 11-12.30

Chairman: R.A. Scott, Secondary School Inspectorate.
11-11.20 Mr N.R. Edmonds, Auckland Technical Institute.
"Future Developments in Technical Institutes".
11.20-11.40 Professor B.J. Welch, Department of Chemical and Materials Engineering, University of Auckland.
"Blending the Science and Engineering of Chemical Processing".
11.40-12 Dr D.J. Daniels, University College of Arts, Science and Education, Bahrain.
"Chemical Industry and the School Curriculum".
12-12.30 General Discussion.

August 27: 11-12.30
Chairman: Professor J.F. Duncan, Victoria University of Wellington.
11-11.20 Dr A.F. Wilson, NZ Forest Products Limited.
"Industrial Careers for the Chemistry Graduate".
11.20-11.40 Dr A.G. Langdon, Waikato University.
"Technology in University Chemistry Courses".
11.40-12 Dr J.G. St.C. Buchanan, BP (NZ)
"The Management Option".
12-12.30 General Discussion.

Industry And Environment

August 27: 11-12.30

SESSION 1.
Chairman: Dr B. Graham, Environmental Health Laboratory, Department of Health, Auckland.
(a) "Is Society At Risk?"
Dr C.A.I. Goring, Global Technical Director, The Dow Chemical Company, USA.
(b) "Interaction Of Industry With The Environment"
Dr R. Bellamy, Department of Cell Biology, University of Auckland.
(c) "Integrated Pest Management — Progress And Prospects"
Dr C.H. Wearing, Entomology Division, DSIR, Auckland.

August 28: 11-12.30

SESSION 2

Chairman: Mr J.G. Fletcher, Director, Heavy Engineering Research Association, Auckland.
(a) "What Value On Pollution Control?"
Mr T.S. Laird, Environmental Engineer, NZ Forest Products Ltd, Kinleith.
(b) "The Fate Of Arsenic Released Through Geothermal Activities In The Waikato Catchment"
Dr J. Aggett, Department of Chemistry, University of Auckland.
(c) "Environmental Effects Of The Wine Industry"
Mr J. Adams, Cooks New Zealand Wine Co. Ltd., Auckland.

Food

August 27: Beverages

Chairman: Mr G. Strachan, Auckland, D.H.P.
2-2.05: Chairman
2.05-2.25: "Tea Infusion" - M. Spiro, London, Imperial College
2.25-2.45: "Phenolics in Fruit Juices" - E. Wilson, Auckland, DHP.
2.45-3.05: "Utilisation of Dairy Products in Beverages" C. Towler, Palmerston North, D.R.I.
3.05-3.25: Discussion and Questions.
3.25-3.30: Chairman.

August 28: Toxic Residues
Chairman: Mr J. Fraser, Wellington, Health Dept.
9-9.05: Chairman
9.05-9.20: "Pesticide Immunopathology" — R.T. Baker, M.A.F. Auckland.
9.20-9.30: "Polyaromatic Hydrocarbons" - J. Love, Chem. Div. DSIR, Christchurch.
9.35-9.50: "Mycotoxins" - J. Mitchell, Chem. Div. DSIR, Auckland.
9.50-10.05: "Nitrosamines" - R. Weston, Chem. Div. DSIR, Wellington.
10.05-10.25: Discussion and Questions.
10.25-10.30: Chairman.

observations it was proposed[5] that the enzymatic hydrolysis of native cellulose required two steps catalyzed by components C_1 and C_x. The polymer is disaggregated by C_1 rendering it susceptible to hydrolysis by C_x. In this context C_x is a -1,4 hydrolyzing activity acting on amorphous chains. Neither C_1 nor C_x is able to solubilize a crystalline cellulose, such as cotton fibre to a significant extent, but when combined they act synergistically, and effectively solubilize crystalline cellulose.

Native cellulose $\xrightarrow{C_1}$ Reactive cellulose $\xrightarrow{C_x}$ cellobiose

The synergism betwen C_1 and C_x types of enzymes for crystalline cellulose degradation is not yet fully understood, but it is conceivable that their alternate action might improve access by opening up the fibre structure. The C_1 component has little or no effect on soluble derivatives of cellulose such as carboxymethylcellulose (CMC). Enzymes classified as C_x can hydrolyze amorphous cellulose or soluble or partially degraded celluloses producing higher cellooligosaccharides, glucose or cellobiose, and consist of exo- and endo- -1,4-glucanases.

For twenty years confusion has existed on the exact nature and role of the C_1 and C_x components. The function of the C_1 component was thought to be that of swelling the cellulose and disrupting the hydrogen bonds thereby disaggregating the chains and converting the crystalline regions to amorphous regions. In some systems (e.g. *Trichoderma reesei* and *T. konigii*) the C_1 is a cellobiohydrolase, and it is the C_x components (the endocellulases) that attack the cellulose first and open-up chain ends where the exo-enyzme (C_1) can act[6 7 8].

In 1975 Wood[9] called for the C_1-C_x concept to be abandoned and redefined the mechanism of cellulase action in new terms. These are, "that crystalline cellulose is effectively rendered soluble by the cooperative action of endoglucanase and exoglucanase enzymes; the exo-glucanase being of a special type that acts by removing cellobiose from the end of the cellulose chain". An example of such synergism was provided by the endo- and exo-glucanases of *T. reesei* which Hofsten[10] illustrated by means of the model shown in Figs. 1-4.

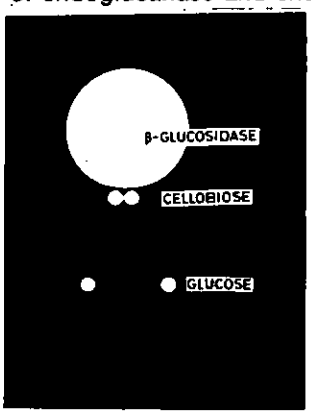

Fig. 1. Model of a β-glucosidase molecule hydrolysing cellobiose.

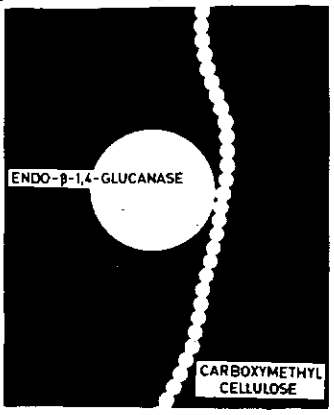

Fig. 2. Model of an endo-β-1,4-glucanase molecule on a carboxymethyl cellulose chain.

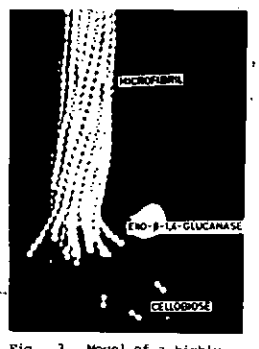

Fig. 3. Model of a highly crystalline microfibril of the type presumably occuring in Avicel and an exo-β-1,4-glucanase hydrolysing cellobiose from the end of one of the forty glucosidic chains in the microfibril.

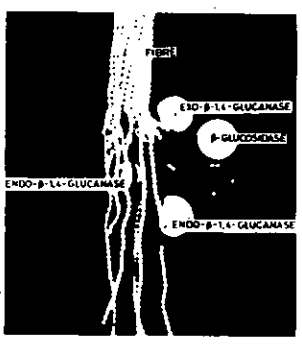

Fig. 4. Proposed mode of action of different cellulolytic enzymes from *T. viride* on part of a cellulose fibre.

Recently, the concept of C_1 being a specific enzyme for the initital attack on cellulose has been revived. Eriksson and his colleagues have reported the isolation of two new enzymes, cellulose oxidase (which they obtained free from endo- and exo-glucanases) and cellobiose quinone oxidoreductase[11 12]. Cellulose oxidase, a hemoprotein, which oxidizes cellobiose to cellobionic acid, is important for cellulose degradation, since the degradation is approximately doubled when it is present in addition to C_1 and C_x enzymes[12]. The enzyme was shown to be oxidative in character and oxidized the hydroxyl groups in the sixth position in the glucose unit to a carboxyl group. It was suggested that the oxidation of glucose units of cellulose to uronic acid moieties by the enzyme caused swelling of the cellulose chains, which, in turn, caused disorder in crystalline cellulose, thereby making the crystalline parts more accessible to enzymes. This is fully in accord with the old hypothesis of Reese et al[5] that cellulose is first activated, so that its accessibility to hydrolytic enzymes is increased.

Cellulase Production

Organisms that have been reported to elaborate high yields of cellulases include *T. reesei*[13], *T. koningii*[14], *Sporotrichum pulverulentum*[6], *Sclerotium rolfsii*[15] and the thermophiles *Thermoascus aurantiacus*[16] and *Thermoactinomycetes*[17]. The two mutants of *T. reesei*, QM9123 and QM9414[18] are currently the best sources for the production of cellulases. Cellulases are believed to be inducible in most microorganisms and indeed sophorose (-D-glucopyranosyl-1,2-D-glucose) induces cellulase-production in several organisms.

Future Developments

With the advent of genetic engineering techniques we can confidently predict that strains of microorganisms will be constructed capable of breaking down the lignocellulose complex and degrading both the cellulose and the hemicellulose. The lignin itself will ultimately be a valuable source of aromatic chemicals. By using thermophiles rapid continuous fermentation at high temperatures can be developed that would harness the heat of fermentation to distil the ethanol. Many microbial products including proteins, polysaccharides and organic acids will be produced more cheaply than petrochemicals and then a new class of biopolymers, biosolvents, and biodetergents will evolve. In the proposed EEC biomolecular engineering project, emphasis is to be placed on enzyme technology, gene transfer and control of gene expression[25]. In the 1990s we could well see columns containing bound cells capable of converting cellulose pulp to ethanol. Then a really balanced alternative to our oil-based economy will have arrived.

Conclusions

The natural abundance of cellulose and its rate of renewal make it attractive as an energy source. The preceding discussion on the structure of cellulose highlighted the inert nature of the lignocellulose complex. However, the recent developments in biotechnology and fermentation techniques should solve the major problems of delignification, pulping and degradation. As more high yield cellulase mutants become available the rate of cellulose degradation will continue to improve. In the conversion of cellulose to glucose and then to alcohol only a small quantity of the chemical energy is lost. For New Zealand alcohol would appear to be a perfectly satisfactory alternative fuel and the DSIR has recently developed a carburettor conversion kit which allows the present fleet of cars to run as efficiently on alcohol as on petrol. In addition, the use of alcohol will result in a

significant improvement in air quality in cities through lower emissions e.g. of lead. Many studies indicate that oil prices will rise substantially in the later 1980s and 1990s. This will exacerbate New Zealand's terms of trade and at that time transport fuels from Biomass must become a much more attractive proposition.

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THE INDUSTRIAL POTENTIAL OF ENZYMES FROM EXTREMELY THERMOPHILIC BACTERIA

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The thermal regions of the central North Island of New Zealand are some of the most extensive in the world. In addition, they are readily accessible and contain a diversity of ecological habitats, including a large number at 100°C. These areas are regarded as an important tourist attraction, and as a source of geothermal power. It is now clear that they also contain an important and unique genetic resource.

