The ANALYST

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THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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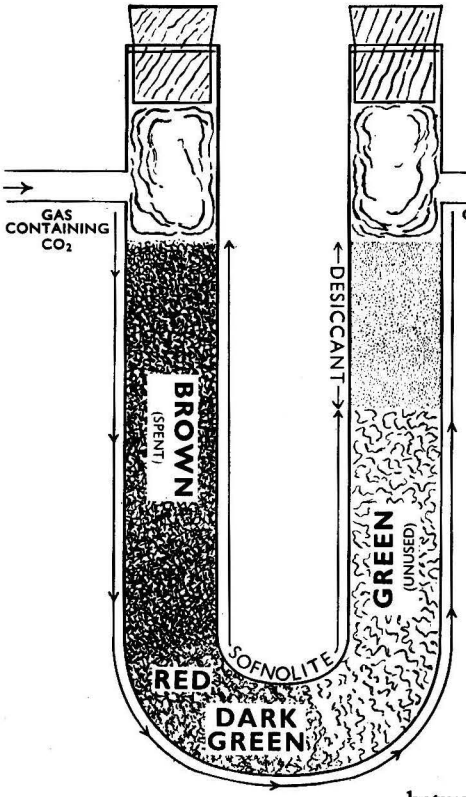

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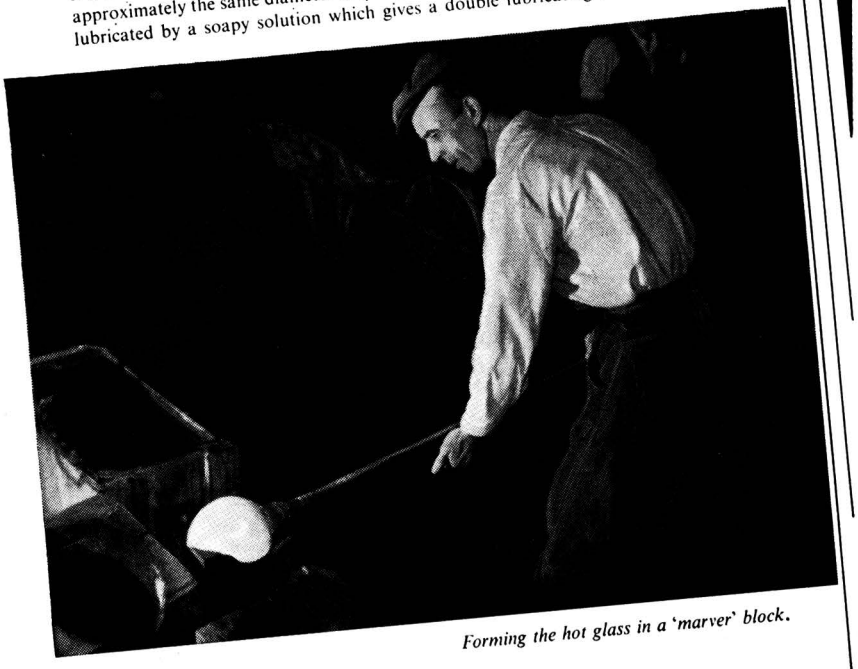

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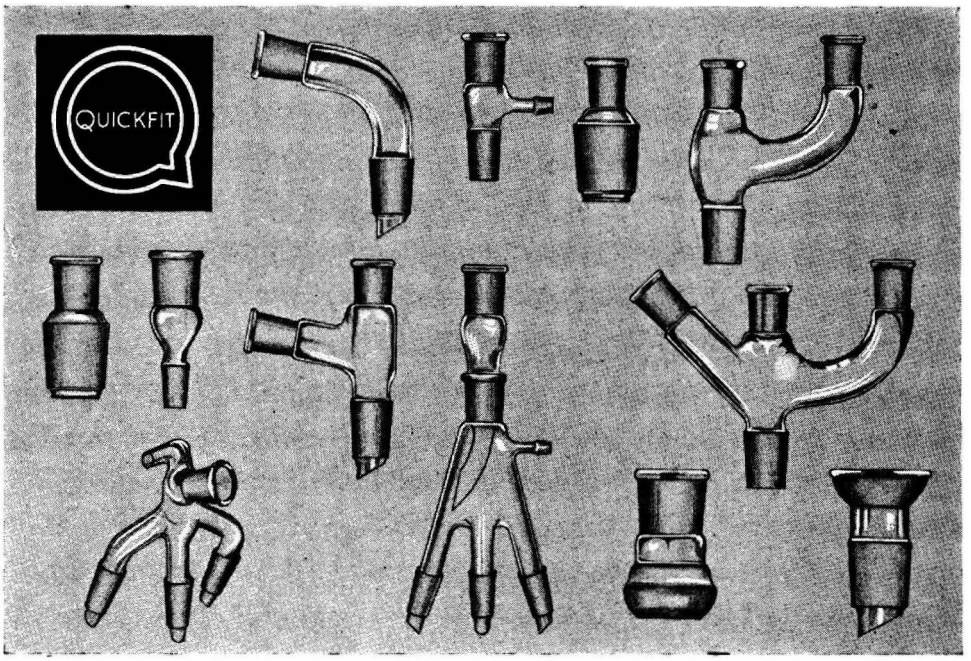

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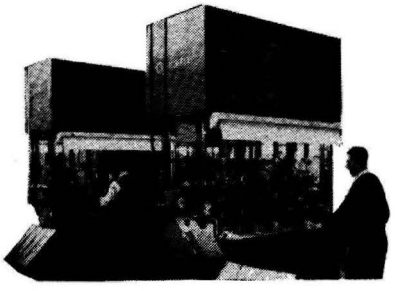

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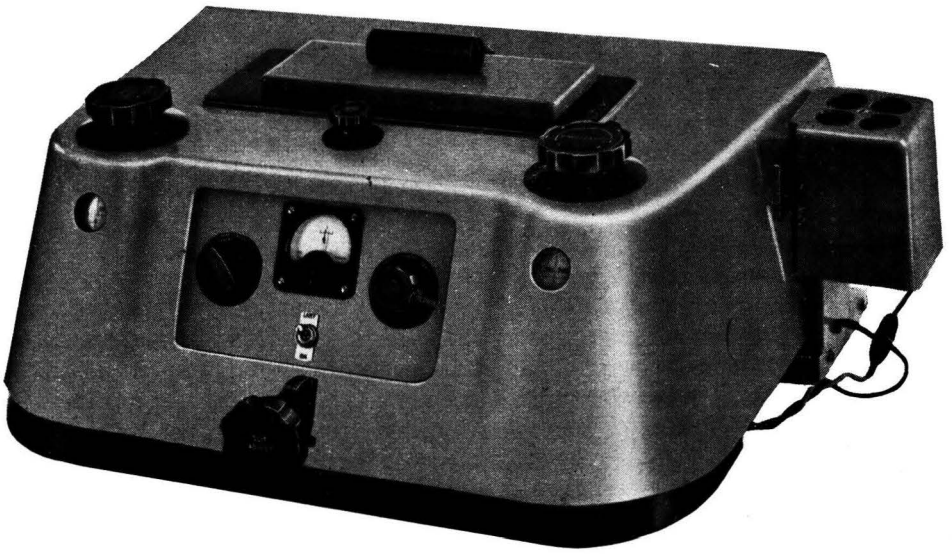

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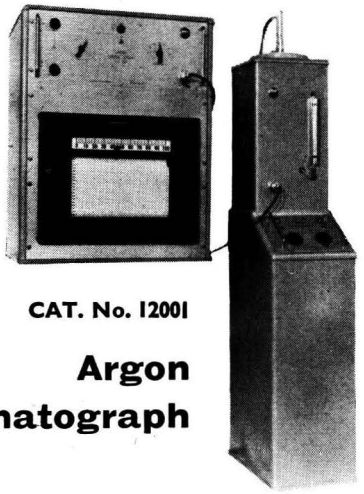

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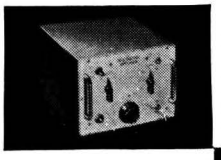

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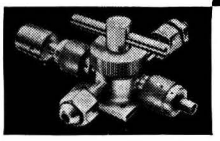

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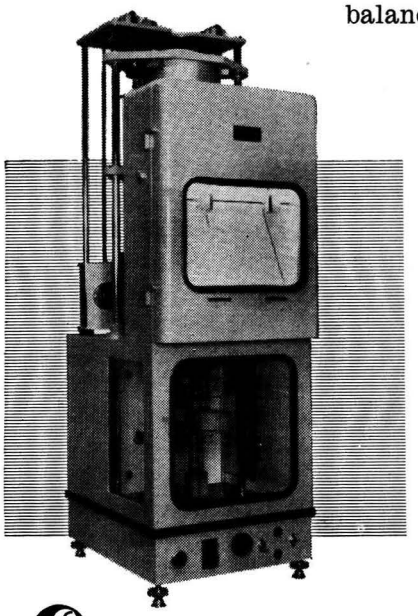

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ANALYST:

The Determination of Exchangeable Sodium, Potassium, Calcium and Magnesium in Soils by Atomic-Absorption Spectrophotometry. *David, D. J.* **85,** 495 (1960)

The Application of Atomic Absorption to Chemical Analysis. A Review. *David, D. J.* **85,** 779 (1960)

Atomic-Absorption Spectrophotometry with Special Reference to the Determination of Magnesium. *Allan, J. E.* **83,** 466 (1958)

Determination of Zinc and Other Elements in Plants by Atomic-Absorption Spectroscopy. *David, J. E.* **83,** 655 (1958)

The Quantitative Determination of Some Noble Metals by Atomic-Absorption Spectroscopy. *Lockyer, R., Hames, G. E.* **84,** 385 (1959)

Determination of Calcuim in Plant Material by Atomic-Absorption Spectrophotometry. *David, D. J.* **84,** 536 (1959)

Determination of Zinc in Metallurgical Materials by Atomic-Absorption Spectroscopy. *Gidley, J. A. F., Jones, J. T.* **85,** 249 (1960)

NATURE:

Atomic-Absorption Spectrophotometric Determination of Molybdenum and Strontium. *David, D. J.* **187,** 1109 (1960)

Determination of Nickel and Cobalt by Atomic-Absorption. *Allen, J. E.* **187,** 1110 (1960)

Determination of Silver in Lead Concentrates by Atomic-Absorption Spectroscopy. *Rawling, B. S. et al.* **188,** 137 (1960)

Determination of Magnesium in Blood Serum by Atomic-Absorption Spectroscopy, *Willis, J. B.* **184** (4681), **187** (1959)

Some Atomic Reactions by Absorption Spectroscopy. *Broida, H. P., Schiff, H. I., Sugden, T. M.* **185,** 759 (1960)

Determination of Calcium in Blood Serum by Atomic-Absorption Spectrosopy. *Willis, J. H.* **186** (4720), 249 (1960)

ANALYTICAL CHEMISTRY:

Atomic-Absorption Spectroscopy. (Survey). *Robinson, J. W.* **32,** 17A (1960)

A Study of Atomic-Absorption Spectroscopy *Menzies, A. C.* **32,** 898 (1960)