Micro-organisms which survive and grow at elevated temperatures (above 45°C) are loosely termed thermophiles. A more rigid definition requires that their growth optimum should be above 50°C[1]. Extreme thermophiles have growth optima above 65°C, and this group is comprised exclusively of bacteria. These are the organisms which are the subject of our research at Waikato. Under currently used laboratory conditions no bacterium has been isolated with a growth optimum above 80°C, and little growth occurs above 85°C. Since many of these extreme thermophiles are isolated from hot springs at 100°C it is quite possible that both the growth optimum and the upper temperature at which growth occurs are appreciably higher under natural conditions than in the laboratory. There seems to us to be no good microbiological or biochemical reason why, providing sufficient free water is available, bacterial growth should not take place up to 100°C.

We have isolated a variety of extremely thermophilic bacteria from the Rotorua area. Apart from the factors enabling their growth and survival at high temperatures (e.g. protein stability, membrane melting point), as a group they exhibit no unusual features.

Roy Daniel is Senior Lecturer in Biochemistry at the University of Waikato. He graduated in Chemistry, B.Sc. (Hons) at the University of Leicester in 1965 and Ph.D. in Biochemistry in 1968 at the same university. After working as a Postdoctoral fellow at the Agriculture Research Unit for Nitrogen Fixation at the University of Sussex and the Division of Plant Industry, CSIRO, Canberra, he took up a lectureship in the Department of Cell Biology, University of Glasgow in 1971. He has been at Waikato since 1975.

Don Cowan is a Postdoctoral researcher with the thermophile group at the University of Waikato. He graduated B.Sc. from Waikato in 1975, M.Sc. (Hons) in 1977, and D.Phil. in 1980. His D.Phil. research involved characterisation of a thermostable extracellular protease from an extreme thermophile.

Hugh Morgan is Senior Lecturer in Biological Sciences at the University of Waikato. After graduating B.Sc. (Hons) in Agricultural Botany at the University of Wales, Aberystwyth in 1965, he completed his Ph.D. in Soil Microbiology at the University of Guelph, Canada in 1970. He was awarded an ICI Postdoctoral fellowship to work at the Macaulay Institute for Soil Research Aberdeen prior to taking up the lectureship at Waikato in 1972.

Enzyme Stability

An early suggestion that growth and survival at high temperatures were achieved by rapid enzyme turnover rather than stability[2,3] has now been discredited, and it has been shown that enzymes isolated from extreme thermophiles are in general stable at the optimum growth temperature of the organism even in the purified state[4]. A number of hypotheses have been advanced to account for such stability. One of the first thermostable proteins characterised, an α-amylase from *Bacillus stearothermophilus*, was found to have a low molecular weight and an unusual tertiary structure[5,6]. This led to predictions[7] that thermostability might be a product of small rigid molecular structures, where conformational flexibility was sacrificed for stability. Other hypotheses, based on increased disulphide bonding[8], hydrogen bonding[9], hydrophobic interactions[10], or salt bridging (ionic interactions)[11] being responsible for thermophilic stability, have since been proposed. The fact that many thermophilic enzymes, like their mesophilic counterparts, demonstrate allosterism suggests that molecular flexibility is not seriously curtailed[4]. Nor has any general reduction in the molecular size of thermophilic enzymes been observed[4]. Several detailed comparative studies of

similar proteins from mesophiles and thermophiles have failed to find any consistent changes in amino acid composition, and in particular, no bias towards amino acid residues responsible for any one type of intramolecular bonding[12,13]. (This work is reviewed in refs 1, 12, 14-16).

Current evidence suggests that, since the net free energy of stabilisation of proteins is small, (the result of a delicate balance between the large destabilising forces due mainly to chain entropy and the large stabilising forces due mainly to hydrophobic interactions) one or more quite small changes in protein structure may result in large changes in thermostability[17]. For example, it has been shown that the substitution of a histidine for the arginine 96 of lysozyme from bacteriophage T4 lowers the melting temperature of the enzyme by 14°C[18]. A study of the X-ray electron density map of the enzyme at 0.24nm resolution showed that, apart from this substitution, no detectable changes in the tertiary structure had taken place.

Enzymes in Industry

There are a number of advantages in using enzymes as industrial catalysts. Firstly, they are usually highly specific, and the undesirable side-products sometimes encountered in chemically catalysed reactions are rarely a problem. Effluent from processes using enzymes is usually less toxic than that from alternative processes. Enzymes are generally more active and more efficient than chemical catalysts. Whereas conversion by chemical means will often be limited by the restraints of the equilibria laws, high levels of conversion can result from catalysis by enzymes with high substrate affinities.

The industrial conversion of glucose to fructose[19] provides an example of some of the advantages of enzymic over chemical catalysis. Alkaline conversion at high temperatures results in side reactions which form quantities of materials such as the ketohexose, psicose, and objectionable coloured by-products (which are costly to remove[20]). Consequently, chemical conversion of glucose to fructose has not been economically viable. Progress in enzyme isolation and characterisation since the late 1950's has yielded a number of glucose isomerase enzymes (GI's) from various micro-organisms. The more suitable of the GI's are capable of a 50% conversion of glucose to fructose in 95% glucose syrups, with little production of undesirable by-products. (Commercial processes such as this, however, utilise vast quantities of enzyme: 17-20 tons of immobilised GI consumed per 10,000 tons of 42% fructose syrup produced — one month's production[21]).

These advantages are reflected in the diversity and rapid growth of the enzyme industry. Enzymes are used in the food and beverage industry (a market currently worth nearly $100 million per year), in detergents, and in medical diagnostics. Growth of the industry as a whole is probably 10-15% per year, but as new processes are developed this could rise dramatically. As an example, the development of processes for producing high-fructose corn syrup from starch, using the enzymes amylase, amyloglucosidase, and glucose isomerase, was largely responsible for the sales of these enzymes in the U.S.A. rising from $26 million (36% of the U.S. enzyme market) in 1975 to about $76 million (55% of the U.S. enzyme market) in 1980. The most significant new developments are likely to be in the field of clinical diagnostic enzymes, in cellulose hydrolysis, and in the immobilisation of enzymes. In diagnostics, the absolute specificity of many enzymes is utilised to measure the concentration in biological fluids of important metabolites such as glucose, urea, cholesterol, and of other enzymes. One clinical kit used for assaying the level of creatine kinase, a muscle enzyme whose blood level rises after a heart attack, utilises two other enzymes, hexokinase and glucose-6-phospate dehydrogenase, so that the end product is $NADPH_2$, which can conveniently be measured with an ultraviolet spectrophotometer.

$$\text{ADP + Creatine Phosphate} \xrightarrow{\text{Creatine Kinase}} \text{Creatine + ATP}$$

$$\text{ATP + Glucose} \xrightarrow{\text{Hexokinase}} \text{ADP + Glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate + NADP+} \xrightarrow{\text{Glucose-6-phosphate Dehydrogenase}} \text{6-phosphogluconate + NADPH + H+}$$

The kit contains the two enzymes, ADP, creatine phosphate, glucose and NADP and the rate of formation of $NADPH_2$ is proportional to the creatine kinase activity in the blood sample assayed. Several clinical kits and analytical systems utilise the almost complete specificity of glucose oxidase for D-glucose.

The second important growth area centres on the enzymic hydrolysis of cellulose to glucose. The exciting commercial feature of this research is the low, or even negative cost of the starting material (in the sense that the material may be a waste product which is currently costing money to dispose of), its ready availability, and its renewable nature. A major end use of such glucose might be ethanol or single cell protein production. However, although enzymes which digest cellulose have been much investigated, so far no cost-effective process has been developed.

The recent development of enzyme immobilisation technology (the linking of enzymes to insoluble, often inorganic, support matrices) is likely to further widen the application of enzymes to industrial catalytic processes. Immobilisation often increases the stability of the enzyme, and may modify the properties of the enzyme in other ways, such as shifting the pH optimum. Immobilised enzyme preparations are suitable for recovery and reuse in batch processes, and have permitted the development of highly efficient continuous-flow reactor systems.

But although enzymes have many advantages, those derived from mesophilic organisms are generally unstable, as illustrated by the consumption figures for glucose isomerase, and expensive. The cost of commercial enzyme production arises from low yields of enzyme per unit of biomass, the expense of extraction and purification, and by losses of active enzyme during purification, storage and handling. Running costs are high because of the short lifetime of most enzymes. Some improvement in the yields of many enzymes has resulted from the work of geneticists in isolating high-producing mutant organisms, and more recently from genetic engineering. In addition, the stability of enzymes can often be improved by chemical manipulations and in particular by immobilisation. However, it is in the area of enzyme stability that thermophilic (and more particularly extremely thermophilic) micro-organisms can provide a significant improvement to existing biotechnology.

Advantages Of Enzymes From Extreme Thermophiles

Some enzymes from mesophilic sources exhibit extraordinary thermostability, but as might be expected, as a class enzymes from extremely thermophilic bacteria are more thermostable than those from thermophiles, which are in turn more thermostable than those from mesophiles*The differences in thermostability are often so large that it is difficult to obtain strictly comparable data since for convenience enzyme thermostabilities are usually measured at temperatures which give half lives of 5-200 minutes: but the half-life of a typical enzyme from a mesophile will be of the order of seconds at 75°C, while the half-life of an enzyme from an extreme thermophile may be of the order of days. It would be much easier to compare data if the thermostabilities were expressed as the temperature at which an enzyme had a given half-life, say one hour, but of course this value would be much less convenient to obtain.

★ **Table 1:**

Thermal stability of enzymes from mesophiles, thermophiles, and extreme thermophiles.

		Half-life (Hours)		
	Temperature (°C)	Mesophile	Thermophile	Extreme Thermophile
Asparaginase	55°C	0.3 (a)	1.4 (b)	-
	75°C	0.3 (d)	-	70 (c)
α-Amylase	90°C	0.005 (e)	0.4 (b)	0.3 (f)
β-Galactosidase	50°C	0.3 (g)	>3 (h)	-
	65°C	-	-	>>750 (i)
	80°C	-	-	> 20 (i)
Protease	60°C	0.25 (i)	-	-
	75°C	-	1 (j)	> 150 (c)
	85°C	-	0.2 (k)	5 (c)

Organisms and growth temperatures:

(a) *Bacillus coagulans* (37°C);[23]
(b) *B. stearothermophilus* (55°C)[23]
(c) *Thermus* T-351 (75°C)[24 25]
(d) *Escherichia* coli (37°C)[24]
(e) *B. subtilis* (37°C)[23]
(f) *B. caldolyticus* (72°C)[26]
(g) *Neurospora crassa* (25°C)[27]
(h) *Mucor pusillus* (25°C)[28]
(i) *B. subtilis* (37°C)[29]
(j) *Malbreachea pulchella* (45°C)[30]
(k) *B. thermoproteolyticus* (55°C)[31]
(l) Thermophile 4-1A (75°C)[33]

Arising from this thermostability, and from other factors, enzymes derived from extremely thermophilic bacteria have a number of economic advantages:

1. The large scale growth of thermophilic bacteria is likely to be cheaper than that of mesophilic bacteria. Part of this is as a result of a reduced capital outlay, since the heat exchange/refrigeration equipment required for the cooling of mesophilic cultures will not be needed. (The additional costs of insulation, and heating and heat recovery equipment are not likely to outweigh this saving). Furthermore much of the cost of mesophile growth vessels is associated with preventing or reducing microbial contamination. Since such contamination is not a problem above 70°C, much simpler and cheaper vessels can be used to grow thermophilic bacteria.