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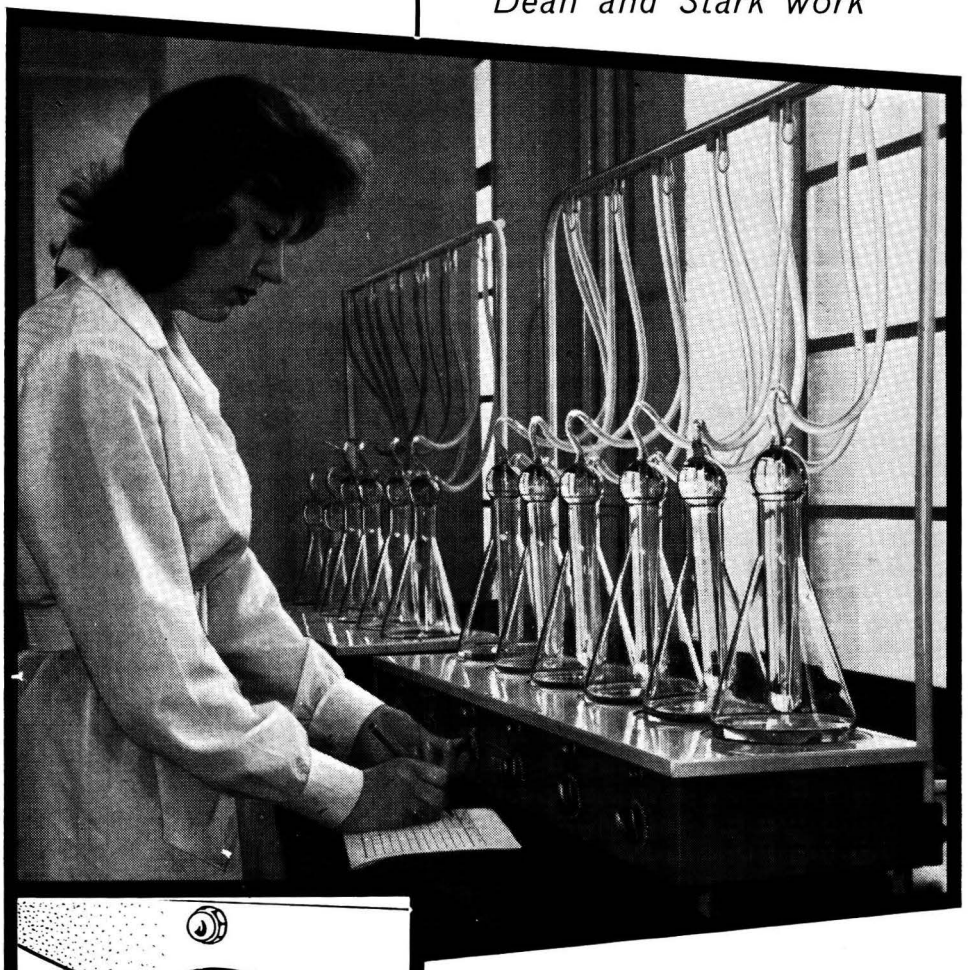

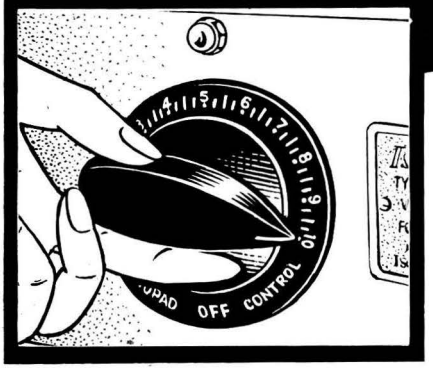

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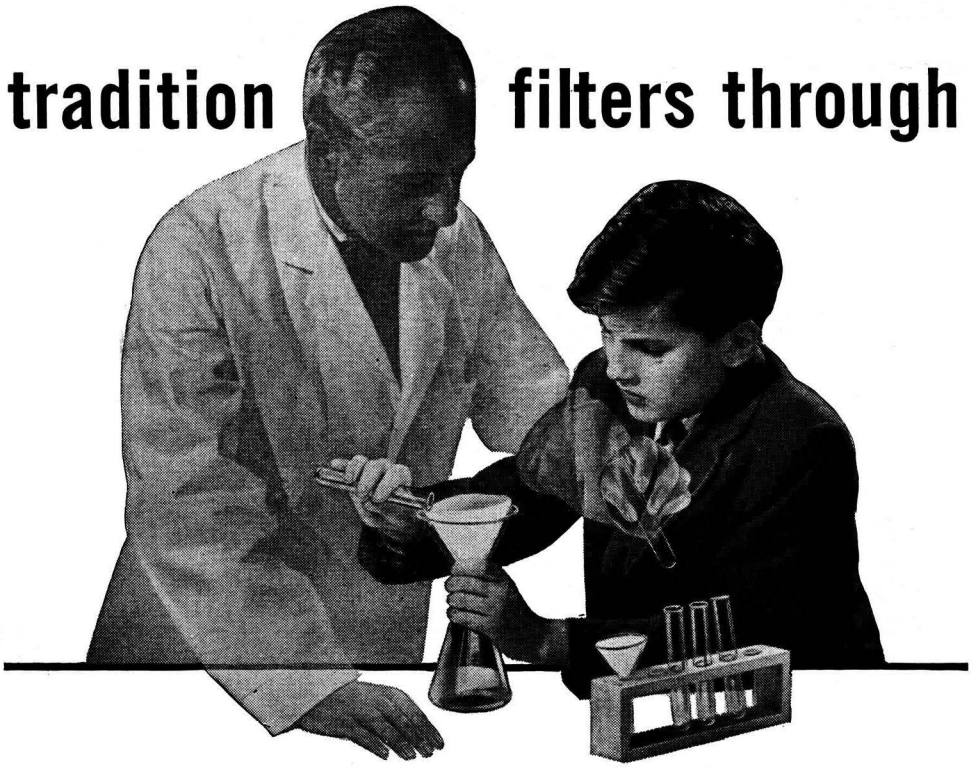

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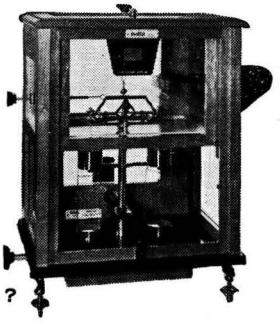

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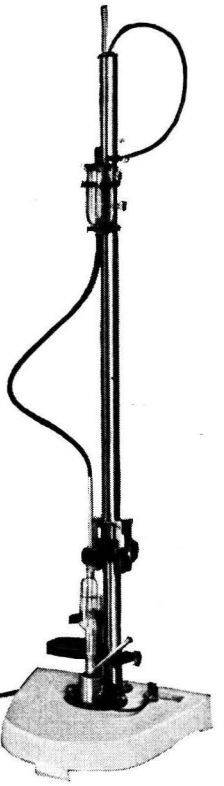

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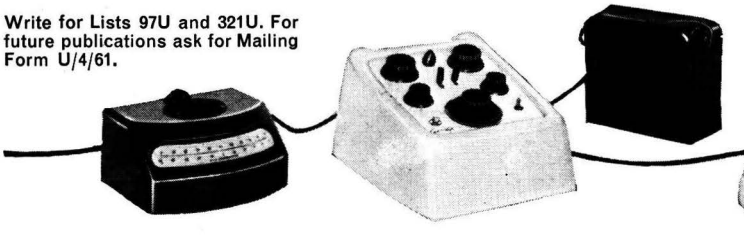

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CONTENTS OF VOL. I—PART I

Proceedings of the Radioactivation Analysis Symposium, Vienna, 1959. Recommended Test Substances for the microdetermination of carbon and hydrogen. Report on calibration of wavelength and photometric scales of non-recording spectrophotometers. Report on the standardization of pH and related terminology. Terminology for scales of working in microchemical analysis. Recommendations for terminology to be used with precision balances. Preliminary recommendations on nomenclature and presentation of data in gas chromatography.

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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

DEATH

We record with regret the death of

Albert Arthur Henly.

SCOTTISH SECTION

A Joint Meeting of the Scottish Section with the Glasgow and West of Scotland Section of the Royal Institute of Chemistry was held at 7.15 p.m. on Friday, February 24th, 1961, in the Royal College of Science and Technology, George Street, Glasgow, C.1. The Chair was taken by the Chairman of the Section, Mr. A. F. Williams, B.Sc., F.R.I.C.

The following papers were presented and discussed: "Applications of Ion-exchange in Inorganic Analytical Chemistry," by R. A. Wells, B.Sc., F.R.I.C., M.I.M.M.; "Ion-exchange in the Electroplating Industry," by V. E. Gripp, B.Sc., A.R.C.S., A.R.I.C. (see summaries below).

Applications of Ion-exchange in Inorganic Analytical Chemistry

Mr. R. A. Wells discussed the fundamental properties of ion exchangers, ion-exchange equilibria and ion-exchange kinetics in the context of inorganic analytical chemistry. The various ways in which the inorganic analyst might employ ion-exchange were illustrated by examples. He proposed possible further extensions of the use of ion-exchange in analytical chemistry, with particular reference to the use of modified cellulose exchangers, liquid ion exchangers, ion-exchange membranes and selective resins.

Ion-exchange in the Electroplating Industry

Mr. V. E. Gripp discussed the elementary principles of ion-exchange and gave a brief review of their historical development and their use in large-scale plants for the purposes of water treatment and so on. This industrial usage of the ion-exchange processes was further amplified by reference to specific applications in the electroplating industry. The process had found extensive practical use in the metal-finishing trade from two entirely different stand-points, namely the purification of metal-treatment liquors, *e.g.*, chrome anodising baths, and also as a means of decontaminating the emergent swill waters from metal-finishing shops. The purification of electroplating solutions represented an important economic advantage that had been well proved in practice, and the speaker amplified this theme by reference to several specific examples, during which he conveyed some idea of the scale upon which they were practised. The treatment of dilute swill waters from, *e.g.*, chromium-plating processes, permitted several advantages to be gained at the same time by using a demineralising technique. Water was largely recovered in a purified condition for re-use, and the capital cost of effluent treatment was greatly reduced because the volume of the plant outflow was drastically curtailed. Several particular points of technical interest arose during the consideration of swill-water treatment, and he enlarged upon these during the lecture.

WESTERN SECTION

THE sixth Annual General Meeting of the Section was held at 5.45 p.m. on Thursday, January 12th, 1961, at the University of Bristol. The Chair was taken by the Chairman of the Section, Dr. G. V. James, M.B.E., F.R.I.C. The following appointments were made for the ensuing year: *Chairman*—Dr. G. V. James. *Vice-Chairman*—Dr. F. H. Pollard. *Hon. Secretary and Treasurer*—Dr. T. G. Morris, Brockleigh, Clevedon Avenue, Sully, Glamorgan. *Members of Committee*—Dr. R. G. H. B. Boddy, Mr. R. E. Coulson, Mr. S. Dixon, Mr. E. A. Hontoir and Mr. R. F. Stephens. Mr. C. H. Manley and Dr. W. J. Williams were appointed Hon. Auditors.

The Annual General Meeting was followed by a Joint Meeting with the Bristol and District Section of the Royal Institute of Chemistry at which the Chair was taken by the Chairman of the Bristol and District Section, Dr. R. Woodcock, F.R.I.C. A lecture on "The Analysis of Plastics" was given by J. Haslam, D.Sc., F.R.I.C.

A JOINT Meeting of the Western Section and the Bristol and District Section of the Royal Institute of Chemistry was held at 7.30 p.m. on Thursday, March 2nd, 1961, at the Technical College, Brunswick Road, Gloucester. The Chair was taken by the Chairman of the Bristol and District Section, Dr. R. Woodcock, F.R.I.C.

The following paper was presented and discussed: "Titrations in Non-aqueous Media," by E. Minshall, M.Sc., F.R.I.C.

A JOINT Meeting of the Western Section and the South Wales Section of the Royal Institute of Chemistry was held at 6 p.m. on Friday, March 10th, 1961, in the Large Lecture Theatre of the New Chemistry Building of University College, Singleton Park, Swansea. The Chair was taken by Mr. P. F. Ellis, M.B.E., B.Sc., A.R.I.C., past Chairman of the South Wales Section.

The following paper was presented and discussed: "Vapour-phase Chromatography," by A. Verdin.

MIDLANDS SECTION

A JOINT Meeting of the Midlands Section with the Association of Clinical Biochemists was held at 6.30 p.m. on Thursday, February 16th, 1961, in the Lecture Theatre, General Hospital, Steelhouse Lane, Birmingham, 4. The Chair was taken by the Chairman of the Midlands Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

The following papers were presented and discussed: "The Use of Vapour-phase Chromatography in the Study of Human Lipid Metabolism," by H. G. Sammons, B.Sc., Ph.D.; "Some Recent Developments in Paper Chromatography," by C. S. Knight, M.Sc., Ph.D., A.R.I.C.

AN Ordinary Meeting of the Section was held at 6.30 p.m. on Wednesday, March 8th, 1961, at the Technical College, Park Square, Luton. The Chair was taken by the Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

The following paper was presented and discussed: "Some Newer Reagents in Analytical Chemistry," by Professor R. Belcher, Ph.D., D.Sc., F.R.I.C., F.Inst.F.

MICROCHEMISTRY GROUP

THE Seventeenth Annual General Meeting of the Group was held at 6.45 p.m. on Friday, February 24th, 1961, in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1. The Chair was taken by the Chairman of the Group, Mr. F. Holmes, B.Sc., A.R.I.C. The following Officers and Committee Members were elected for the forthcoming year: *Chairman*—Mr. C. Whalley. *Vice-Chairman*—Miss M. Corner. *Hon. Secretary*—Mr. D. W. Wilson, Department of Chemistry, Sir John Cass College, Jewry Street, Aldgate, London, E.C.3. *Hon. Treasurer*—Mr. G. Ingram. *Members of Committee*—Mr. S. Bance, Mr. C. B. Dennis, Miss J. P. Dixon, Mr. F. Holmes, Dr. J. A. Hunter and Mr. H. C. J. Saint. Dr. L. H. N. Cooper and Mr. H. Childs were re-appointed as Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Group at which the Chair was taken by the new Chairman of the Group, Mr. C. Whalley. The following paper was presented and discussed: "The Determination of Metals by Organic Reagents," by F. Holmes, B.Sc., A.R.I.C. (see summary below).

The Determination of Metals by Organic Reagents

Mr. F. Holmes reviewed briefly the factors influencing the formation of metal complexes and then described how the nature of the complexes affected their solubility; for insoluble complexes the effect of pH was also mentioned. He then surveyed the possibilities of chelate ring formation in the light of the ligand atoms involved and the flexibility of the rings formed; both bidentate and polydentate ligands were dealt with.

After this introduction the lecturer passed on to a consideration of unselective sequestering and precipitating agents. These were surveyed in general terms, then EDTA and structural modifications of it were considered, from both the practical and theoretical points of view. He pointed out the consequences of changing the molecule in various ways in attempts to make it more selective, or its complexes more stable.

Following this he made some mention of nitrogen- and sulphur-containing ligands, which were more selective for transition metals and B-sub-group metals. The relationship between stability and configuration were indicated for different types of co-ordination and different ligand molecules, and he discussed the limitations imposed by the Irving - Williams order.

The rest of the lecture was devoted to a consideration of two highly selective groups of reagents—those based on two oxime groups and used for the gravimetric determination of nickel and palladium, and those containing the —N=C—C=N— group and used for the spectrophotometric determination of ferrous, cuprous and other reducing cations. He gave an indication of the charge-transfer spectra associated with these latter reagents. Mr. Holmes dealt at some length with modern work on substituted bipyridines and *o*-phenanthrolines and discussed the properties of the chelates formed by these compounds. He concluded by saying that, although these properties might be understood once they were known, it was not generally possible to predict them quantitatively.

PHYSICAL METHODS GROUP

The Seventy-fifth Ordinary Meeting of the Group was held at 6 p.m. on Tuesday, February 28th, 1961, in the Cuthbert Wallace Lecture Room, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2. The Chair was taken by the Chairman of the Group, Dr. G. W. C. Milner, F.R.I.C., A.Inst.P.

The subject of the meeting was "Physical Methods of Analysis Used in Medical Research" and the following papers were presented and discussed: "Gas Chromatography and Anaesthetic Research," by D. W. Hill, M.Sc., A.Inst.P., A.M.I.E.E.; "The Measurement of the Oxygen Tension of Blood by Means of a Covered Platinum Electrode," by J. M. Bishop, M.D., M.R.C.P.; "Application of Infra-red Spectroscopy to Some Problems of Medical Science," by A. E. Kellie, B.Sc., Ph.D., A.R.I.C. (see summaries below).

The meeting was preceded at 3.15 p.m. by a visit to the Research Departments of the Royal College of Surgeons of England.

Gas Chromatography and Anaesthetic Research

Mr. D. W. Hill said that the outstanding advantage of gas chromatography in anaesthetic and respiratory research lay in its versatility. He concentrated on the various techniques that had been employed rather than on the applications.

In anaesthetic research, in particular, one was faced with the need to analyse a considerable variety of gases and vapours. These included oxygen, nitrogen, carbon dioxide, nitrous oxide, cyclopropane, diethyl ether, halothane, chloroform, trichloroethylene and methoxyfluorane.

Using packed columns, the anaesthetic vapours were separated on a short column of dinonyl phthalate on firebrick, run at 80° C. In parallel with this was run a 20-foot column of dimethyl sulphoxide on firebrick, at room temperature, to separate oxygen, nitrous oxide and carbon dioxide. An electrically driven sampling valve was used.

In addition to the use of packed columns, he described an experimental capillary-column gas chromatograph with a cathode-ray tube display.

The flame-ionisation detector had been extensively used by Dr. Butler of the Research Department of Anaesthetics for the analysis of anaesthetic concentrations in whole blood. This technique offered considerable promise for use in pharmacological investigations.

The Measurement of the Oxygen Tension of Blood by Means of a Covered Platinum Electrode

Dr. J. M. Bishop said that the measurement of the oxygen tension of blood had considerable value in physiological studies. Owing to the shape of the oxyhaemoglobin dissociation curve, the oxygen tension was a more precise measure of the state of oxygenation of arterial blood in normal subjects, and deviations from the normal in disease were more readily detected. The oxygen tension of venous blood might be of importance in a study of the metabolism of a region of the body.

Estimation of oxygen tension indirectly from the dissociation curve was inaccurate for the arterial blood of normal subjects. The microtonometer method of Riley was a difficult technique and was not suitable for tensions greater than 90 mm Hg. Polarographic measurement therefore offered many advantages, providing a direct estimate and theoretically being suitable for all oxygen tensions.

Unfortunately many complicating factors arose, and for a long time they had not been satisfactorily overcome. The dropping-mercury electrode was unpredictably influenced by the presence of red cells, so that estimates had to be made on separated plasma. Bare platinum electrodes were similarly affected, and when covered with cellophane or agar they were still sensitive to most of these difficulties. An instrument employing this type of electrode was described, together with the results of tonometer experiments to establish its accuracy and reliability.

Application of Infra-red Spectroscopy to Some Problems of Medical Science

Dr. A. E. Kellie said that for the development of a practical infra-red spectrometer in the early forties they were largely indebted to the recognition of the value of infra-red spectroscopy in the oil industry. During the following decades improvements in instrumental performance as a result of developing techniques—double-beam operations, multiple-pass systems, gratings and grating replicas, as well as improvements in detector response—had kept the field in a state of continuous change, yet almost from the outset the uniqueness of the steroid series as a medium for the application of infra-red spectroscopic techniques was appreciated, and under the direction of the late Dr. Dobriner spectroscopic methods had been applied with outstanding success to the study of the metabolism of endogenous steroid hormones.

In the animal kingdom, many somatic cells possessed the ability to biosynthesise cholesterol, which could be considered as a major precursor of the animal steroid hormones. Steroids of the pregnane (C_{21}), androstane (C_{19}) and oestrane (C_{18}) series formed in the endocrine glands—adrenal cortex, placenta, ovary and testes—were secreted into the blood system, which carried them round the body to centres of biological action. They exerted their influence on the metabolic processes of the body, exercising control over carbohydrate metabolism (gluco-corticosteroids), electrolyte balance (mineralo-corticosteroids), the development of secondary sex characteristic in males (androgens) and females (oestrogens), and were responsible for changes that were essential to the maintenance of pregnancy (progestins). The survival of these biologically active compounds in the blood stream as measured by their biological half-lives was short, and they underwent multiple changes leading to the formation of many new compounds, which were eventually excreted as a complex mixture in the urine. By reference to specific examples, Dr. Kellie illustrated how chromatographic techniques used in conjunction with infra-red spectroscopy contributed largely to the formidable task of separating and identifying the components of this mixture.

The application of infra-red methods to other problems of medical biochemistry had been less striking. He used the failure to make equal progress in these fields to illustrate the limitations and difficulties of the method.

BIOLOGICAL METHODS GROUP

AN Ordinary Meeting of the Group was held at 7 p.m. on Wednesday, February 15th, 1961, in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1. The Chair was taken by the Honorary Assistant Secretary of the Society, Mr. S. A. Price, B.Sc., F.R.I.C.

The following paper was presented and discussed: "The Use of Enzymes in Analysis," by C. J. Threlfall, B.Sc.

Obituary

WILLIAM HERBERT SIMMONS

WILLIAM HERBERT SIMMONS was born in North London on May 24th, 1880, and died at his home in Addiscombe, Surrey, on December 2nd, 1960. He received his early education at the Grocers' Company's School, Hackney Downs. He later studied chemistry and physics at Finsbury Technical College, Birkbeck College and University College, taking his degree of B.Sc. in 1904. In the meantime, in 1899, he had entered the laboratory of Dr. Samuel Rideal as a pupil and, after serving for three years, became assistant chemist to the soap firm of Vinolia Co. Ltd., later succeeding as chief chemist, and in this period also taking up an appointment as consulting chemist to Ozonair Ltd. It was this branch of chemistry, soap and essential oils, that was to become his life work, but, nevertheless, in 1907 he took up an appointment as research chemist to Messrs. Thomas Morson & Son Ltd., manufacturers of pharmaceutical chemicals, and remained with this firm until, in 1911, he established a private consulting practice in Victoria Street, London, specialising in the chemistry and analysis of soap, fixed oils, waxes and essential oils. In this field he became an internationally acknowledged expert and continued as a consultant until his retirement through ill health in June, 1959. In 1917 he was elected to the Fellowship of the Institute of Chemistry and somewhat later he included the field of petroleum and petroleum products in his consulting practice, becoming a Member of the Institute of Petroleum in 1934.

For many years W. H. Simmons was Lecturer in Soap Manufacture, Waxes and Lubricating Oils at Battersea Polytechnic. In 1914 he was appointed Examiner in Soap Manufacture to the City and Guilds of London Institute, and later was, for over 20 years, a member of the Advisory Committee of the same Institute on Petroleum and Petroleum Products. He also served on a number of the Technical Committees of the British Standards Institution and Sub-Committees of the British Pharmacopœia Commission and of the British Pharmaceutical Codex Revision Committee.

Simmons joined our Society in 1904 and was first elected a Member of Council in 1918, serving many terms thereafter, including a Vice-Presidency in 1938–9. For 29 years, from 1923, he was a conscientious member of the Publication Committee, but his most outstanding contribution to the work of the Society was that of Honorary Secretary to the Standing Committee on the Uniformity of Analytical Methods from the time of its formation in November, 1924, until the name was changed in 1935 to the Analytical Methods Committee, and thereafter until 1946. Twenty-one years as Honorary Secretary to one of our most important Committees is indeed a fine record, but, not content with this, Simmons served as Chairman of the Essential Oils Sub-Committee from 1933 until 1956, when, in recognition of his long service to the Society, he was appointed Honorary Member of the Analytical Methods Committee.

Simmons was sole author of a book entitled "Soap: its Composition, Manufacture and Properties" (4th edition, 1936) and joint author with H. A. Appleton of "The Handbook of Soap Manufacture" (1908); with C. Ainsworth Mitchell of "Edible Fats and Oils" (1911) and with G. H. Hurst of "Textile Soaps and Oils" (3rd edition, 1921). Most of his original papers were contributed to *The Perfumery and Essential Oil Record*; upwards of 40 appeared over the years and many were abstracted in *The Analyst*, including a useful one on the Detection of Petroleum in Cassia Oil (*Analyst*, 1936, **61**, 51). One of Simmons's special interests was the use of formic acid in essential-oil analysis, and in this connection he contributed an important paper to *The Analyst* on the Determination of Alcohol in Geranium Oils (1915, **40**, 491) in which, for the first time, many valuable data were presented. The

formylation method has been widely used and was recommended in a recent A.M.C. Report on The Determination of Linalol in Essential Oils (*Analyst*, 1957, **82**, 325) and was only amended last year in favour of the Fiore acetylation method (*Analyst*, 1960, **85**, 165).