Running costs may also be lower, since refrigeration is unlikely to be required, and most of the heat needed can be supplied by the exothermic growth of the organism, and heat recovery. Procedures for the sterilisation of the vessel and of the growth media can be less strict, or possibly even omitted completely for growth at 75°C or above.

2. There is evidence that higher yields of purified enzymes can be obtained from isolation procedures when thermophilic micro-organisms are used as the source[22 23]. This is a function of the greater stability of their enzymes.
3. Being more stable, thermophilic enzymes have a longer useful life in industrial enzyme reactors than their mesophilic equivalents, increasing their cost-effectiveness.
4. Reactors using thermophilic enzymes can be operated at temperatures sufficiently high to prevent microbial contamination. This would permit reactors to operate for longer periods between sanitising procedures, and without the necessity of presterilisation or any addition of antimicrobial agents.
5. Our work suggests that some enzymes from extremely thermophilic bacteria are resistant to the denaturing effects of detergents and of organic solvents, compared with those from mesophiles. For example we have observed that the apparent activity of a proteolytic enzyme from an extreme thermophile increases tenfold in the presence of low concentrations of detergent because of an effect on the casein substrate, but that mesophilic proteases are denatured and inactivated by the detergent[32]. This stability could minimise losses during cleaning of reactors utilising immobilised enzymes. It might also enable the use of mixed solvent systems to improve the solubility of substrates or products.
6. In high-temperature bioreactors, volatilisation of reaction end products could occur. This could be valuable either as a recovery step or as a means of removing a potentially inhibitory end-product.
7. The activity of enzymes from extremely thermophilic bacteria at ambient temperature is usually less than 2% of that at the optimum temperature. Reactions can be easily stopped simply by cooling.
8. There are a number of general advantages in operating industrial processes at higher temperatures. Viscosity is reduced, solubilities are higher, diffusion is accelerated, and less effort need be taken to dissipate the waste heat inevitably generated during industrial processes.

Many of the advantages referred to here for thermophilic enzymes, and some additional ones, apply to the use of whole extremely thermophilic bacteria to replace mesophilic microorganisms in existing processes. The advantages of an ethanol fermentation carried out near the boiling point of ethanol are fairly obvious for example. Extreme thermophiles are of course non-pathogenic, since by definition they will not grow at body temperatures.

All the techniques, such as immobilisation and genetic engineering, currently being developed to enhance the usefulness of enzymes from mesophiles are also applicable to enzymes from extreme thermophiles. We have been able, for example, to further increase the stability and to modify the properties of an extracellular protease and an intracellular asparaginase from an extreme thermophile by immobilisation (Table 2). Because of the impossibility of extreme thermophiles being pathogenic, the use of recombinant DNA techniques carried none of the potential hazards normally associated with these.

Table 2:

Effect of Immobilisation on Enzymes from *Thermus* T-351.

	D-asparaginase[24]		Protease[32]	
Property	Free Enzyme	Immobilised to Sepharose 4B	Free Enzyme	Immobilised to Sepharose 4B
$t_{\frac{1}{2}}$ (minutes) at 85°C	30	600	360	1060
$t_{\frac{1}{2}}$ (minutes) at 95°C	-	-	28	125
pH optimum	No change		7.0	6.0
K_m (mM)	20.0	28.5	No change	
Substrate inhibition	-	-	Present	Absent

Furthermore, we have no evidence that the metabolic activities of extremely thermophilic bacteria are in any way limited, or that any enzyme currently found in a mesophilic bacterium will not also be available from an extreme thermophile.

Conclusion

There are few areas where the use of enzymes from extreme thermophiles will not be cheaper and more efficient. We believe that not only will they eventually replace most enzymes from mesophiles used in existing processes, but that their advantages will greatly widen and increase the industrial use of enzymes.

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Guest Speakers At Jubilee Conference

Prof. Robin Stokes

The name of Robin Harold Stokes is known to all who have studied physical chemistry in the past 20 years, as co-author with R.A. Robinson of the definitive work 'Electrolyte Solutions', first published in 1959 and still widely used by students and research workers for its wealth of precise detailed information on the thermodynamic and transport behaviour of ions in solution. He has to his credit two other books and nearly 100 original papers on electrolyte solutions.

Prof. Stokes is no stranger to medals and honours. In 1940, upon completion of his M.Sc. degree at the Auckland University College, he was awarded the Sir George Fowlds Memorial Medal and has acquired no less than 5 university scholarships. In 1946, shortly after his appointment to the Department of Chemistry of the University of Western Australia, he was awarded the Rennie Medal of the RACI. In the same year he was the Medola Medallist of the Royal Institute of Chemistry, and was awarded the H.G. Smith Memorial Medal of the RACI in 1953. He was awarded a medal in the Queen's Silver Jubilee Awards.

Last year he was honoured in his retirement from the University of New England by the Electrochemistry Division, RACI, which struck the R.H. Stokes Medal of which he was the first recipient.

Throughout his career, Prof. Stokes has been a dedicated and enthusiastic experimentalist. During World War II he was chief chemist with the Colonial Ammunition Co., Auckland, but in the evenings and weekends he pursued his research work on solution chemistry.

Many papers on such subjects as the measurement and interpretation of diffusion, activity and osmotic coefficients followed, laying the foundation upon which much of modern electrochemistry is built.

Prof. Stokes' visit to the Conference is financed by the NZ Section of the Royal Society of Chemistry.

Prof Max McGlashan, Head of the Dept of Chemistry at University College, London, will be one of the key speakers at **Golden Jubilee Conference** in Auckland in August. Max has not been too forthcoming in supplying information but **Prof Parton,** under whom he studied at Canterbury, has kindly supplied the following:

1. His research field is non-electrolytic solutions — the thermodynamics and statistical mechanics of such systems, which he entered under **E.A. Guggenheim** some 30 years ago.
2. He is extremely interested in chemical thermodynamics and has written a book with that title (Academic Press 1979) in which he lays great (and justifiable) stress on experimental methods. He says "The great charm of thermodynamics to me is that at best it can be both rigorous in the formulation of its algebra, and rigorous in its experimental demands." (This of course makes it repellant to many chemists.) He edits the Journal of Chemical Thermodynamics.
3. Another major interest is the accurate use of the 'language' of the physical sciences. Hence his membership of such bodies as the Commission on Physico-Chemical Symbols, Terminology and Units, and the Royal Society Symbols Committee, and the tireless advocacy of SI units. His RIC Monograph on Physico-chemical Quantities and Units (1968) — reviewed by **Geoff Malcolm** of NZIC — is very good. He was a member of the UK Metrication Board from 1969 until **Mrs Thatcher** abolished it with its job incomplete.
4. His current post at University College has a distinguished lineage back through **Nyholm, Hughes, Ingold, Donnan, Ramsay** to **A.W. Williamson** of ether fame, 150 years ago.

Which is typically Parton!

Obituary

BRUCE FRANK CAIN

1930 - 1981

Ph.D. (Auck.), D. Phil. (Oxon), D.Sc. (Auck.), FRSNZ, FNZIC.

Bruce Cain (photo courtesy Auckland Star)

Bruce Frank Cain, Director of the Cancer Society's Cancer Chemotherapy Research Laboratory, University of Auckland School of Medicine, and Honorary Professor in the University of Auckland, died suddenly from a heart attack at his home in Kumeu on January 9, 1981. His unexpected death at the age of 50 was a tragedy for his family and friends, for his scientific and medical colleagues in NZ and around the world, and was also an enormous loss for NZ science and for world-wide cancer research.

Bruce Cain was born in Auckland on May 15, 1930, and was educated at Mangere East School, Otahuhu College and the University of Auckland. While excelling in the formal study of science, his early extracurricular activities in chemistry in a laboratory in the backyard of his parents' home reflected the enthusiasm and dedication which were always to be his distinguishing characteristics. At Auckland University, he was awarded in 1951 the G. Phillips Prize for heading the final year in B.Sc. chemistry. The following year he gained the M.Sc. degree with 1st. class honours in chemistry, doing research under the supervision of Prof. L.H. Briggs into the occurence and characterization of terpenes and alkaloids in NZ native plants. These studies were continued as a University Research Fund Fellow in 1953-1954, and he was awarded the Ph.D. degree. He inherited from Prof. Briggs a lasting knowledge of the NZ flora and a deep interest in its chemistry that he was to put to good use in later years.

In 1955 he travelled to Oxford, where he worked in the Dyson Perrins Laboratory under Prof. Sir Robert Robinson and received the D. Phil. degree.

Back in Auckland in October 1956, he joined the staff of the Auckland Division of the Cancer Society of NZ as Senior Cancer Research Fellow in chemistry. He worked initially at the Albert St laboratory of the Chemistry Dept. of the University, and later at the hospital in Cornwall Park. During the early part of this time much of his work was on extraction of NZ plants, with over 1500 species being collected, extracted, and the extracts fractionated and tested for anti-tumour activity both in Auckland and overseas. This work was a logical application of the training and experience he had gained to the search for new drugs for cancer chemotherapy. It required much hard work for relatively little return, but that has always been the nature of this particular type of research.

These studies resulted in a series of papers, and in three new drugs that were extensively tested in experimental tumour systems but did not reach the clinic. They were also instrumental in shaping Bruce Cain's thoughts along the lines that were to result in so much later success. While he never discounted the use of research aimed at the detection of anti-tumour compounds from natural sources, quoting the many useful drugs which have been obtained this way, he sensed that it was not where the future lay. He saw also that, because of the limited return for a large amount of work, it was not an appropriate type of research for a small laboratory to be fully engaged in. In an intellectual sense also, it seemed more satisfying to use chemical and biological knowledge and intuition to design and modify drugs to enhance their activity, rather than to search patiently through all of Nature's flora for compounds — evolved for some other purpose — that might incidentally have anti-tumour activity.

When he became Director of the laboratory in 1963 this general philosophy was put into practice. Work with Graham Atwell and later also with Ralph Seelye on several series of bisquaternary compounds with high anti-tumour activity led in 1967-1968 to the formulation of two basic principles that were to guide much later drug design both in his laboratory and in other laboratories around the world. The biological activity of a series of similar compounds (differing only in the length of alkyl sidechains) was shown to be related to their overall hydrophobicity in a characteristic manner, reaching a maximum level and declining again. Drug biological activity is dependent in this way on hydrophobicity (which can be expressed in terms of the partition coefficient of a drug for solubility in water and lipid) because the drug needs to be transported from the site of administration to the site of action, and it is this phenomenon which is hydrophobicity-dependent.