The foregoing account indicates that Simmons was a man completely devoted to his profession, but nevertheless, as a freemason, he held London Grand Rank, was an active member of the University of London Graduates Association and a zealous member of the Methodist Church. W. H. Simmons was highly respected by all who knew him; his dominant characteristics were complete loyalty to all persons and causes, coupled with extreme modesty of outlook, these qualities being ever embellished with gracious courtesy.

NOEL L. ALLPORT

DONALD FORD PHILLIPS

DONALD FORD PHILLIPS, whose tragic death in February of this year came as a great shock to all his friends, was born at Stourbridge and educated at Bablake Secondary School, Coventry.

He held various appointments in the metallurgical industry, and I first met him in 1943, when he was Chief Chemist at High Duty Alloys Limited, Slough, and was enthusiastic enough to travel from Slough to Yorkshire every week to attend my evening course in microanalysis. This formed the basis of an intimate friendship which lasted until Phillips's death.

In 1949 he joined the Chemical Inspectorate, Department of Atomic Energy, Springfield, as Senior Experimental Officer, where he was in charge of a group of laboratories responsible for the analysis of uranium and ancillary materials by spectrographic, radiometric and fluorometric methods. He held this position until his death.

In 1947, Phillips and I were selected as B.I.O.S. investigators to study developments in microanalysis in Germany during the war; this involved living together for about a month, sometimes under very primitive conditions. Throughout this time, Phillips's sense of humour transformed what could have been a penance into an enjoyable outing. In 1949 he became Honorary Secretary of the Microchemistry Group and in 1957 its seventh Chairman. In both capacities he worked tirelessly to promote the activities of the Group, which still bears the stamp of his efforts.

Phillips had two main interests outside chemistry: one was transport and, in particular, railways. He published various articles on the subject in transport journals. His other main interest was languages; he spoke French and German fluently and had an astonishing knowledge of their idioms.

Phillips was not only a good friend; he was that rarity, a loyal friend, as those who were privileged to know him remarked many times. He was generous and kind; his good humour became proverbial and nothing ever made him lose his temper.

Those of us who knew him well will miss him sorely; but such was his character that he would not have us mourn him.

One can say of him as of Chaucer's knight:

"And of his port as meek as is a maid
He never yet no villiany had said
In all his life unto no manner of wight"

R. BELCHER

THOMAS TICKLE

THOMAS TICKLE died in London on October 29th, 1960, in his ninetieth year. He was born on June 11th, 1871, and was the only son of a Devon farmer. He started school in the small village of Patchacott and then proceeded to Shebbear School in Devon as a boarder. Upon leaving school he was apprenticed to a pharmaceutical chemist in Exeter, and in 1892 he entered the School of the Pharmaceutical Society as Jacob Bell Scholar. Here he had a highly creditable record, gaining a bronze medal in botany, silver medals in chemistry and pharmacy and also the Pereira Medal awarded for the most meritorious performance in the Society's "Major" qualifying examination.

Tickle was awarded a Salters' research fellowship for the period 1896–98, when he made a study of γ-pyrone at University College, London, the results of his work being published

in joint authorship with the late Professor J. Norman Collie. He then graduated as a B.Sc. of London University, became an Associate of the Institute of Chemistry in 1900, and in 1901 he passed the examination of the Institute in the branch which, at that time, covered Food and Drugs, Fertilisers and Feeding Stuffs, Soils and Waters, as well as Therapeutics, Pharmacology and Microscopy. He was elected a Fellow of the Institute in 1903.

It was in 1901 that Tickle was appointed Public Analyst for the City of Exeter, where he established a laboratory and began to practise as an analytical and consulting chemist. In the following year he was appointed Public Analyst for the County of Devon, and later also for the City of Plymouth and the Borough of Torquay. He was also Agricultural Analyst for Devonshire, Exeter and Plymouth.

Tickle, always affectionately referred to by his many friends as "Tommy" Tickle, joined our Society (then the Society of Public Analysts) in 1902, and he was a Member of Council in 1914–15. He also served the Institute of Chemistry in a similar capacity in 1916–19. Although he was an inveterate experimentalist (he would not take anything on trust) and faithfully recorded the results of his work in his laboratory note-books, he did not publish much of it; but in 1907 he contributed a paper to the *Pharmaceutical Journal* on the assay of morphine, and in 1921 published a note in *The Analyst* on the determination of lead in peaty waters.

In view of Tickle's training it is not surprising that outside the work arising from his official appointments, his professional interests had a strong pharmaceutical bias. Much of his work involved analysis for the National Pharmaceutical Union of samples taken under the National Health Service drug-testing scheme, and his work in this connection resulted in the amendment of a number of official standards. In 1947 he took into partnership Dr. C. V. Reynolds, who had been his chief assistant for nearly 20 years and who now carries on the practice. Tickle served for many years as a member of the Board of Examiners of the Pharmaceutical Society, and it has been placed on record that in this capacity "he showed a characteristic sympathy, fairness and wide outlook which earned him the respect of all his colleagues." He was a member of the British Pharmacopoeia Commission, 1932–48, and in 1948 he gave the inaugural address at the opening of the 107th session of the School of Pharmacy, which was the first session under the aegis of the University of London.

A great lover of the out-of-doors, Tickle found relaxation from his work in swimming, rowing and sailing. Up to within a year of his death he regularly bathed in the sea at Dawlish, and as a young man, he knew Dartmoor well. He was a Past Master of the Galen Lodge of Freemasons, London, of which he was a member for over sixty years.

Thomas Tickle always found much pleasure in his family, to whom he was devoted, and at the time of his death he was visiting his son in London in order to be present at the christening of his great-grandson. However, that was not to be. Two days before this event he arranged a dinner party for his relatives and some old friends, and, after spending a most enjoyable evening with them, he returned to his son's home in Wembley and happily retired to rest. That night he died in his sleep.

Tickle had been a widower for 25 years. He leaves two sons and two daughters. This tall, upright, bearded, sharp-featured and keen-eyed gentleman, with his warm pleasant personality, will be much missed by those members of the Society for Analytical Chemistry who were privileged to know him.

STANLEY DIXON

Biological Standardisation and the Analyst

A Review*

By J. W. LIGHTBOWN

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Summary of contents

Most quantitative analytical procedures are based on the assessment of known chemical reactions or physical constants; this necessitates a precise knowledge of the chemical structure and properties of the substance under examination. In a few instances the analyst has to estimate amounts of a substance of unknown or complicated structure, perhaps in a complex mixture for which chemical and physical methods cannot be used. Two examples illustrate the problems that then arise.

A solution of dextrose prepared for therapeutic use can be analysed and the concentration of dextrose determined precisely. If, however, it has been carelessly prepared and is administered by injection it may produce an undesirable rise in body-temperature, owing to the presence of pyrogen, a material of bacterial origin with complex but uncertain structure. The presence of pyrogen cannot be determined by any known chemical or physical means. Blood serum contains a complex mixture of proteins, which can be fractionated chemically and physically. The globulin fraction of certain sera has the property of combining specifically with toxic materials elaborated by bacteria and neutralising the toxicity. A particular globulin fraction may contain many different antitoxin proteins, but these cannot be identified and determined by chemical and physical means. As the use of antitoxic sera is often the only method of treatment for certain toxaemias, their reliable standardisation is desirable.

Many other useful therapeutic agents, such as the enzymes hyaluronidase and fibrinolysin, the hormones insulin and corticotrophin and the antibiotics nystatin and neomycin, pose similar problems to the analyst. Some of these drugs are extremely active and have only a small margin of safety between the useful and toxic doses, so that the activity must then be precisely controlled. In the future, chemical and physical methods for analysing these substances may become possible, but at present other methods must be used.

One property common to all these materials is their biological activity; they are all capable of modifying in some way the activities of living organisms. Usually, the effects produced by them are specific: hyaluronidase causes the hydrolysis of hyaluronic acid, a material important in maintaining the structural organisation of certain tissues; insulin, when introduced into the blood-stream of an animal, produces a decrease in the amount of glucose in the blood; nystatin interferes with the metabolism of yeast cells and prevents

* Reprints of this paper will be available shortly. For details please see p. 275.

their growth; neomycin behaves similarly towards bacteria. In each substance we have a specific property producing an effect that can be measured quantitatively. The measurement of biological activity so as to quantify the substance producing the effect requires the use of biological standardisation by one or other of several techniques based on well established principles.

Direct measurement of biological activity

When measurement of a physical property, such as optical rotation, is used in analysis, it is possible to define conditions for agreement among different operators; the specific rotation of glucose is the same in London and New York. Experience has shown that conditions for measuring specific biological activity cannot be so defined.

Insulin may be highly purified, but any attempt to define the decrease in blood-sugar produced by a given dose of insulin when injected into a rabbit would be unsuccessful. If a group of rabbits of the same age, weight and sex maintained under uniform conditions were all injected with a fixed dose simultaneously, the responses would be variable. Even the mean response for a large group of animals varies between experiments.

When the concentration of an antibiotic that inhibits the growth of a given micro-organism is determined, many millions of individual cells are exposed simultaneously to the antibiotic, and a reliable mean value might be expected. In practice, the values obtained on successive occasions may still vary greatly.

Variability is a characteristic of living organisms; variation in characters such as shape and size is self-evident, but variation in degree of response to certain stimuli is not always so obvious. The response of a living organism to the stimulus of a biologically active drug is the result of complex interaction of a series of related processes, *e.g.*, absorption into the animal, transport to the sensitive site and penetration into the cell and components of the cell. Variation at each stage will occur between animals and even in the same animal at different times. Some small success can be obtained in standardising the biological system by using closed colonies of mice or standard bacterial strains, but the level of a given response still varies with changes in environment, such as temperature and nutrition, and also with other undefined variable factors; effective standardisation is therefore impracticable in terms of a biological response.

Comparative measurement of biological activity

Ehrlich was the first to show how these difficulties could be overcome in standardising diphtheria antitoxin. Attempts had been made to standardise preparations of antiserum directly on the basis of the amount of diphtheria toxin they neutralised, but preparations of toxin were variable and unstable, and results varied from day to day. Ehrlich chose one particular sample of antiserum, which he designated as the standard reference preparation, and all other preparations of antiserum were compared directly with it. If the abilities of standard and unknown to neutralise a toxin preparation were compared directly in a single experiment, all variables affected both estimates equally, and a reproducible ratio for the activities of test and standard could be obtained. Ratios of the activities of a number of different preparations with the standard were directly comparable with each other. By defining the activity of the standard preparation in arbitrary units, the potencies of the unknown preparations could be stated in terms of these same units. This principle of calibrating the test system at every determination is now universally practised in biological assay. Standard preparations are available nationally and internationally for many biologically active substances. Each has its activity defined in terms of units, and each standard preparation is a yardstick for measuring the activity of a particular substance.

The use of a standard of comparison in analysis is not confined to biological assay. Many assays based on colorimetry involve use of this principle, as do determinations of arsenic and lead. The fundamental difference is that in the physico-chemical assays the standard curves of samples are prepared with reagents of known identity and purity, whereas in many biological assays the purity and even the identity may be unknown. If agreement between laboratories is to be obtained under these conditions one unique standard preparation must be used, although working standards calibrated against the master standard may be used in routine analysis.

In a few instances biological assay may be applied to materials of known structure and readily obtainable in a pure condition, perhaps because biological assay is more sensitive

than a chemical or physical method or more convenient. Determinations of a number of vitamins and amino acids are often made in this way; a unique standard preparation is then unnecessary. Whether or not a unique standard preparation is required, the principles involved in designing and interpreting the assay are the same.

Comparison between non-biological and biological assay methods

All assays, including chemical and physical ones, are based on standards, and certain assumptions are made. The constants used to interpret effects such as light absorption, optical rotation and refractive index have been previously recorded for pure samples; as certain assumptions were made in estimating the purity, the constants have no absolute validity. The specificity of physical methods may vary greatly. In chemical assays, the specificity of the method also varies, and once again the determination is usually based on a standard of assumed purity, except in gravimetric determinations, which are referred ultimately to the standard gram. The assumptions made about the purity of the standards used in chemical and physical assays can for most purposes be ignored.

Assumptions about the purity of material being assayed are also commonly made in routine analysis. Pharmacopoeial monographs are specifically designed to control impurities likely to be present, *e.g.*, the assay described in the British Pharmacopoeia for tartaric acid is a determination of free acid and in itself is completely non-specific. Only when considered with tests for identity and with limit tests can it be considered to provide a valid quantitative measurement. Even then the assumption is made that the tartaric acid has been prepared in a normal way, thereby limiting the kinds of impurities that might be present. A sample of tartaric acid could contain 10 per cent. of citric acid and still comply with the B.P. requirements for tartaric acid.

Although tartaric acid is produced biologically, it is chemically a relatively simple substance, and the impurities likely to be present after purification are known. For many naturally occurring substances that can only be assayed biologically, there cannot be the same certainty as to the efficiency and specificity of the purification processes used. Often the substances occur naturally in families of closely related compounds in different proportions, *e.g.*, the penicillins and the neomycins. It may be possible to control the fermentation by choice of a suitable strain and growth medium, so that only a single member of the family is produced, as in the manufacture of penicillin, when mainly benzylpenicillin is obtained. Sometimes this control may be more difficult, and selection of a single component can only be obtained by chemical and physical fractionation, *e.g.*, in the manufacture of neomycin.

The difference in the assumptions that can be safely made in assaying materials by the different methods can be seen from the designs of the assays. This is illustrated by the methods of assay used for penicillin, which originally could only be assayed biologically, but may now be assayed by all three means.

(*a*) Physical assay is conveniently carried out by determining the optical rotation at a single concentration. The result is interpreted by reference to a published value for reputedly pure material.

(*b*) Chemical assay may be made by measuring the amount of iodine that combines with the penicilloic acid produced by hydrolysis of a known weight of penicillin. The nature of the reaction with iodine is not fully understood, and, since the amount of iodine combining with a fixed weight of penicilloic acid varies with conditions such as temperature, pH and exposure to light, it is necessary to calibrate the assay by means of an authentic sample. Assays of the unknown sample and the standard are made at a single concentration, as the number of atoms of iodine combining with one molecule of penicilloic acid is independent of the concentration of this acid. Penicillin may also be assayed by forming a coloured hydroxamate after hydrolysis, the intensity of the colour being measured photometrically. The assay is calibrated with a sample of known purity by plotting a graph relating optical density to concentration. Two concentrations of the standard are sufficient to demonstrate adherence to Beer's law. The activity of unknown samples examined at a single concentration may be read from the line; it is a safe assumption that test and standard behave identically if conditions are standardised.

All these methods estimate total penicillin, and the results are calculated on the assumption that only benzylpenicillin is present. If a sample contained 10 per cent. of

heptylpenicillin this would not be apparent from the results, but similar results would be obtained in different laboratories. Molecule for molecule the different penicillins react in the same way.

(*c*) Biological assay could be carried out by means of a design similar to that of the hydroxamate method, *i.e.*, with two concentrations of a standard preparation to define the calibration graph and a single concentration of the unknown sample. This procedure, which might be expected to measure total penicillin, is unsound. Many factors, mostly ill defined, govern the shape of the calibration graph, which may be complex, and, although a portion of the line may be straight under certain conditions, these factors may be difficult to control. This is particularly so since one important factor may be the presence or absence of heptylpenicillin. Since it cannot be assumed that the standard and test will react with the test organism in an identical fashion, each assay must be constructed so that its validity can be assessed from its own internal evidence.

Requirements for valid biological assay

Three requirements that must be satisfied for valid biological assays have been discussed by Jerne and Wood[1] and, with special reference to closely related antibiotics, by Miles.[2] The first is that differences between responses in the several dose groups of an assay are wholly caused by differences in dosage and by random sampling; the random sampling applies both to the variable test organism and to variations in environment that cannot be controlled. The second is that the response must be a determinable function of the dose; usually a transformation of the response is sought, which gives a straight line when plotted against the logarithm of the dose. Thirdly, the response of the standard and test materials must be due to a single active principle. If more than one active principle is present, the proportions in test and standard must be the same. This last requirement applies also to materials exerting an antagonistic or potentiating effect or affecting stability of the active principle.

When a biological assay is constructed with three dose levels each of standard and test and with a sufficient number of test organisms or measured responses in the several dose groups, its validity can be checked statistically, and the potency ratio is obtained from the distance between the two dose-response lines.

If the requirements described are met, valid bioassays can be obtained, and the potency expressed in international units per unit of weight or volume will be a measure of the content of biologically active principle as well as of the biological activity. If, however, any one of the requirements is not satisfied, then statistically valid assays may still be obtained or they may not. If they are obtained, then the potencies found for the unknown may vary from assay to assay. The potency in international units per milligram in these circumstances is not a measure of content of active principle, but only of the biological activity under the specific conditions of each assay.

Biological assay of heterogeneous material

The requirement for valid bioassay that standard and test substances shall be homogeneous is often not met in practice. Even when standardised procedures of isolation and purification are used, a variable product must be expected, since the starting material, being of natural origin, is itself variable in composition. The standard and test material may contain less than 1·0 per cent. of contaminating active material, as with certain antibiotics, hormones and vitamins, or the heterogenity may be much greater. The implications should always be remembered, particularly when biological and chemico-physical assays are being compared.

These implications may be illustrated by reference to neomycin B. Two neomycins have been identified, B and C; their chemical structures are similar, although not yet fully defined, and both are biologically active, but their relative activities differ for different test organisms. Neamine, or neomycin A, which is a degradation product of either material may also be present, as may other hitherto unidentified biologically active components. This complex, normally called "neomycin B" may contain anything from 80 per cent. upwards of neomycin B, perhaps up to 15 per cent. of neomycin C, 1 or 2 per cent. of neamine and traces of the other biologically active components resembling neomycins. The biological assay of such material poses quite a problem; in fact, an accurate and reproducible assay is fundamentally impossible. If the standard of comparison has exactly the same composition

as the material being tested, valid comparisons can be made, but the biological assay would not be necessary were this fact known in advance.

The standard preparation for neomycin currently used in Great Britain has a composition similar to that described above; commercial material assayed against it may vary in composition, and this will become obvious in the results obtained by bioassay. Each biologically active component of the complex contributes to the over-all activity; the activity of each component is affected to different degrees by changes in environment, *e.g.*, pH, Eh, composition of medium and temperature, and also by the age of the test organism. When comparisons are made against the standard, the result obtained will therefore vary from assay to assay, and difficulty is experienced in obtaining agreement between successive assays in either the same or different laboratories. There is also difficulty in getting statistically valid assays with parallel dose-response lines. Chemical and physical methods of control are not specific and may not show up the variations in composition, so that disagreement arises between chemical and biological findings. The inability of bioassayists to obtain valid and reproducible assays from time to time and from place to place is too often interpreted as a failure in technique, and much wasted time and effort is directed to correcting this. The shortcomings of the assay method are, however, fundamental and cannot be overcome by manipulating the technique. By carefully controlling some of the variables known to be important, such as strain of test organism and composition of medium, precise and reproducible results may be obtained for a while, but this situation cannot last. If it were possible to control the assay in this way, then the potency of the drug could be measured directly in terms of the response of the biological system, without reference to a standard; such an approach has always proved abortive. The attempts to standardise assay conditions so that the biological activity of unlike substances can be reproducibly compared cannot be better illustrated than by the attempts that have been made for over 30 years to define conditions for the Rideal - Walker test, so that a wide range of disinfectants could be compared with phenol. It is a safe prediction that attempts will still be in progress after a further 30 years.

The discrepancies in bioassay of such materials as current neomycin have to be accepted. Instead of trying to ignore the implications, it should be recognised that these very discrepancies afford valuable evidence. When two samples of neomycin are found to have non-parallel dose-response lines, it shows that they have different compositions. This difference is not eliminated by adjusting conditions so that the dose-response lines are parallel.

The correct approach is to develop methods of separating the active components before determining them. Here, the bioassayist is dependent on the chemist. Too often this seems to be a challenge that is not met or even recognised. During attempts to develop methods of separation and purification, biological assays, with their internal evidence, probably give a much more sensitive indication of progress than do changes in chemical and physical criteria. To be able to obtain reproducible and precise biological comparisons of two samples under varied conditions of environment and test organism is probably as good evidence of their common identity as is a mixed melting-point.

An obvious conclusion to be drawn is that, when "perfect" biological assays can be obtained, the material will be so pure that there will be no need to use them, and it will be possible to exert analytical control by chemical and physical methods. It has been suggested that the ultimate aim of bioassayists concerned with analysis should be the abolition of bioassay. As will be seen later, this situation has arrived for certain of the vitamins and almost for certain antibiotics.

When this stage has not been reached, biological assay must be used, whatever its limitations, since it is needed to control the quality of material bought and sold and to ensure that substances used clinically have a defined activity. In defining quality for the purposes of the Therapeutic Substances Regulations or for conformity to B.P. standards, account is taken of the difficulties in assay when the acceptable minimum potency is laid down. The limits of error acceptable in the assay are also related to the difficulties of assay; thus, in the British Pharmacopoeia, fiducial limits of 80 to 125 per cent. for the stated potency of penicillin are recognised, whereas for corticotrophin, fiducial limits of 50 to 200 per cent. of the stated potency are considered satisfactory. For heterogeneous materials controlled biologically and assayed against a heterogeneous standard, it must be recognised that there is no true potency for any particular sample. A sample will have a family of potencies depending on the conditions of assay. These may be distributed about a mode, but the modal value has

no intrinsic superiority over any individual value. Probably, the potency obtained under conditions of assay most closely resembling the conditions under which the material is to be used would be the most useful. These conditions are, however, virtually indeterminable for biological products intended for use in man. They might theoretically be determined for products assayed on the whole animal, but with antibiotics there is little guide to the true physiological conditions under which the drug acts *in vivo*; even such factors as pH and oxygen tension cannot be defined, and both these factors could markedly affect the potency obtained in a biological assay of, for example, neomycin.

When products giving variable potencies on assay are to be bought and sold, obvious difficulties will arise if the unit is used directly as a unit of quantity instead of a unit of activity. The drug should be dealt with on a weight basis, with the specification that it should meet certain minimum requirements. These should be framed so as to take into account the possible variation, as with B.P. minimum requirements.

International co-ordination of biological standardisation

Ehrlich's original standard reference preparation was distributed internationally by him up to 1914, thereby allowing agreement to be reached between different national laboratories and hence rationalising development and research in this field.

After the first World War it became obvious that similar standard preparations were necessary for other drugs, and the League of Nations accepted responsibility for organising their collection and distribution. The service of developing and supplying the standards was based mainly on two laboratories, The State Serum Institute, Copenhagen, which was made responsible for international standards for sera and antigens, and the National Institute for Medical Research, London, which was made responsible initially for hormones and later for other non-immunological products. International reference preparations for certain arsenical drugs were maintained by the Frankfurt Institute in Germany until the withdrawal of Germany from the League of Nations in 1935, when these standards were transferred to the Department of Biological Standards at the National Institute for Medical Research.

When the League of Nations was disbanded, the responsibility for international biological standards was taken over by the World Health Organisation, which formed an Expert Committee on Biological Standardisation. The committee has met annually in Geneva since 1947. It has no fixed membership, but draws each year from a panel of international experts, chosen according to the subjects under consideration. The committee decides what International Standards are needed and initiates their setting up. It has permanent administrative offices in Geneva, but no laboratory facilities. The practical work is organised by the two departments at Copenhagen and Mill Hill, as in the past, with the same division of interests.

Classification of international biological preparations

International Standards—

These are preparations to which an international unit has been assigned on the basis of extensive international collaborative studies. Each standard is a unique preparation defining the international unit and is intended primarily to be used for calibrating National Standards.

International Reference Preparations—

These are preparations that originally served as standards in limit tests for toxicity and minimum therapeutic activity, but, at its twelfth session in 1958 the Expert Committee re-defined[3] the preparations as follows: "An International Reference Preparation is a preparation to which an International Unit has not been assigned. The reason for this may be that the completion of a full international study, which must precede the establishment of an international standard, would delay the availability of an international reference preparation for which there is an immediate demand. The reason may also be that an international unit would not serve a useful purpose or that extensive laboratory studies have failed to provide a satisfactory assay method." When the preparations are likely to be used in biological assay, each is assigned a provisional potency by the department distributing them, usually on the basis of information supplied by the manufacturer.