He also deduced from the general topology of the drugs of these series that they would fit neatly into the minor groove of double-helical DNA, and proposed this as the biologically-important binding site. Thus the biological activity of a particular compound is also dependent on its detailed geometry, which dictates how well it fits the DNA binding site. Considerable later work in his laboratory and elsewhere proved the correctness of these postulates and validated their worth as guiding principles for the design of new drugs for cancer chemotherapy. Application of these principles and other considerations led to the development of the 9-anilinoacridine class of anti-tumour agents, from which the AMSA (acridinylaminomethanesulfonanilide) drugs evolved.

In 1974 the laboratory was moved to the DSIR Plant Diseases Division at Mt. Albert when the old Cornwall Hospital was finally demolished. While a final home for the laboratory in the new Pathology Building of the Medical School had been decided on some time earlier, it was clear that years would elapse before this building was ready. In partial anticipation of the move to Mt. Albert, Bruce Cain had purchased a home in the area, and was able to walk to work. The writer remembers this as a very pleasant time for the small group in the laboratory and for Bruce Cain. Working for the first time in reasonably large quarters, his struggle to survive and have his ideas and work accepted was largely past, with national and international recognition beginning to come. One compound of the AMSA series (m-AMSA or amsacrine) was steadily moving through the US National Cancer Institute tests towards clinical trial. On the other hand the interests of the laboratory were still somewhat narrowly focussed, and the extra work and pressure which were to come with the clinical success of m-AMSA and the management of a larger and much more diverse group of scientists were not yet evident. It was the time for having an idea at morning teatime, going to the bench to turn it into reality over the next few days, and having an answer back from the animal tumour screen in a week or so.

While thoroughly enjoying this environment, Bruce Cain was always aware that effective drug design in the cancer chemotherapy field was a truly interdisciplinary project, and was always keen to enter into collaboration with other people, to acquire new skills himself and to urge his staff to do so. In this regard, he looked forward to the move to the Medical

School site, not only because of the larger and better-equipped laboratory, but because of the wider opportunities to develop new collaborative efforts with people of different scientific and medical skills. The move was made in late 1978, and roughly coincided with the move of Bruce Cain's family home to Kumeu, the clinical introduction of m-AMSA, the enquiries by a number of overseas drug companies about obtaining marketing rights, and considerable publicity. The combination of these factors put a tremendous load on him, which he took up in his typical enthusiastic manner. His only (and often-aired) complaint was that he was no longer able to do as much bench work as he wished. There were other compensations, however. He was able to see the laboratory expand, with an increasing biological emphasis, due in large part to the mutually-beneficial collaboration developed with the Warner-Lambert company. The Medical School laboratory was the fulfilment of his long-held view that effective cancer drug development could only be carried out by an interdisciplinary group of chemists and biologists, working together as a group and in close association with clinical personnel. The real tragedy of his early death is that it occurred so shortly after he had, by 25 years of constant effort, built up exactly such a research group in the type of environment he had always envisioned.

Bruce Cain received many awards and honours, and was in demand as a speaker both within NZ and overseas. He was a Fellow of the Royal Society of Chemistry, the Royal Society of New Zealand, the New York Academy of Sciences and the American Chemical Society. Since 1972 he had served as a member of the Medicinal Chemistry panel of the American Chemical Society. In 1974 he was invited to be a World Health Organization delegate to an international conference on Tumour Models and Screening Systems. In 1978 and 1979 he was an invited speaker at Gordon Research Conferences in New Hampshire on the chemotherapy of experimental cancer. In 1980 he was guest lecturer at the first World Conference on Clinical Pharmacology and Therapeutics in London. In 1979 he was appointed an honorary professor of the University of Auckland, and very recently was awarded the D.Sc. degree by the University.

Bruce Cain was a Fellow of the NZIC for many years, served on several of its committees, and always took a keen interest in its affairs. Thus it is appropriate to record here the above factual details of the scientific life and achievements of a fellow chemist. They clearly illustrate a brilliant and expanding career cut short in full bloom, and for that we are profoundly sad. Yet the NZIC is fundamentally an association of people who happen to be chemists. While we all benefit professionally from the image that Bruce Cain's work brought to chemistry, perhaps we can also benefit in a more personal way from an appreciation of the individual qualities which enabled him to achieve so much. The following portrait is necessarily a subjective one, drawn from the writer's daily association with Bruce Cain over the last 10 years, and from conversations with others who knew him. His early basic characteristics of enthusiasm and determination were later allied with an enormous capacity for hard work and a vast knowledge of chemistry and the other subjects that came within the field of expertise of the laboratory. During the time the laboratory was at Mt. Albert, with his home just up the road, it was almost impossible to arrive at work before him or leave after him. Even at the Medical School, when he had to travel to Kumeu, he used to avoid the rush hour by arriving early and leaving late, usually with a pile of reading for the evening. He was a skilled practical worker, with an uncanny feeling for just how long to attempt something before deciding that another route would be more efficient. If you were stuck at a particular point in a synthesis and mentioned it to Bruce, he was quite likely to turn up in a few days with the intermediate you needed, having taken time off from his own schedule to try a different synthetic method.

His enthusiasm, especially after return from an overseas trip, was always like a tonic. Instead of being exhausted, and perhaps envious of the facilities he had seen, he was instead always overflowing with ideas that would send him immediately to the bench to try out. He was always willing to take the time to explain to people of all backgrounds what he was doing, at the level which was appropriate. He was equally willing to listen to and help his staff with their work, and kept well on top of all that was going on. The leadership was always there; by his own unconscious example and from his infectious enthusiasm for the projects underway. He was ruthlessly honest with himself and fair to others; he always gave his own best efforts and expected the same from others. He detested affectation, sloppiness and laziness, and could be hard on such lapses, but basically he led by example. In spite of the limelight of later years, he was a modest man, with a clear awareness of the potential for misunderstanding in press reports about cancer research; and a determination not to permit such about any of the work done in his laboratory.

He maintained a lifelong interest in horticulture and all aspects of viticulture, especially the end result. He would often claim that years of exposure to laboratory chemicals had so damaged his sense of smell that all he could detect was boiling pyridine, yet he had a wide knowledge and appreciation of local and overseas wines. The visit of an overseas scientist or the need to provide for a local meeting or conference was a welcome chance to take a tasting tour of the Auckland vineyards.

Bruce Cain met his wife Pat in Oxford, and they had a son Richard and a daughter Elizabeth. His family, home and garden were always his main escape and relaxation from a busy scientific life. His wife and children always gave him and the laboratory great support, and their help was instrumental in his success.

Bruce Cain was always proud to be a New Zealander, and his life work stands as an example to us all that New Zealanders working in New Zealand can still make great and lasting contributions to world science, and in so doing honour their country together with themselves.

W.A. Denny

(A memoir on Bruce Cain, with a list of his publications will appear in a forthcoming volume of the Proceedings of the Royal Society of NZ.)

Readers Write

The Editor,
Sir,

HAZARDOUS LIQUID TRANSPORT

It is pleasing to see that on the front cover of the 1980 "Chemistry Year Book" you have again drawn attention to the responsible attitude displayed by Freightways in their bulk liquid handling fleet. Their distinctive vehicles are no doubt familiar to most chemists in this country and it is very obvious that great care has been taken to identify liquids being carried.

Many of these liquids are, of course, potentially extremely hazardous in the event of their being in the wrong place at the wrong time.

However, I must admit to being rather surprised at their being the recipient of international awards for their safety attitudes and practices if the unit you have featured is typical of their road fleet. The track-mounted tank apparently contains oleum, which we all know to be a very potent liquid indeed. There is some doubt as to what the trailer-mounted tank contains; the sign on the rear indicates oleum, but the one on the right hand side says it is empty. It is scarcely necessary to point out that there could be occasions where it is vital to be absolutely certain what it contains, and the driver may not be available for enlightenment on this point.

I have no doubt that the material from which the tanks have been manufactured is fully resistant to the range of chemicals they may contain and that welding and other operations have been very carefully performed in order to ensure sound vessels. But oh, those unprotected valves, pipelines and manholes! They are simply begging to be shorn off if the vehicle were to become involved in an accident, particularly if rolling ensued.

Couldn't we have some form of cover which projected from the tank and shrouded these vulnerable fittings so that they are much less likely to release the tank contents in times of trouble? If an accident to one of these units should occur, the consequences can be of almost trivial concern, as long as the liquid cargo is safety retained.

If it is not, the result could be lasting damage to the public image of the chemical industry, in addition to the more obvious effects.

C.W. Harland
(Fellow)

BRANCH NEWS

Manawatu

The first meeting of 1981, held jointly with the Chemistry, Biochemistry and Biophysics Department of Massey University, was addressed by **Prof. Andreas C. Maehly,** Director, Swedish National Laboratory of Forensic Science, Linkoping. Since 1972 about 50 research papers have been presented by the laboratory on the analysis of shoeprints, gunshot residues, fuses and electrical shorts.

Dr Kevin Brown (Chemistry Division, DSIR, Gracefield) presented this year's energy lecture on "Liquid Fuels from Coal — An Option for the Future". Dr Brown discussed aspects of the "Energy Crisis" and the importance of coal as an energy source. The chemical properties of coal were described, followed by methods for the conversion of coal to liquid products. Problems in mining and processing of coal, from the social disruption of communities to potential pollution of the atmosphere, the soil and artesian water supplies, were discussed.

The first of the Branch lectures to Manawatu high school students was presented on April 6 by **Prof. David Cullwick,** Professor of Marketing, Department of Business Administration, Victoria University of Wellington. Prof. Cullwick's topic, "Our Chemical Future", covered a wide range of industrial activities that could lead to increased employment opportunities in the future. Many new industrial processes are being developed that will require chemists and biochemists to control many aspects of the work. Close links between scientists, technologists and marketers will be required, so that scientific training and experience will increasingly become an entry point for careers in management, according to Prof. Cullwick.

Taranaki members are reminded of the New Plymouth meeting on June 29 that will b e addressed by Branch chairman **Dr Cecil B. Johnson** (Applied Biochemistry Division, DSIR, Palmerston North) on "Tallow: Its Composition and Uses — Present and Potential". Further information may be obtained from **Dr Keith Sewell,** Ivon Watkin-Dow Ltd, New Plymouth.

Next month's (July) Hawkes Bay meeting will be addressed by **Dr Max Turner** (Department of Soil Science, Massey University) on "Trace Elements in Soil". Further information may be obtained from **Mr Ted Fletcher.**

Members and their associates in the Hawkes Bay area organised a meeting at the Leopard Brewery on March 2. The meeting took the form of a conducted tour of the plant followed by a "subjective analysis of the product". Details of future meetings may be obtained from **Messrs Ted Fletcher** (Ph. 777-769), **Colin McDonald** (Ph. 53-399) or **Hugh Gahagan** (Ph. 56-807) of Napier.