AUTHOR'S PREPARATIONS—

At its fifth and sixth sessions in 1951 and 1952, the Expert Committee on Biological Standardisation decided to set up a new class of substances, "Author's Preparations," as defined in the Eighth Report.[4] It was held that there was a need for international reference preparations to facilitate research and development in certain fields involving biologically active substances that might eventually prove useful clinically, particularly in the antibiotic field. It was considered impractical to set up international standards for these substances because of the volume of work involved, but it was decided that the International Centres for Biological Standards would hold and distribute Author's Preparations. These were to be supplied to the Centres by the authors, already filled into ampoules and in a stable form. It was expected that the authors would also define the unit. Although this type of preparation was set up as the result of a number of requests, only two substances were submitted up to 1958, namely, primisterin and dextran sulphate, and in 1958 this category was discontinued when the class of international reference preparations was re-defined.

AUTHENTIC CHEMICAL SUBSTANCES—

The Expert Committee on Biological Standardisation at its fifth[5] and sixth[6] sessions in 1951 and 1952 made recommendations that led to the setting up of a new class of international preparations designated as Authentic Chemical Substances. This collection, which is held and issued by the World Health Organisation Centre for Authentic Chemical Substances, in Stockholm, is the responsibility of the Expert Committee on the International Pharmacopoeia. The collection includes substances that, although they can be completely characterised by chemical and physical tests, are in demand as authentic chemicals or as convenient standards for biological assay. A number of international biological standards that have been discontinued, *e.g.*, oestrone, progesterone, vitamin A and tubocurarine, were transferred to the collection at Stockholm, as was the international reference preparation for chloramphenicol, and these are now available as Authentic Chemicals. These materials clearly do not define a unit of activity; they are simply authentic specimens having a high degree of purity.

PREPARATION OF AN INTERNATIONAL STANDARD

When a new standard is to be set up, a suitable sample is chosen as a result of preliminary testing. Occasionally, several samples prepared in different laboratories may be blended, *e.g.*, insulin or digitalis. Usually these samples are presented by industrial laboratories or individual research workers. The material is distributed in small amounts into individual glass containers and dried in a vacuum over phosphorus pentoxide; the containers are filled with pure dry nitrogen, sealed by fusion of the glass and stored in the dark at $-10°$ C. The material is thus protected as far as is practicable from oxidation, moisture and light and is kept at a low temperature—conditions conducive to the highest stability.[7] The new standard is then distributed to a number of laboratories in different countries for examination and for comparison with any existing national or laboratory standard. As a result of this study, the proposed International Standard is assigned a potency, and the international unit of activity is defined as the activity contained in a given weight of the standard. Other samples can then be compared with the International Standard and their activity expressed in international units per unit of weight or volume. The comparison may be direct or performed through a secondary national standard previously calibrated against the International Standard. The International Standards are usually limited in amount and are normally issued only to national laboratories with the intention that they should be used to establish and check national standards. However, it is hoped to set up part of the new International Standard for corticotrophin to be freely available to all laboratories as an International Working Standard.

The choice of sample for use as International Standard is not always easy. Should the standard consist of the purest sample available or should it be a sample corresponding more closely in composition with the material to be assayed against it? In practice both types of standard are used. The International Standard for vitamin D[8] serves as a standard for determining vitamin D in a wide range of extremely complex natural materials. The International Standard in this instance was the purest sample available at the time it was set up. The International Standard for bacitracin,[9] on the other hand, is a complex mixture of bacitracins. It would have been possible to obtain a purer sample of a single

bacitracin, but most commercial bacitracin, at the time of setting up the standard in 1953, consisted of mixtures of approximately the same composition as the standard. There did not appear to be any immediate likelihood of one particular bacitracin becoming freely available, and therefore the sample chosen for use as an international standard resembled current production material. Supplies of this particular standard will be exhausted in a year or so, and for the second International Standard a sample is being sought that will contain a greater proportion of a single bacitracin, since such material is now more readily available. Successive International Standards for insulin have increased in purity, and a third International Standard for corticotrophin, now being set up, will be much purer than the first and second.

International collaborative assays

It is of interest to consider the international collaborative assays carried out over the last 15 years in establishing new standards, whether the setting up of a first International Standard or of a replacement. The activities of two samples were compared in a number of different laboratories, often by a variety of methods. One of the samples was often the purest that could be currently obtained; the other may have been of a similar degree of purity or, if it had been produced at an earlier period, less pure. The laboratories chosen to take part in the assays were all highly experienced and used techniques with which they were familiar. Of eighteen collaborative studies organised for this purpose and dealing with "Pharmacological Substances," only five produced results that were homogeneous between laboratories. In the other collaborative studies, the variation occurring between laboratories had different degrees of significance. The cause of these variations could not be defined. In the assays on antibiotics, such as penicillin and dihydrostreptomycin, which were highly precise, small weighing and dilution errors may have been sufficient to account for the discrepancies. In the collaborative assay of the International Standard for penicillin, for example, the limits of error for the final potency were less than ± 1 per cent.[10]

Collaborating laboratories are always asked to carry out their assays in a way such that weighing and dilution errors may be assessed, but unfortunately the results received do not always comply with this request. In many collaborative assays the discrepancies are probably due to some degree of heterogeneity between the two samples being compared. This was obviously so in the collaborative study of dextran sulphate, since an attempt was made to assay this material against the International Standard for heparin, both substances being anticoagulants, but chemically unrelated.[11] Perhaps the most interesting result of this study was that a proportion of the assays carried out were statistically valid, with no significant departures from parallelism of the two dose-response lines. In a number of collaborative studies, *e.g.*, on thyrotrophin[12] and heparin,[13] the variations were greater than would be likely from weighing and dilution errors, and it seems possible that there was heterogeneity between the samples being compared, in spite of the fact that statistically valid assays were obtained within each laboratory. As has been pointed out by Miles,[2] statistically valid assays of penicillin G against penicillin K or of streptomycin against dihydrostreptomycin are possible, but the potency ratios obtained may differ with different test organisms.

When the results of a collaborative assay are heterogeneous, the choice of a potency for the proposed International Standard is difficult and arbitrary. Formally, the collaborative assay is invalid and should be rejected; in practice, this course is usually avoided. There is often no reason to believe that a further collaborative study would be more successful, and the difficulty in reaching agreement between laboratories emphasises the need for an International Standard. The value chosen is therefore a compromise arrived at in various ways according to the particular conditions.

Even if the collaborative assay is statistically valid, the potency is only estimated within certain limits, and an arbitrary choice of a particular value has to be made. In either event, once the potency of the proposed International Standard has been agreed on and the international unit defined, the uncertainties of the collaborative assay have only a historical interest.

Development of international biological standardisation

The development of international biological standardisation up to 1939 was reviewed by Dale[14] and up to 1945, in much greater detail, by Gautier.[15] This later review, which gives

a complete bibliography, describes the work carried out by the Permanent Commission on Biological Standardisation of the League of Nations from 1935 until the functions of the Commission were taken over by the World Health Organisation. It discusses the practical difficulties that arose in this period, many of them, as might be expected, being due to heterogeneity in the materials to be standardised. Much useful and fundamental knowledge came directly from attempts to obtain satisfactory standard preparations and to rationalise the assay procedures. As a direct result of the attempts to standardise gas-gangrene antitoxins the nature of many of the toxins involved was elucidated and their relative importance *in vivo* was determined. This work would have had much greater importance during the period of the second World War had it not been for the development of penicillin. The establishment of an International Standard for penicillin was one of the last functions of the League of Nations Commission on Biological Standardisation in 1944.[16]

When International Biological Standards were reviewed by Dale[14] in 1939, there were thirty available. There are now fifty-three established Standards with defined international units of activity, together with some fifty-three International Reference Preparations, which do not involve a unit of activity.[17] In 1939 most International Standards were for immunological products, vitamins and hormones. These groups are still represented, but thirteen International Standards and Reference Preparations have been discontinued since 1939, mainly because these substances can now be characterised by chemical and physical means. These include provitamin A, vitamin B (thiamine), vitamin C (ascorbic acid), vitamin A, vitamin E (α-tocopherol), oestrone, oestradiol monobenzoate, androsterone and progesterone. There is now only one vitamin with a unit of activity defined in terms of an international standard, *i.e.*, vitamin D_3. An International Reference Preparation for vitamin B_{12} was established in 1959.[18] It was originally to be set up as a standard for biological assay, but this is no longer needed, as Vitamin B_{12} can readily be standardised by chemical and physical means. The preparation will probably be considered for transfer to the collection of Authentic Chemicals.

The only standard antiserum discontinued has been staphylococcus B antitoxin. The advent of penicillin and other antibiotics active against *Staphylococcus* diverted attention from immunological products for the treatment of *S. aureus* infections. In recent years the problems produced by antibiotic-resistant strains of this organism have re-focused interest on the staphylococcal toxins and antitoxins, and the Expert Committee on Biological Standardisation is re-considering the need for International Standards and reference preparations for staphylococcal products, including leucocidins and antileucocidins.[19] In 1939 there was only one standard antigen, namely, tuberculin; now there are nine. They include the International Standard for pertussis vaccine, the first International Standard for a vaccine. The standardisation of potency of vaccines is particularly difficult, although it does not normally concern the analyst; great strides have been made in this field in the last 20 years, much of the success being due to work in the United Kingdom. More than half of the reference preparations are antisera, most of which have been set up recently in an attempt to rationalise diagnosis of certain infectious diseases in man.

A new class of International Standards that has come into existence since 1939 is for antibiotics, a rapidly expanding group. There are at present ten standards for antibiotics, and nine International Reference Preparations, three of which it is expected will shortly be established as International Standards. Two of the antibiotics, nystatin and amphotericin, are specifically active against mycoses. The twenty or so antibiotics dealt with are only a minority of those discovered since the introduction of penicillin; the policy in the past has been to set up standards only for antibiotics firmly established and widespread in their use.

Among the hormones four new standards have been created since 1939. One of these, heparin, was established in 1942; the others are thyrotrophin, growth hormone and corticotrophin. The last-named has presented many difficulties of standardisation. The first International Standard, set up in 1950, was replaced in 1955 by the second International Standard,[20] there being little difference in purity between these two preparations. A third standard is now in course of preparation; it has appreciably greater purity and should help to improve the assays of this hormone. An International Reference Preparation for human menopausal gonadotrophin has also been established.[21]

Among the miscellaneous international preparations are those reference preparations serving as standards in limits tests for toxicity or minimum therapeutic activity. Two of the earliest international preparations established were of this nature, for neoarsphenamine

and sulpharsphenamine; for neither was a unit of activity defined. Similar preparations now exist for the melaminyl trypanocides Mel B and MSb, established in 1954,[22] and for dimercaprol, established in 1952.[23] It is perhaps surprising that, in spite of modern analytical techniques, biological methods are still necessary to control the purity of such chemicals. In 1951 an International Standard for tubocurarine was established[24]; however, this was discontinued by 1955, since the material could be controlled by chemical and physical means.

In 1958 an International Reference Preparation for pyrogens was established.[25] Limit tests for pyrogens are widely carried out, and their inadequacies are well appreciated. In 1953 a small-scale collaborative study was organised by the World Health Organisation. Two samples of pyrogenic material were compared in a number of laboratories. Great difficulties were experienced in obtaining reasonable dose-response curves or reproducible results from day to day and from laboratory to laboratory. It was impossible to make a valid quantitative comparison of the activities of the two preparations; this was probably due to heterogeneity between the samples, one of which was prepared from *Proteus vulgaris* and the other from *Serratia marcescens*. It was soon recognised that the standards for toxicity of neoarsphenamine and sulpharsphenamine could not be used for other arsphenamines, although they were fairly closely related chemically. It is perhaps not surprising that even crude comparisons of different pyrogens proved impossible. The International Reference Preparation for pyrogens remains, however, as a common reference point for workers in different laboratories. The preparation has no defined unit of activity and is made available in the hope that those laboratories using the material will submit information and comments about its use. The results so accumulated may eventually point the way to better methods for controlling this activity.

A list of the available International Standards, Reference Preparations and Authentic Chemicals is given in the Appendix to this review (p. 229).