Wellington

Prof. R.D. Batt, Head, Department of Chemistry, Biochemistry and Biophysics at Massey University spoke on "Alcohol consumption in NZ" at the March meeting. Prof. Batt is the head of the Alcohol Research Group at Massey University which is financed primarily by the Medical Research Council of NZ to carry out research on alcohol and acetaldehyde metabolism in human volunteers. He pointed out that alcohol consumption per head of population in NZ was now at the highest level recorded since official statistics were first published in 1892 and he discussed the effects of alcohol intake on the health of drinkers and in relation to the drinking-driving legislation.

The April meeting was addressed by **Dr R.W. Henley,** Chemistry Division, DSIR, Wairakei on "Applied geochemistry in the development of geothermal resources". He discussed the problems of waste water injection back into geothermal reservoirs associated with the Broadlands-Ohaki power project, particularly the potentially serious and costly problem of silica scale formation in pipelines and in the reservoirs. An application of thermodynamics based on experimental studies of high temperature solution chemistry was described, with reference to the avoidance of silica scale formation.

As part of the Jubilee activities for this year the Branch has also held "Careers in Chemistry" seminars for secondary school students at the Central Institute of Technology in Upper Hutt and at Victoria University in Wellington, both during March. The seminars were run by a group of NZIC members representing government, industrial and educational occupations.

Canterbury

The first meeting for 1981 (February) was addressed by **Prof. Don Stedman,** University of Michigan, who spoke on "Photochemical Smog".

The March meeting was addressed by **Prof. A. Maehly,** Director, National Laboratory of Forensic Science, Linkoping, Sweden. Prof. Maehly spoke on examples of casework and the research which is carried out in his laboratory.

The April meeting heard **Prof. Leon Phillips,** Canterbury Chemistry Department, speak on "The Chemistry of Venus's Atmosphere". This talk covered the present knowledge of the Venusian atmosphere and also relevant research work presently in progress in various laboratories.

Otago

Mr Adrian C. Wrigglesworth has won the 1980 Inglis Memorial Prize for the best student in Advanced 2 Chemistry. This award is funded by both the Otago branch and the University.

Branch members attended a joint meeting with the Department of Chemistry on April 10 to hear an address by **Dr William Horwitz,** Deputy Associate Director for Toxicological Sciences, Bureau of Foods, FDA, Washington. He spoke on some aspects of "Regulatory Analytical Chemistry".

The branch meeting on April 22 was preceded by light refreshments following which an address was given by **Mr M. Farrier,** Safety and Environmental Superintendent, NZ Aluminium Smelters Ltd, Invercargill. His topic was "The Chemistry of Aluminium Production". The lecture was aimed at bridging the gap between the apparent simplicity of the reduction of alumina and the actual complexity of the commercial process. The development of electrolytic cell technology was discussed in relation to chemistry, the efficient use of energy, and environmental factors.

Waikato Marks NZIC Jubilee

To mark the NZIC's Golden Jubilee year, the Waikato Branch has organised a display at the Ruakura Open Day on June 10. Stands have been organised with working exhibits demonstrating the importance of chemistry to agricultural research and development. Emphasis will be on analytical chemistry.

Each stand will be backed by a large board outlining the **principle** of the method and highlighting an important agricultural **application.** A 10-min. slide/tape show will also be run every 30min. to give an introduction to analytical chemistry and its importance to agricultural research.

The following exhibits have been included:

Milk testing for fat and protein — IR Milk analyser; Teat Sanitiser residues in milk — Iodide selective electrode; Meat tenderness — Gelelectrophoresis separation of muscle proteins; Ryegrass staggers — purification of mycotoxins by thin layer chromatography; Facial Eczema — determination of sporidesmin by high performance liquid chromotography; Pesticide Residues on Crops — gas chromatography of kiwifruit residues; Major element analysis of soil for Quick test — K, Ca and Mg using the automated 4 channel flame photometer; Trace Element Analyser — Mg levels in blood using atomic abosrbtion spectroscopy; Selenium status of stock and humans — determination of Selenium in blood with autoanalyser/fluorometry; Energy value of feeds — bomb calorimetry; Methane evolution by ruminants — radioactive counting.

Assessment Of Laboratory Practice

Suggested as an aid to facilitate assessment of chemical laboratories, report no. CD 2303 "Guide Questionnaire to Assess Laboratory Practice" (author: **Wolfgang J. Passl**) is available free of charge from the Librarian, Chemistry Division, DSIR, Private Bag, Petone. It lists important and optional aspects of Good Laboratory Practice (GLP) expressed as questions in a suitable order followed in the course of an inspection.

The questionnaire was prepared both for the external as well as for the internal assessment of laboratories. Particular consideration was given to TELARC requirements as well as the Good Laboratory Practice for "Non-clinical" Laboratory Studies laid down in the US Federal Register Vol 43, No. 247, part 58, December 22, 1978.

NZIC Fellows Named To Senior DSIR Appointments

NZIC Fellows, **Dr Bruce Miller** and **Mr Ian McDonald,** have been named as two of the three Chief Directors of DSIR, newly created senior positions to co-ordinate scientific investigation in the broad areas of NZ's natural resources, manufacturing and agriculture.

As a result of their promotions **Drs Gordon Leary,** FNZIC, and **Michael Leamy** were named Directors respectively of the Chemistry Division and Soil Bureau.

Dr Miller, MA, MSc, Agrl (Sweden), FNZIC, FNZIAS, is 58. He is to take up the responsibility for the Resources Group of DSIR divisions — Botany, Ecology, Geological Survey, Geophysics, Oceanographic Institute, Science Information and Soil Bureau. Dr Miller brings to his new task nearly 40 years' experience in soil science and science adminstration.

Mr McDonald, MSc, FNZIC, ARIC, is Dominion Analyst, effectively NZ's top Government chemist. Earlier in his career Mr McDonald, 55, produced a succession of techniques for purifying natural materials, notably for the timber and paper industries. His new duties umbrella DISR's Industrial group of divisions — Applied Mathematics, Auckland Industrial Development, Chemistry, Christchurch Industrial Development, Industrial Processing, Institute of Nuclear Sciences, Physics and Engineering Laboratory, and Wheat Research.

McDonald

Miller

Leamy

Leary

Dr Leary was head of Chemistry Division's toxicology and forensic sections. He also becomes Dominion Analyst.

Born in 1940 in Southport, Britain and educated at Dulwich College, London, Buller High School, Westport and Christchurch Boys' High, he graduated from Canterbury University in 1961 BSc, 1962 MSc and gained his PhD in 1965 when he joined DSIR's Chemistry Division.

In 1967-69 Dr Leary did post-doctoral studies as a fellow of the Royal Institution, London. He has also held a BP post-doctoral fellowship, and was a visiting scientist at the Swedish Forest Products Research Institute, Stockholm, 1977-78. Dr Leary is author of over 35 scientific papers of considerable note.

Dr Leamy, formerly chief pedologist of the bureau, was educated at St Patrick's College, Wellington, and Victoria University. In 1956 he graduated with an MSc in geology and in 1976 he gained his DSc. Dr Leary has been on the staff of the Soil Bureau since 1949, and has had extensive overseas experience.

He has worked on soil surveys in Fiji, Hobart, Brisbane, Malaysia, South Africa, Tonga, the Cook Islands, and Samoa. From 1965 to 1966 he was Colombo Plan soil correlator in Malaysia, during which time he wrote and had published a soil survey manual for use in the field in that country.

More recently his close involvement with soil taxonomy, the American system of detailed soil classification, which is currently being investigated for NZ conditions at the Soil Bureau, has led to his being made chairman of the International Committee of Classification of Andisols (ICOMAND).

He is author and co-author of more than 60 scientific papers, and many articles for NZ newspapers and magazines.

He is also past-president of the NZ Society of Soil Science, and a member of the International Society of Soil Science, the Wellington Branch of the Royal Society of NZ, the Geological Society of NZ and the NZ Institute of Agricultural Science.

News From Govt. Departments

DSIR

Applied Biochemistry Division

Dr David Greenwood of ABD's Organic Chemistry Group, returned in late March. He has been in the Biochemistry Department, University of Liverpool, since September 1977 studying for a Ph.D. degree while he was on an NRAC Post-Graduate Fellowship. His area of research, with **Dr H.H. Rees,** was the hydroxylating mechanisms of the insect moulting hormones, ecdysteroids, relative to insect-plant relationships.

Chemistry Division

Following the appointment of **Dr G.J. Leary** as Director, **Dr H.P. Rothbaum** has been made Deputy Director and will also be responsible for the Instrument section.

Mr E.R. Cairns has returned to Chemistry Division following a year's work at Sydney University. Mr Cairns intends to use radioimmunoassay methods for some toxicological analyses.

Mrs Linda Parker, a recent graduate from Canterbury University, has joined the Inorganic Materials section.

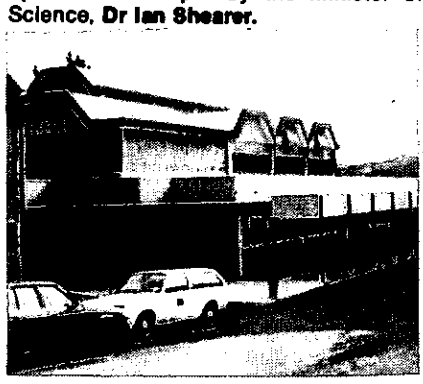
The new Chemistry Division Geothermal laboratory in Taupo was officially opened on 10 April by the Minister of Science and Technology **Dr Ian Shearer.** Energy Minister **Mr W.F. Birch** was also a guest at the opening.

Recent seminars organised by the Division have included "Regulation of Food Additives and Toxicants" by **Dr W. Horwitz,** U.S. Food and Drug Administration and "Chemistry Division in the 1980's" by **Mr I.R.C. McDonald,** recently appointed a Chief Director of DSIR.

Dr Bill Swallow will be taking up a DSIR Study Award at the Chester Beatty Research Institute, London, for 13 months from July. While in UK he will be working on the metabolic activation of polycyclic aromatic hydrocarbons to give carcinogens in association with **Prof. P. Sims.**

Industrial Processing Division

Stage 1 of the IPD laboratories at Gracefield (illustrated) was officially opened on 1 April by the Minister of Science, **Dr Ian Shearer.**



Institute of Nuclear Sciences

Dr N.E. Whitehead attended an IAEA meeting on nuclear methods of trace element analysis in biological materials at Bombay. This was followed by a trace element symposium in which he presented a paper on **in vivo** x-ray fluorescence analysis. Both conferences were at the Bhabha Atomic Research Centre. They were very well run, but he notes that the lino squares in one lecture theatre are still not glued down, since his last visit in 1978!