Expression of biological activity in weight units

The concept of a unit of biological activity based on a defined weight of a unique preparation was generally accepted as both meaningful and useful by analysts concerned with biological products at the time that the first antibiotic standard was developed. The unit of activity for penicillin was defined in the traditional way and permitted research, control and therapy in the penicillin field to develop without confusion. With the advent of streptomycin came the use of the "microgram equivalent" notation to express the potency of the product. This method of expressing activity, which has developed widely in the U.S.A. in particular, has produced much confusion, not least in the analytical field. The aim was to express the purity of a sample of antibiotic in terms of the number of micrograms of theoretically pure material contained in 1 mg of the sample. Estimates of purity were made in a biological assay by comparison with a standard preparation, this standard preparation having its own potency expressed as micrograms of theoretically pure material per milligram of standard. In the U.S.A., the standard preparation is usually a unique preparation held by the Food and Drug Administration Laboratories or the United States Pharmacopoeia. Its "potency" is defined on the basis of the best estimate of purity made by chemical and physical means, either directly on the standard itself or on a previous standard, perhaps a manufacturer's Master Standard, used to establish the first U.S.A. National Standard.

The assessment, by chemical and physical means, of the purity of the standard preparation is often difficult; after all, it is precisely because of this difficulty that a biological standard is required. However, an assessment is made, sometimes when the structure of the antibiotic is not fully known, and the material may only be available in an amorphous form. A sample of the highest activity obtainable is taken, determinations are made of known impurities, such as salts, water and solvent, and it is then assumed that the remainder is pure antibiotic. Thus, if the total of known impurities is x per cent., the sample contains $10(100 - x)$ μg of pure antibiotic per mg.

The Expert Committee on Biological Standardisation at its third session[26] discussed the question of alternative methods for expressing potency; it was stated "that the expression of potency in gram-equivalents is valid, though not always desirable, when the active principle in the standard preparation is known to be homogeneous and free from inert material, and the active principles in preparations to be assayed may be heterogeneous." However, this nomenclature has been little used, and in fact the microgram notation used in the U.S.A.

is based on a different concept. The potency of an unknown sample may be expressed in terms of a standard reference preparation as x microgram equivalents per mg, indicating that 1 mg of the unknown contains the activity of x μg of the standard preparation. This notation has meaning; if the unit of activity is defined as the activity of 1 μg of the standard reference preparation, then 1 unit is equivalent to 1 microgram equivalent. Both are measures of activity. In the U.S.A., potency is expressed not in microgram equivalents per milligram, but in micrograms per milligram. The potency has become a measure of weight content, not activity, and the weight measure is of a theoretical substance, not of a standard preparation. The difficulties of assessing the purity of the standard in absolute terms are considered relatively unimportant in the U.S.A. If it becomes obvious that a mistake has been made, as when samples are found on assay to have a higher than "theoretical" content, then the potency of the master standard is adjusted in the light of the new knowledge.[27] One of the aims of unit notation is to maintain continuity; 1 unit of the present International Standard for diphtheria antitoxin has the same activity as 1 unit of the first International Standard for this substance, established in 1922. The activity of "1 μg" may be changed from year to year in the hope that each change will be the final one. It is argued that in most instances the first estimate is usually close enough for all practical purposes and that, if re-evaluations have to be made, they are insignificant in clinical importance. This is probably often so, but it does not justify the resultant ambiguity and confusion.

Further complications arise from the fact that no uniform policy was adopted in choosing content of base, acid or salt to express the potency of the standard preparation of several antibiotics. In some instances the "potency" of the standard is expressed in terms of theoretical base content, *e.g.*, streptomycin, dihydrostreptomycin and oxytetracycline, or acid, *e.g.*, novobiocin. In others it is expressed in terms of content of a particular salt, *e.g.*, aureomycin hydrochloride and tetracycline hydrochloride. In the last-named instance this leads to figures being quoted for the potency of "theoretically pure" base, which are difficult to comprehend; thus, the potency of theoretically pure tetracycline base is 1082 μg per mg and of chlortetracycline base is 1076 μg per mg.[28] The relationship between the international unit and the "microgram" for the International Standards and Reference Preparations is shown in the Appendix to this review (Tables III and IV, respectively).

When a statement is made that a sample contains 1600 units of penicillin per mg or a solution 100 units per ml, the meaning is clear and unequivocal, whereas the statement that a sample contains 650 μg of neomycin per mg or a solution 100 μg per ml may give rise to doubt. Does this refer to a weight of neomycin sulphate or neomycin base? Does it refer to a weight of neomycin B or C or a mixture of both? To add to the confusion, it was realised at one stage that the potency of the U.S.A. Standard for neomycin was based on a false assessment of the purity, and its value in micrograms per milligram was changed in 1953. Much of the value of Waksman's monograph on neomycin[29] is lost because of the difficulty of knowing in a particular context what is meant by x μg of neomycin. One microgram has an internationally accepted meaning, *viz.*, one millionth part of a gram; it is a unit of weight. Although the aim of "microgram" notation is to express the content of active ingredient of an antibiotic on a weight basis, this is impossible when the active ingredient cannot be defined, and so the unit of weight is being used as a unit of activity; in such circumstances only the author of a statement relating to amounts of this antibiotic can know what he means by x μg.

The main reason put forward in favour of "microgram" notation for expressing potency of antibiotics is that the physician is used to prescribing on a weight basis and is confused by units. The procedure used in the British Pharmacopoeia in certain instances, however, allows dosage in units of weight, so accommodaing the physician, but exercises control of quality in terms of units of activity. The minimum permitted potency is expressed in units per milligram, *e.g.*, 900 units per mg for chlortetracycline hydrochloride and erythromycin. The dosage is expressed in weight of B.P. material.

This method may be used when the antibiotic is homogeneous and when the minimum permitted potency represents a high degree of purity.[30] It is indeed possible that a given dose may vary in its content of active ingredient, but a variation of 5 or even 10 per cent. is therapeutically unimportant. This would be the maximum variation that could exist with tablets of chlortetracycline or oxytetracycline of B.P. quality.

When it is possible to define quality in terms of weight of a defined chemical substance, then the need for a biological standard with a defined unit of activity no longer exists. This

situation has been reached for phenoxymethylpenicillin. The B.P. monograph for this antibiotic does not make use of bioassay and defines the minimum permitted quality in terms of $C_{16}H_{18}N_2O_5S$; control is exercised through chemical and physical tests.

Expression of biological activity in international units

It is common practice to refer to the potency of a biologically standardised drug in units per milligram or millilitre without any qualification. The Therapeutic Substances Regulations require that, in the United Kingdom, units of activity shall be the international units when these exist, but it is rare for a labelled potency to be stated in international units or with the recognised abbreviation, i.u. In the U.S.A., potencies are often expressed in U.S.P. units; these are again usually identical with the appropriate international unit, but this is not always made clear. Some of the advantages of having an international unit are lost in this way, and it would be worth-while for units to be described as international units when appropriate. The expression of activity simply in the form of "international units" abbreviated to i.u. is in itself not particularly satisfactory. There are many international units, *e.g.*, for weight, temperature, heat, electrical resistance; these all have specific names, so that confusion is avoided. In biological standardisation we deal with international units of activity; unfortunately, the qualification "of activity" is often omitted, and reference is usually made to international units of the substance, implying a quantitative measurement of the material. It would obviously be difficult to use a specific name for international units of activity, since all the units of activity are unrelated, but the recognition of an abbreviation i.u.a. instead of i.u. would make the position clear.

The International Union of Biology and the International Union of Pure and Applied Chemistry have formed an International Enzyme Commission, one of whose terms of reference is the definition of international units of activity for enzymes.[31] The international unit is to be the amount of an enzyme that will under certain standard conditions bring about the conversion of 1 μmole of substrate per minute. It seems unlikely that it will be possible to define units of activity in this way for most of the enzymes used therapeutically, but the possibility can readily be foreseen of confusion arising if two international units of activity are defined—one on the basis of a material standard and the other on the basis of standard conditions—the two being unrelated, but both abbreviated i.u. or u. Such a situation might arise with trypsin and chymotrypsin. Although it would be difficult to use a specific name for international units based on material standards, since these are not related to one another, it might be desirable to introduce a specific name for the international unit of activity proposed by the Enzyme Commission, as these units will be directly comparable one with another.

Future developments

In the past 10 years, International Standards for many vitamins and hormones have been discontinued, and it is likely that a number of antibiotic standards will soon follow suit. In 1959 the Expert Committee on Biological Standardisation considered[32] a French proposal that the international unit of activity for benzylpenicillin should be discarded as a basis of dosage for this material.[33] It also considered the possibility of discontinuing the International Standard for benzylpenicillin and transferring it to the collection of Authentic Substances. Of eleven different National Control Laboratories whose opinions were sought on this point, only five were in favour of discontinuing the Standard, and the Committee decided not to make a change.

Even when an International Standard becomes unnecessary, biological assay may still be used, because of its greater sensitivity. It it unlikely, for example, that chemical or physical methods for estimating antibiotics in animal feeding stuffs or in blood serum will be a practical possibility in the near future. It might be expected, however, that the use of the international unit would cease under these conditions and that dosage and so on would be expressed in terms of weight. Unfortunately, the use of units may be so firmly established that such a change is difficult. This has been the situation with vitamin A, so that, at the request of the International Union of Pure and Applied Chemistry, the Expert Committee on Biological Standardisation re-defined the unit of activity for vitamin A as being the activity of 0·000344 mg of pure all-*trans* vitamin A acetate.[34] This therefore became the first international unit of biological activity defined solely in terms of the pure substance and not in terms of a Standard Preparation. This procedure, which can only be justified

on grounds of expediency, might cause confusion, and it is to be hoped that official publications, such as pharmacopoeias, will change from the use of units as soon as this is possible.

New methods of biological assay are continually being developed, some of which may be used routinely for analytical purposes in the future. Naturally occurring anti-tumour substances can be assayed by plate-diffusion techniques with selected mutant strains of bacteria that are reputed to resemble tumour cells.[35] Anti-viral agents, such as interferon, may also be assayed by a similar technique, in which the test organism is a layer of tissue cells infected with virus.[36] Of more immediate interest to the analyst are the biological methods of assaying insecticide residues in foodstuffs, recently the subject of a report by Needham.[37] These methods are more sensitive than available physico-chemical methods, and there is promise of obtaining specific assays by the use of strains of test organisms rendered resistant to selected insecticides.

In the biological standardisation of antisera, basic methods have changed little since the first International Standard was established. However, methods of analysis based on immuno-electrophoresis, which are currently used in immunological research, may well make possible the quantitative measurement of specific antibodies by essentially physical methods.[38]

Although new methods of biological standardisation should extend the scope of the analyst, practice of even well established assay techniques is often neglected. Most analysts using bioassay techniques in this country are probably working in laboratories associated with the manufacture of the drugs, and many of the methods now widely used have originated in these laboratories.

The procedures of biological standardisation in analysis have been avoided by most laboratories dealing with control of the quality of drugs under the Food and Drugs Act, probably because of high cost and because the discipline is strange to the traditional analyst. In particular, the need for statistical evaluation of the results seems to create a barrier restricting the use of these methods. Many biological-assay methods are expensive, particularly those requiring the use of experimental animals, but others, *e.g.*, antibiotic assays, are not. The precision that can be obtained in bioassay of antibiotics is as great as that obtainable in analysis of most drugs by chemical and physical means. The methods of statistical evaluation required are relatively simple and well documented.

The control of quality of those biologically active drugs scheduled under the Therapeutic Substances Act is adequately exercised. Certain similar drugs supplied under the National Health Service are also carefully controlled, but many important drugs requiring biological assay are sold to the public without any safeguard, except the integrity of the manufacturer.

Legislation relating to the control of drugs is at present under review, but this deficiency in existing control is due not so much to inadequacy of the present laws, as to inadequacy of existing laboratories responsible for testing under the Food and Drugs Act. It would be unreasonable to expect every such analytical laboratory to be equipped to deal with the full range of biological assays, but there is no reason why regional laboratories should not perform this function, each specialising in a different field.