Plant Physiology Division

Dr Roger Slack returned from a visit to UK where he attended a symposium on Food, Nutrition and Climate, sponsored by the Rank Prize Fund. At the symposium Dr Slack received the 2nd Rank Prize for nutrition jointly with **Dr Hugo Kortlchak** of Hawaii and **Dr Hal Hatch** of Plant Industry, CSIRO, Canberra. The award was made for the elucidation of the C4 pathway of photosynthesis which Drs Hatch and Slack studied together whilst at the Colonial Sugar Refining Co. Laboratories in Brisbane.

Soil Bureau

The Director, **Dr Bruce Miller,** who has just been appointed as a Chief Director of DSIR, recently attended an expert meeting on World Soil Policy convened by UNEP in collaboration with FAO, UNESCO and the ISSS (International Soil Science Society). The meeting, held in Rome, formulated a policy for conserving and improving soil resources and proposed a plan of action for its implementation, at both international and national levels.

Dr Norm Wells has returned from 4 weeks in Malaysia and Singapore. He spent some time at the Malaysian Agricultural Research and Development Institute (MARDI) at Serdang, near Kuala Lumpur, with two trips to the east coast to look at problem soils of sandy ridges and depressions and to make land use recommendations.

Dr Cyril Childs reports that a Mossbauer spectrometer has now been commissioned at the Bureau. It will be used largely to investigate the nature, genesis and weathering sequences of iron compounds in soils.

Invermay Agricultural Research Centre

Dr David Forss has recently returned from visits made to food research laboratories in Sydney, and dairy research laboratories in Melbourne.

NZ Dairy Research Institute

Recent appointments to the staff include **Messrs Kevin C. Palmer** (Milk Powders and Drying Section) and **Rodney J. Bennett** (Casein and Related Products Section).

Dr Terry D. Thomas attended an International Dairy Federation (IDF) Symposium at Ede, Netherlands in April to present a key address on "Proteinases of Starter Streptococci". He also visited a number of research centres in UK and USA.

Dr Wayne B. Sanderson attended an IDF International Symposium in Luxembourg on the Application of Dairy Ingredients in the Food Industry. At the same time, he attended a meeting of one of the IDF Commission Permanent Committees of which he is a member.

Dr Lawrie K. Creamer recently returned from USA after spending 12 months' sabbatical leave at the University of Wisconsin where he studied the chemistry and properties of milk proteins.

University News

Massey

On Saturday, March 21, Massey University held an "Open Day" for the public of Palmerston North and surrounding districts. A wide variety of University activities were on display. Various aspects of chemistry and biochemistry were illustrated in the different disciplines. These ranged from industrial processing in the Food Science and Biotechnology Department's displays to alternative tractor fuels (e.g. seed oils and tallow and their derivatives) by the Agricultural/Horticultural Section of the Agronomy Department. Prominent displays in the Chemistry, Biochemistry and Biophysics Department included that of alcohol research, lipid metabolism in plants, lipoproteins and heart disease, the iron binding protein (lactoferrin) in human milk and protein sequencing. A "chemical magic" show was appreciated by the audience.

Dr Ian Shearer, Minister of Science and Technology (left) with NZIC President-elect,, Dr Stan Simpson, following the Minister's visit to the April Council meeting.

Dr Eric Ainscough of the Department of Chemistry, Biochemistry and Biophysics, recently presented a most interesting lecture on "Heavy Metals in Medicine" in the Department's Science Seminars series. He discussed aspects of the applications of compounds of mercury, platinum and gold to various clinical conditions. Although many different compounds containing these elements have been in use over a number of years, biochemical studies of their action is almost a virgin field of endeavour, according to Dr Ainscough.

Dr Mary Earle (Department of Food Science) recently received a 3-year research contract from the DSIR. The contract will assist the Food Technology Research Centre in its work to increase the processing of NZ's biological raw materials for export markets. This work will cover basic and applied research involving new technologies and product development for a wide range of materials and processes. It will also provide a necessary advisory newletter for industry.

Canterbury

There are three Visiting Lecturers for 1981 in the Chemistry Department. **Miss**

Jill Ussher (an Auckland graduate) from Westland High School, Hokitika, will spend the year in the department and is principally associated with first-year classes. **Dr Ron Baumgarten,** an organic chemist from the University of Illinois, Chicago Circle, is in the department until the end of June. **Prof. J.T. Spence,** Head of Chemistry, Utah State University, will be at Canterbury for 5 months from July. Prof. Spence has wide research interests in inorganic and bio-inorganic fields as well as a teaching interest in analytical chemistry.

Two other visitors are, or will be, in the Chemistry Department on leave from their home institutions during 1981. **Prof. Don Stedman,** University of Michigan, Ann Arbor, is an atmospheric chemist working in both the Departments of Chemistry and of Atmospheric and Oceanic Science, where his research group carries out experiments on the chemistry of both clean and polluted air. While in NZ he is carrying out measurements of background nitrogen oxide levels in the troposphere. **Prof. W.C. Agosta,** Rockefeller University, New York, will be a visiting Erskine Fellow from mid-July. Prof. Agosta's main research interests lie in the general field of synthetic organic chemistry with a particular emphasis on photochemical processes.

Visitors who have recently passed through Canterbury and given seminars include **Prof. Heinrich Vahrenkamp,** Freiburg, West Germany ("Some aspects of metal-metal bonding") and **Prof. B.R. McGarvey,** New South Wales ("Magnetic Properties of Iron II Complexes").

Prof. Leon Phillips recently attended a symposium on atmospheric chemistry at the Australian Academy of Sciences, Canberra and **Dr Colin Freeman** the ANZAAS Congress at Brisbane.

Christchurch Polytechnic

Dr Selwyn Malster is spending the year on exchange at Norwich City College, England. His position in Christchurch is being occupied by **Gordon Livingstone,** who has had past teaching experience in grammar schools, colleges of further education and polytechnics.

Canterbury Junior Chemical Society 1981 Field Day

54 seventh-form members of the Junior Chemical Society recently spent the day in the University Chemistry Department on their annual "field day". The participants (under the fatherly guidance of **Dr Ward Robinson**) examined the topic "Computers in Chemistry" and used a micro-computer to experiment with models of various chemical systems.

Canterbury Science Teachers Association

A recent Chemistry Sectional meeting was devoted to the topic "Computers in the Teaching of Chemistry". About 40 members were present at the meeting which was addressed by **Prof. Bruce Penfold** and **Dr John Blunt** and followed by practical exercises.

Otago

Chemistry Department

Dr M. Woods was a recent visitor to the department. He is from Birkbeck College, London and he gave a talk on "Recent Aspects of Phosphorus and Nitrogen Chemistry". Another visitor was **Prof. B.R. McGarvey** from the University of Windsor, Ontario who spoke on "esr, nmr, and Mossbauer studies of Fe(II) complexes.

Dr B.M. Peake has recently returned from UK where he delivered a plenary lecture at the Royal Chemical Society International esr Conference held at Lancaster University.

Biochemistry Department

Dr I.T. Forrester has been awarded the prestigious Claude McCarthy Fellowship. He will be undertaking a year of full time research on spermatozoa viability, working within the department, with the Invermay Animal Research Station, and including a period in Canada.

Wellcome Institute

Dr D.B. Myers is on sabbatical leave at the Institute of Medical and Veterinary Sciences in Adelaide.

Textiles Department

Prof. R.H. Peters, head of the Department of Polymer and Fibre Science, University of Manchester Institute of Science and Technology has accepted a William Evans Visiting Professorship offered by Otago University. Prof. Peters is an international figure in the field of Textile Chemistry and an acknowledged world authority on the physical chemistry of dyeing processes.

Central Institute of Technology

Seminars recently held included: "An Industrial Pharmacist's View of our Pharmaceutical Industry" by **Mr N. Baxter,** May and Baker Ltd, "Bile Acids Clue, Curse and Curve" by **Dr D. Collins,** Wallaceville Animal Research Centre, and "Frusemide and All That — First Flush of Success" by **Dr M.Kingsford,** Chemistry Division, DSIR.

Cover Story

Perkin-Elmer Unveils Sigma 115 Gas Chromatograph And Data Handling System

The Sigma 115 was introduced by Perkin-Elmer at the Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy this year. It has evolved from refining and improving the Sigma 1B.

For about $25,000 it is said to offer sophistication in capabilities unmatched by other currently marketed data handling/gas chromatography systems.

In its simplest form, the SIGMA 115 is a standard chromatographic analyzer with integral microprocessor control from the console. The console is capable of printing 56 characters/sec. and has a device which provides self-aligning paper. BASIC 2 programmability is standard. Memory expansion for on-line storage can be increased to a total of 32K bytes. Speed data transfer and receipt has been doubled.

The most interesting change is the addition of the Level 3 chromatography communications and control. Standard features of the Level 3 option include 16K bytes of memory for on-line storage, the RS-232C interface, a modem eliminator for phone link-ups and the most sophisticated version of SIGMA BASIC providing automatic communication with, and control of, data-handling peripheral devices. This means, say the NZ distributors, Warburton Franki Ltd, that the SIGMA 115 Level 3 is the most sophisticated data handling/gas chromatography system available today.

SIGMA BASIC Level 3's flexibility allows users to replot chromatograms, fix a drifting baseline and plot baseline-corrected chromatograms (the optional digital plotter can be used for replotting chromatograms). The software supplied with the plotter, accesses time-slice and peak file data and prompts the analyst through the replot/reintegrate routine. The analyst can select a portion or the whole of the chromatogram for replot; chromatograms can be labelled with retention times and the peaks can be named if desired.

Floppy disk storage, CRT and keyboard in the 3600 Data Station are available if required.

The keyboard and CRT can be used as a terminal to the SIGMA 115 for BASIC program writing, editing or running. A chromatogram can be called up from floppy disk storage and displayed on the CRT; software provided with the system allows users to manipulate the displayed chromatogram as required and for as long as needed.

Flexibility doesn't end with chromatogram manipulation. A chromatogram can be displayed on the Model 3600's CRT **in real time,** while another is being plotted on the console's printer/plotter.

Further information can be obtained from Elizabeth Dengate-Thrush (Ph. Wellington 698-272) or Josina Van Houtte (Ph. Auckland 770-924).

C 082 For further details, use Reader Service Card

Equipment Update

The Modern Gas Chromatograph

ADVANCED ELECTRONICS LTD

Tracor Instruments Group

Tracor manufactures four different gas chromatographs. Three of these, Models 560, 550 and 222, are designed for general purpose GC operation, while the Model 150G is for trace gas analysis.

The Model 560 is a dual column chromatograph, with all temperature and timing functions under digital control. Up to 3 detectors can be mounted, while any two can be operated simultaneously.

Models 550 and 222 can have up to 4 detectors mounted. All 3 instruments have independent heating for each detector and each inlet. There is a choice of 8 detector systems: FID, ECD, NPD, FPD, Hall Electrolytic Conductivity (HECD), Photo Ionisation (PID), TCD and Ultrasonic.

The Model 150G trace gas analyser uses either TCD or ultrasonic detectors.

Examples of all these chromatographs and detector systems are in use in NZ.

C 083 For further details, use Reader Service Card

WARBURTON FRANKI LTD

Perkin-Elmer

Perkin-Elmer is well known for its Sigma range of gas chromatographs. Within the series there are 4 instrument families, each offering varying degrees of complexity but all based on a common unit, the 'Chromatographic Analyzer' which contains all the essential chromatographic equipment.

The Sigma 4B gas chromatographs are the simplest, being dedicated isothermal instruments designed for single or dual channel operation. There are six different detectors available, namely FID, NPD, FPD, ECD, TCD and a new WPA water pollution analyzer for the analysis of trihalomethanes in drinking water.

The Sigma 3B models are intended for single ramp temperature programmed operation. Injector, detector and column oven temperatures are controlled by a micro-processor with keyboard entry of all parameters and visual display of set and actual values. Permanently resident in the memory of the Sigma 3B is a service diagnostic chip.

The more complex Sigma 2B is a dual ramp temperature programmed microprocessor-controlled instrument.

The Sigma 115 is the latest and most sophisticated of the range with the chromatograph operated from a free-standing data module, the Sigma 15. SIGMA BASIC is standard and allows bi-directional intelligent communication with large computers, and can serve as a terminal for a computer. LEVEL 3 with optional digital plotter enables the analyst to replot and reintegrate selected peaks in chromatograms. Perkin-Elmer offers a full complement of data handling peripherals, the Model 981 Intelligent Mini-disk for bulk storage, the Model 550 Visual Display Unit which provides a screen and second keyboard for program writing and editing. For both floppy disk storage and CRT, Perkin-Elmer offers the Model 3600 Data Station.

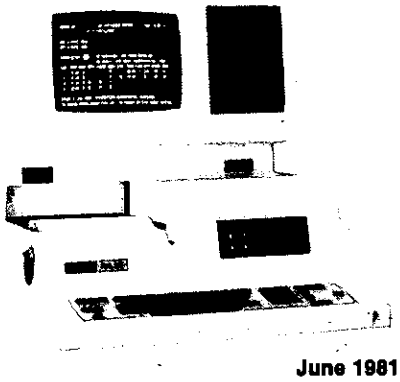
Sigma accessories include AS100B auto sampler, HS-6 head space sampler, MS41 Solid sampler, CDS-190 pyroprobe, gas sampling and column switching valves, for back flushing and peak cutting.

C 084 For further details, use Reader Service Card

WILTON SCIENTIFIC LTD

Varian

The Vista 401 is the data system at the heart of the new chromatography from Varian, and is described as the most powerful, innovative and intelligent data system currently available to the Chromatographer. It features 232 K Memory, built in dual printer/plotter, 12" CRT and interactive keyboard, expandable to 1 to 4 channel operation with optional remote 2 channel printer/plotter for full simultaneous 4 channel capability. Other options include on-line single or dual floppy disk(s) for additional capability and memory storage. The Vista 401 (illustrated) does data acquisition, calculations and reporting common to chromatography and can communicate with peripheral computers.



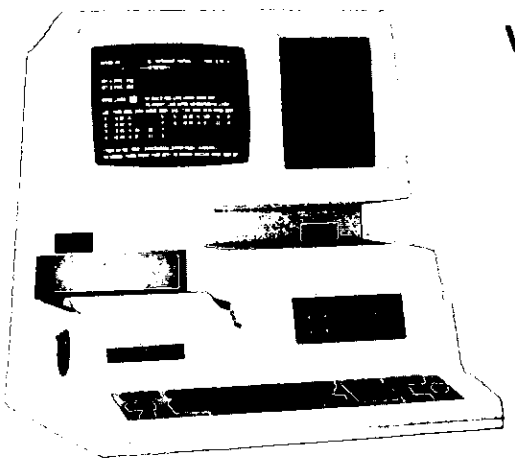
VISTA the new chromatography from Varian

You can choose a Varian chromatography system that will meet your needs today and tomorrow and tomorrow and tomorrow...

No matter what gas and/or liquid chromatography capability you need, Varian's VISTA Series lets you structure a system that makes the best sense for your requirements.

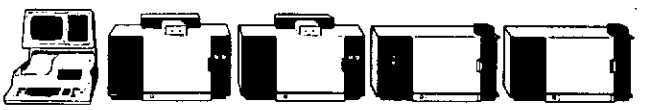
VISTA systems are modular and compatible with one another and you can use them to automate the gas and liquid chromatographs already in your lab. As your chromatography needs change and grow, your VISTA System will grow with you.

The new Varian VISTA Chromatography Systems...



VISTA 401 Chromatography Data System

VISTA 401 provides sophisticated data handling and automatic instrument control for up to four VISTA chromatographs in a wide variety of configurations (see example below). It will handle the data from any four GC's and/or LC's already in your lab. Performance features include: friendly interactive CRT-and-keyboard, large memory to hold methods, on-line floppy disk storage, and a built-in, dual-channel printer/plotter that gives you the analysis report and annotated chromatogram on one piece of paper.



varian

WILTON SCIENTIFIC LTD.

P.O. Box 31044, Lower Hutt
P.O. Box 9071, Auckland
P.O. Box 22705, Christchurch
P.O. Box 1424, Dunedin

Instrument Gases Now Made In NZ

Laboratory gases which have a guaranteed minimum purity are now being prepared in NZ for use in analytical instruments.

The instrument grade gases are specifically produced by NZ Industrial Gases Ltd for use in high precision or complex analytical work. Until now, industrial grade gases — which are not made to analytical accuracy — have been used.

"To get the best results from highly specialised analytical equipment, it is essential that the correct purity of gas be used," says NZIG's product manager for special gases, **Philip Best.**

Many laboratories use oxygen, nitrogen, carbon dioxide and hydrogen in their work, but until now, the only gases available have been industrial grade or high priced imports.

"While industrial grade gases are adequate for their designed uses, they are often not satisfactory for use in precision analytical work," he says.

"Selecting the correct purity of gas — for use in equipment such as gas chromatographs, flame ionization, atomic absorption and the like — can have a great bearing on the success of the project. After all, gases form an integral part of this analytical equipment."

A certificate accompanies all instrument grade gas cylinders to show the trace components analysed.

An example, which compared the purities of the various grades of nitrogen, highlights the accuracy of the new service. Industrial dry nitrogen is about 99.5% pure. Its principal impurities are oxygen, carbon dioxide, hydrogen, neon, helium, argon and water.

Instrument grade nitrogen is guaranteed to be better than 99.9% pure. The impurities are: oxygen (less than 10vpm); carbon dioxide (less than 5vpm); hydrogen (less than 1vpm); neon, helium, argon (together not totalling 7vpm). Water vapour is less than 0.01 grams/m³. This means a frost point of less than -60°F.

As another example, hydrogen usually runs at around 99.7% pure. Instrument grade hydrogen is guaranteed to have:

- less than .1 percent oxygen;
- less than .1 percent nitrogen;
- less than 100vpm of carbon dioxide;
- less than 15vpm of other carbon compounds (measured as carbon dioxide). The frost point could be guaranteed to be less than -60°F.

High purity helium is used in gas chromatographs, which use thermal conductivity detectors. Some more sensitive instruments use ultra high purity — about 99.9999% pure and gases to this standard are also available. Depending on the type of analysis being made, the analyst could use argon, hydrogen, or nitrogen — the normal alternatives, although there are others. Oxygen or air cannot be used as these would burn out the thermal conductivity filaments.

Analysts will certainly obtain better results from their equipment by using the correct purity of gas. But they should also use the correct gas equipment. This should be equipment of an instrument grade nature — not of an industrial grade. The user can then be certain that the instrument grade gas leaving the cylinder to enter the analytical equipment will not be altered by the equipment through which it must pass — which could affect the result of work being undertaken.

C 080 For further details, use Reader Service Card

CHROMATOGRAPH (Cont)

The Vista Chromatography System includes Vista GLC and Vista HPLC instrumentation. Any 4 (mixed) chromatographs may be fully controlled by one 401, including multiple auto sampling devices. Vista systems are modular and compatible with one another; furthermore they can be used to automate gas and liquid chromatographs already in the laboratory. As a user's chromatography needs change and grow the Vista system will grow and adapt to these needs.

The Vista 54 HPLC series is based on the well established Varian 5000 HPLC instruments. These are microputer based, having binary and ternary gradient capability, with a comprehensive range of UV, RI and Fluorescence detectors, automated loop and carrousel sample changes, all fully compatible with the 401.

The Vista 44 instruments are microprocessor based gas chromatographs designed for 401 control. They are derived from the popular 3700 series of GLC's and feature the same comprehensive detector range TCD, FID, ECD, TSD, FPD, HECD as well as simultaneous capillary and packed column capability and optional auto samplers.

A new range of GLC instruments known as the 6000 series are being introduced to the NZ market. These are microprocessor controlled and feature CRT and interactive keyboard. Their physical layout and dimensions are derived from the 3700 series. The 6000 instruments are fully compatible with the Vista 401, yet are capable of stand-alone operation.

The Vista 401 will also interface with the Varian 3700 GLC and 5000 HPLC series and the 401 may be used in conjunction with any other manufacturer's chromatographs.

C 086 For further details, use Reader Service Card

POTENTIOSTAT/GALVANOSTAT FOR ELECTROCHEMISTRY

A new low-cost potentiostat/galvanostat (Model 363) has been announced by EG & G Princeton Applied Research for use in electrochemical applications requiring either potential or current control. This powerful yet cost-effective unit should find broad application in such diverse fields as electro-organic synthesis, battery and fuel cell testing, corrosion measurements and electrochemical thickness determinations, according to local agents ANAC Ltd.

The bipolar potentiostat features plus or minus 1A current capability at up to plus or minus 30V. A 4-digit thumbwheel switch controls the applied voltage or current. The range of potential control is plus or minus 9.9999V, while current ranges from 1μa to 1A are available. The convenient front panel meter allows the user to visually monitor either the current or potential while front panel outputs are available so that current and potential can be recorded. A connector is available on the front panel to accept externally-supplied waveforms such as ramps, triangles, sinusoids, and trapezoids for various types of voltammetry. The built-in electrometer prevents reference electrode loading and provides a low-impedance, unity-gain monitoring point for the working electrode potential.

The unit has been designed for easy interface with other EG & G PARC instruments and accessories.

C 081 For further details, use Reader Service Card

CONFERENCE CHECKLIST

The Canadian Society for Chemical Engineering has called for papers for the 2nd World Congress of Chemical Engineering, to be held in Montreal, October 4-9, 1981. It is expected to draw over 3000 chemical engineers from all parts of the world to discuss on the latest advances in their field. Some 700 presentations are expected under the general theme of "Chemical Engineering for World Development."

Interested attendees or authors should contact: Congress Secretariat, 2nd World Congress in Chemical Engineering, Canadian Society for Chemical Engineering, 151 Slater St, Suite 906, Ottawa, Ontario, Canada K1P 5H3.

★ ★ ★

The 6th International Conference on Chemical Education with the theme "Teaching Chemistry in a Diverse World", co-sponsored by IUPAC, AGS and Unesco, will be held at College Park, Maryland on 9-14 August, 1981. Enquiries to **Prof Henry Heikkinen,** Chemistry Dept, Box 419, University of Maryland, College Park, MD 20742, USA.

★ ★ ★

Preparations are underway for the 4th International Conference on Organic Synthesis, to be held August 22-27, 1982 in Tokyo.

Moisture/Solids Control

M.J. Collins
(from American Dairy Review)

Traditionally the laboratory tests for moisture/solids in the dairy industry have not been rapid or automated enough to provide real time data which could be used for standardization or process control. The older tests generally involve a gravimetric determination using conventional heating. Drying times typically range from 2-20 hours. In order to reduce this time higher temperatures are used but accuracy and precision generally suffer because of sample degradation due to the absence of a sharp end point in terms of weight loss. Also many of these tests become very subjective with the end point based on a visual appearance or odor and as expected, results vary considerably from operator to operator.

Recognizing the importance of faster, more reliable moisture/solids measurements in the dairy industry, there has been and continues to be considerable work aimed at utilizing some of the newer analytical techniques. As an example, there has been extensive development in using infrared spectroscopy particularly for milk analysis. While the technique works well for milk analysis it is generally considered too expensive for routine quality control and is not applicable to the full range of dairy products.

A basic problem in trying to accurately measure moisture/solids in dairy products exists because these are natural products which vary in composition from day to day. Most analytical techniques are affected by these changes in composition and therefore must be continually calibrated. The ideal technique is one which measures the moisture directly and consequently is unaffected by variations in the composition of the solids.

The idea of using microwave drying for the determination of moisture/solids in all types of dairy products has attracted considerable interest in recent years. Theoretically, it offers the potential for rapid and accurate moisture determinations because it selectively heats and removes only the water contained in the product and it is unaffected by the composition of the remaining solids.

Initially attempts involved the use of conventional, domestic microwave ovens for sample drying. Generally, these ovens proved unsatisfactory due to the uneven distribution of microwave energy ("hot spots") and the problem of handling the excess reflected microwave energy. The speed and reliability of the test for process control was limited by the manual external weighings and calculations.

Recognizing the potential of microwave drying as an analytical technique, CEM Corporation launched a major development effort in this area approximately six years ago. This work culminated with the introduction of an analytical instrument designated as Automatic Volatility Computer (AVC) Model MP which provides completely automatic moisture determinations for all types of dairy products using microwave drying. This instrument is now finding wide use in the dairy industry for standardization and process control as well as raw materials testing.

The purpose of this article is to describe the CEM Moisture/Solids Analyzer and discuss some of the ways it is now being used by the dairy industry for standardization and process control.

CEM moisture/solids analyzer. The CEM instrument consists of a microwave drying system, an analytical electronic balance and a microprocessor based digital computer. The system provides for completely automatic moisture/solids determination with .01% resolution. The system is completely self contained and can be used in production areas as well as quality control laboratories. It is extremely simple to operate and can be used by production as well as laboratory personnel.

The determination is accomplished by monitoring weight loss for a sample while it is dried by exposure to microwave radiation. Water molecules absorb the microwave radiation causing an increase in molecular motion (rotational). Due to this absorption of energy, the water is selectively heated and removed through vaporization. The advantages of microwave drying are speed and selectivity. This increased rate of drying is a result of the uniform heating of the water throughout the sample. In conventional drying ovens, the heating is non-uniform from the outside in and therefore much slower. Drying times are reduced from several hours to minutes using the microwave technique.

As indicated earlier, problems exist in trying to use conventional microwave ovens for this type of drying. The major problems include non-uniform distribution of microwave energy within the cavity and reflected microwave energy which can cause serious damage to the magnetron. In earlier systems tubing with water flowing through it, a water loop, was placed in the oven cavity to absorb the excess microwave energy and eliminate the problem of reflected power. The major disadvantage of this system is that the water loop tends to draw the microwave energy away from the moisture in the sample and interfere with sample drying. The water temperature within the loop must be carefully controlled as it affects the absorption coefficient and any variation will result in an effective variation of the microwave energy available for drying. Also, any interruption in the flow of water through the loop could result in serious damage to the magnetron. In the CEM system the water loop is eliminated by using recent microwave technology. The cavity is completely unloaded except for the sample resulting in much more effective drying and eliminating all of the other problems associated with a water loop.

Utilizing microprocessor control, the CEM system is completely automatic and extremely simple to operate. Anyone can be trained to use the instrument in minutes. Simply place a sample on the balance pan, close the oven door and depress the appropriate pushbutton. From this point on, the instrument operates unattended, displaying the moisture/solids result in a matter of minutes.

A pushbutton actuated automatic tare mode is provided which allows the operator to quickly and accurately zero out container weight. The initial sample weight is stored and the microwave drying system is actuated for a period of time to dry the sample. At the end of the drying interval, the microwave system is turned off, the final weight, weight loss and per cent moisture/solids are displayed on a digital panel readout. These data are stored in computer memory so that they may be recalled at any time after the completion of the analysis, prior to the next determination.

Another feature of the system is the "auto project" mode in which the sample is only partially dried and the final % moisture/solids is mathematically projected from the weight data collected during the drying period. This mode of operation is particularly useful for powder samples which may require longer drying times due to physically bound water.

The Automatic Volatility Computer is designed for providing real time data which can be used for process control or product standardization. The instrument is completely self contained so that it may be located in the production area to minimize sam-

pling time. Also, because it is completely automatic, it may be operated by anyone, again to minimize sample turn around time.

Major dairy applications where the system is being used for control purposes are the following:

- ice cream mix — Complete determination for ice cream requires 3-3.5 minutes. Nominal sample size of 3 grams. Mix can be standardized and also raw material ingredients can be tested.
- butter — Salted butter determination in 1.5 minutes (unsalted butter 5 minutes). Precision of .015% (1 sigma). Knowing the salt and curd content the moisture determination can be used to control the butter fat level. Because the system is self-contained and completely automatic, it can be placed near the churn and operated by the production personnel. This allows almost continuous monitoring and control of the churn.
- processed cheese — Drying time required for most processed cheese products is 2-3 minutes. Location of the instrument can be optimized so that sampling time is minimized. Provides on-line control capabilities.
- natural cheese — For most products drying times range from 2-3 minutes. Cheese may be analyzed directly from the vat. By developing the necessary correlations, vat moisture can be used to control final product moisture. Is also being used to check raw material ingredients in cheese manufacturing.

Other applications include milk solids, dry milk powder, cottage cheese, yogurt and eggs. As the industry becomes more familiar with this new analytical technique, new applications will emerge.

C 085 For further details, use Reader Service Card

(The following item is from Prof. Graham Wright who recently returned to NZ after a subbatical at Southampton, England. As President of the NZIC, he took up the Levich case with the Minister of Foreign Affairs in 1978.)

Levich And Kukk: Chemists Under Pressure

The Electrochemistry Group of the Royal Society of Chemistry presented the Electrochemistry Medal for 1981 to ***Benjamin Levich***

at a meeting of Electrochemists at Loughborough in March. It was a heartwarming occasion. Levich had fallen foul of the authorities in his native Moscow in 1972 when he had sought leave to emigrate from Russia. As a result he lost his senior position at the Institute of Electrochemistry and his chair at Moscow University. He was banned from all scientific work and, together with his family, had to endure a long period of frustration until he was finally permitted to leave the USSR in 1979. Renowned for his research on mass transport at electrodes, turbulence near interfaces, charge transfer processes, and the theory of chemical kinetics, Levich is a distinguished theoretical chemist who has pioneered a number of difficult research fields. His book on ***Physico-Chemical Hydrodynamics*** *is the basis of what are now standard experimental techniques in electrochemistry and fluid mechanics.*

The medal presentation ceremony took place at the University of Technology in Loughborough, one of Britain's newest universities with strong emphasis on applied science, engineering and physical education. It is an all-residential University, with a sweeping campus and modern halls of residence; it was an excellent venue for the informal spring meeting of the Electrochemistry Group, with Prof. Levich as the guest of honour. But by a tragic coincidence, on the second day of the meeting, March 27, 1981, another Soviet electrochemist ***Juri Kukk*** *died in a labour camp near Murmansk.*

Dr. Juri A. Kukk, aged 40, was an Assistant-Professor in Inorganic Chemistry at the Tartu State Univeristy in Estonia. His research was concerned with the kinetics of electrode reactions on metals and alloys, including hydrogen overpotential and the discovery of implantation of alkali and alkaline-earth metals in tin cathodes[1]. After a period of research in France at the CRNS Laboratory of Interfacial Electrochemistry during 1975-76, he requested permission to emigrate with his family in May 1979. As a result he was suspended from his post at Tartu University in September 1979 and his position rapidly became difficult. A British electrochemist, ***Prof. Graham Hills*** *met him in Moscow in October 1979. "He was clearly desperate," reported Hills. "It was already being said about him that he was deranged but I formed the opinion that he was simply a tired, persecuted man." During 1980 Juri Kukk was arrested twice by the KGB, and he was harassed and subjected to enforced psychiatric examinations and detention. Finally, in January 1981, he was sentenced to 2 years in a labour camp for anti-Soviet propaganda activities. He began a hunger strike and he was moved from an Estonian labour camp to a camp in the Arctic circle where he died.*

Like most chemists and other scientists I feel uneasy about raising matters concerning human rights, particularly in a professional body like NZIC. Objections are sometimes raised that these are political matters, that the facts are not completely known, and that there is precious little that we can do about it anyway. But these objections can be answered: denial of human rights and injustice to individuals are a personal affront whether they result from politics or any other cause. We are entitled to support a colleague who is suffering personal injustice whatever the origin. As for the facts of each case, it is perfectly true that one can never be sure of knowing the complete story, but it seems worthwhile to put on record as much as we do know, in the hope that any errors or omissions can then be corrected in the future and be put right. The most effective action against alleged injustice seems to be to publicise each case. The Levich affair has shown that sustained publicity throughout the scientific community can be effective — protests and support for Levich from various parts of the world, including NZIC, over a period of 5 years had their effect on the Soviet authorities who had to consider the reputation of Soviet Science in the long term.

One feature of the Levich affair was the apparent involvement of the scientific establishment in cutting off all his opportunities to pursue scientific work, not only banning his publications but also banning references to his papers by other Soviet authors. In an attempt to justify this treatment one Russian scientist stated that Levich had not been doing good scientific work for some years and that he was feeding off other people. Even if it were true, this would be no reason for such treatment. But the last word was from Levich himself, who in his Medallist's address to the Loughborough Conference gave a masterly review of electrochemical research, with many penetrating comments on current theories and ideas for new developments. Conferences do serve a useful purpose after all!

Graham Wright

Papers available in English:
Yu. A. Kurr and V.E. Past, Cathodic injection of alkali-earth metals in tin. *Soviet Electrochem., 7,* 1799 (1971).
Yu. A. Kukk and V.E. Past, Effect of alkali solution composition on hydrogen overvoltage on tin. *Soviet Electrochem., 7,* 1796 (1971).

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