References

1. Jerne, N. K., and Wood, E. C., *Biometrics*, 1949, **5**, 273.
2. Miles, A. A., *Bull. World Health Organisation*, 1952, **6**, 131.
3. Expert Committee on Biological Standardisation, Twelfth Report, *World Health Organisation Tech. Rep. Ser.*, 1959, **172**, 20.
4. ——, Eighth Report, *Ibid.*, 1955, **96**, 18.
5. ——, Fifth Report, *Ibid.*, 1952, **56**, 12.
6. ——, Sixth Report, *Ibid.*, 1953, **68**, 25.
7. Jerne, N. K., and Perry, W. L. M., *Bull. World Health Organisation*, 1956, **14**, 167.
8. Coward, K. H., and Irwin, J. O., *Ibid.*, 1954, **10**, 875.
9. Humphrey, J. H., Lightbown, J. W., Mussett, M. V., and Perry, W. L. M., *Ibid.*, 1953, **9**, 861.
10. Humphrey, J. H., Mussett, M. V., and Perry, W. L. M., *Ibid.*, 1953, **9**, 15.
11. Mussett, M. V., and Perry, W. L. M., *Ibid.*, 1956, **14**, 647.
12. ——, ——, *Ibid.*, 1955, **13**, 917.
13. Bangham, D. R., and Mussett, M. V., *Ibid.*, 1959, **20**, 1201.
14. Dale, Sir Henry, *Analyst*, 1939, **64**, 554.
15. Gautier, R., *Bull. Health Organisation, League of Nations*, 1945, **12**, 1.
16. Commission on Biological Standardisation, *Ibid.*, 1945, **12**, 181.
17. Expert Committee on Biological Standardisation, Thirteenth Report, *World Health Organisation Tech. Rep. Ser.*, 1960, **187**, 26.

18. Bangham, D. R., and Mussett, M. V., *Bull. World Health Organisation*, 1960, **22**, 555.
19. Expert Committee on Biological Standardisation, Thirteenth Report, *World Health Organisation Tech. Rep. Ser.*, 1960, **187**, 19.
20. Mussett, M. V., and Perry, W. L. M., *Bull. World Health Organisation*, 1956, **14**, 543.
21. Expert Committee on Biological Standardisation, Thirteenth Report, *World Health Organisation Tech. Rep. Ser.*, 1960, **187**, 9.
22. ——, Eighth Report, *Ibid.*, 1955, **96**, 16.
23. ——, Sixth Report, *Ibid.*, 1953, **68**, 18.
24. Davies, M. G., Miles, A. A., and Perry, W. L. M., *Bull. World Health Organisation*, 1949, **2**, 65.
25. Humphrey, J. H., and Bangham, D. R., *Ibid.*, 1959, **20**, 1241.
26. Expert Committee on Biological Standardisation, Third Report, *World Health Organisation Tech. Rep. Ser.*, 1950, **2**, 12.
27. Humphrey, J. H., Lightbown, J. W., and Mussett, M. V., *Bull. World Health Organisation*, 1959, **20**, 1221.
28. Grove, D. C., and Randall, W. A., "Assay Methods of Antibiotics," Medical Encyclopaedia Inc., New York, 1955.
29. Waksman, S. A., *Editor*, "Neomycin," Ballière, Tindall & Cox Ltd., London, 1958.
30. Miles, A. A., and Perry, W. L. M., *Bull. World Health Organisation*, 1953, **9**, 1.
31. International Commission on Enzymes, *Nature*, 1958, **181**, 452.
32. Expert Committee on Biological Standardisation, Thirteenth Report, *World Health Organisation Tech. Rep. Ser.*, 1960, **187**, 7.
33. Commission d'Études des Antibiotiques, *Thérapie*, 1959, **14**, 9.
34. Expert Committee on Biological Standardisation, Thirteenth Report, *World Health Organisation Tech. Rep. Ser.*, 1960, **187**, 10.
35. Gause, G. F., *Biol. Rev.*, 1959, **34**, 378.
36. Porterfield, J. S., *Lancet*, 1959, **ii**, 326.
37. Needham, P. H., *Analyst*, 1960, **85**, 792.
38. Grabar, P., *Adv. Protein Chem.*, 1952, **13**, 1.

NOTE—Annual Reports of the Expert Committee on Biological Standardisation of the World Health Organisation can be obtained from Her Majesty's Stationery Office, London. Since the Tenth Report, published in 1957, an Appendix has been included; this gives a complete list of International Biological Standards and Reference Compounds available from the two Control Centres and also contains a complete bibliography relating to these materials.

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APPENDIX

TABLE I

INTERNATIONAL STANDARDS, EXCLUDING ANTIBIOTICS

Antigens—
- Old tuberculin
- Tetanus toxoid, plain
- Diphtheria toxoid, plain
- Diphtheria toxoid, adsorbed
- Schick test toxin (diphtheria)
- Pertussis vaccine
- Swine erysipelas vaccine

Antibodies—
- Tetanus antitoxin
- Diphtheria antitoxin
- Antidysentery serum (Shiga)
- Gas-gangrene antitoxins—
  - *Clostridium welchii*, types A, B and D
  - *Vibrion septique*
  - Oedematiens
  - Histolyticus
  - Sordelli
- Staphylococcus α-antitoxin
- Scarlet fever streptococcus antitoxin
- Anti-streptolysin O
- Swine erysipelas serum (anti-N)
- Antipneumococcus serum, types 1 and 2
- Anti-*Brucella abortus* serum
- Anti-Q-fever serum
- Anti-rabies serum
- Anti-A blood-typing serum
- Anti-B blood-typing serum
- Syphilitic human serum

Hormones—
- Oxytocic, vasopressor and antidiuretic substances (previously named posterior pituitary lobe)
- Prolactin
- Corticotrophin (previously named adrenocorticotrophic hormone)
- Thyrotrophin
- Growth hormone
- Serum gonadotrophin
- Chorionic gonadotrophin
- Insulin
- Heparin

Miscellaneous—
- Vitamin D_3
- Hyaluronidase
- Digitalis

Table II

International Reference Preparations, excluding antibiotics

Antigens—
Cholera antigen (Inaba)
Cholera antigen (Ogawa)
Cholera vaccine (Inaba)
Cholera vaccine (Ogawa)
Cardiolipin
Lecithin (beef heart)
Lecithin (egg)

Antibodies—
Cholera agglutinating serum (Inaba)
Cholera agglutinating serum (Ogawa)
Diphtheria antitoxin for flocculation test
Antityphoid serum (provisional)
Antipoliomyelitis sera, types 1, 2 and 3
Anti-*Leptospira* sera, representing 19 strains

Hormones—
Human menopausal gonadotrophin

Miscellaneous—
Opacity reference preparation
Vitamin B_{12}
Neoarsphenamine
Sulpharsphenamine
Oxoarsphenamine
Mel B
MSb
Dimercaprol
Protamine
Pyrogen

Table III

International Standards for antibiotics

Substance	Defined potency, i.u. per mg	Equivalence of 1 i.u. to American "μg"	Calculated purity of Standard on basis of American "μg," %
Penicillin (sodium salt)	1670	Not used	>99*
Phenoxymethylpenicillin (free acid)	1695	Not used	>99*
Streptomycin (sulphate)	780	1 "μg" of base	97·5
Dihydrostreptomycin (sulphate)	760	1 "μg" of base	95·1
Bacitracin	55	Not used	Not known
Tetracycline (hydrochloride)	990	1 "μg" of hydrochloride	99·0
Chlortetracycline (hydrochloride)	1000	1 "μg" of hydrochloride	100
Oxytetracycline (base dehydrate)	900	1 "μg" of anhydrous base	97·1
Erythromycin (base)	950	1 "μg" of anhydrous base	95
Polymixin B	7874	Not used	Not known

* Independent estimate.

Table IV

International Reference Preparations for antibiotics

Substance	Provisional potency, units per mg	Equivalence of provisional unit to American "μg"	Calculated purity of Reference Preparation on basis of American "μg," %
Amphotericin B	960	1 "μg"	96
Kanamycin (sulphate)	812	1 "μg" of free base	97·4
Vancomycin	1007	Not used	Not known
Viomycin (sulphate)	730	1 "μg" of free base	87*
Neomycin (sulphate)	680	1 "μg" of free base	Not known
Nystatin	2855	Not used	Not known
Novobiocin (sodium salt)	835	1 "μg" of free acid	86·5
Oleandomycin (chloroform adduct)	845	1 "μg" of free base	97·3
Penicillin K†	—	—	—

* On the assumption that viomycin is dibasic.
† No unit; preparation is intended for use as a marker in chromatography.

Discontinued International Standards Available as Authentic Chemicals

Oestrone; progesterone; vitamin A (acetate); tubocurarine (D-tubocurarine chloride); chloramphenicol

The Determination of Citronellol in Admixture with Geraniol

Further Studies of Formylation Reactions by Gas - Liquid Chromatography

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Work undertaken on behalf of the Essential Oils Sub-Commiteee of the Analytical Methods Committee

Previous studies of hot formylation proved tedious and difficult, owing to the lack of a suitable technique for investigating the course of the reactions and the nature and proportions of the end-products. This paper describes experiments in which gas - liquid chromatography was used for that purpose and from which it was concluded that formylation cannot be made the basis of an accurate analytical method no matter what changes be made in working conditions.

WHEN the method known as "hot formylation" was first used, it was supposed that boiling anhydrous formic acid converted citronellol to its formate and geraniol to hydrocarbons,[1] so that the ester value of the formylated material could be used to determine the percentage of citronellol in an essential oil containing both citronellol and geraniol. Later work indicated that these assumptions were not wholly true. Pfau[2] reported that the chief reaction products from citronellol were the mono- and diformates of 3,7-dimethyloctane-1,7-diol. It was also found that polymers of uncertain composition could originate from the alcohols.

A previous communication[3] from the Essential Oils Sub-Committee of the Analytical Methods Committee of the Society for Analytical Chemistry reported results of comparative tests with four variants of the method. Method A, under consideration by the International Organization for Standardization, gave answers nearest to the true figures, but it was judged too inaccurate to justify its recommendation for determining citronellol in essential oils. Changes in working conditions obviously caused significant variations in the results, but it was not possible to deduce from available information whether some specific conditions existed under which a hot-formylation method could be relied upon to give results of acceptable accuracy. The same report mentioned that gas - liquid chromatograms of the formylated samples had shown peaks that might correspond with the substances described by Pfau, although they failed to support his views about their relative proportions.

In this investigation, the first objective was to determine the nature of the compounds giving rise to the principal observed peaks. Gas - liquid chromatograms were then used to trace the effects of variations in reaction conditions and to relate changes in the observed proportions of reaction products to deviations from the correct figures in analytical results. The findings of these experiments were used to decide whether or not the principle of hot formylation is a sound basis for an analytical procedure for determining citronellol in essential oils.

Fig. 1 shows a chromatogram of the more volatile end-products after a mixture of equal weights of geraniol and citronellol had been treated by method A.[3] These were followed by a number of compounds having low volatility, which gave rise to a series of low drawn-out peaks. It was evident from the retention times of the peaks traced that the assumptions that geraniol is converted almost quantitatively to hydrocarbons and citronellol to its formate could not both be true, and consideration of the areas under the peaks in relation to the known composition of the mixture suggested that neither assumption was correct, but that both alcohols undergo complex changes. Consequently, it was decided to study the reactions and reaction products of citronellol separately from those of geraniol. In all experiments, specially purified samples were used, whose content of citronellol or geraniol exceeded 99 per cent., as determined by acetylation and by gas - liquid chromatography. The formic acid used was of analytical-reagent grade.

Citronellol

Reaction products—

A 10-ml portion of citronellol and 20 ml of 98 per cent. formic acid were heated together at boiling-point for 1 hour. The washed product had an ester value of 359, equivalent to 118 per cent. of citronellyl formate. Acid diluted with water to 75 per cent. gave a citronellyl formate equivalent of 93 per cent. Chromatograms of these two reaction mixtures are shown in Figs. 2 and 3. Peaks (1) and (2) have the retention times of citronellyl formate and citronellol. Peaks (4) and (5) may be produced by the mono- and diformates of 3,7-dimethyloctane-1,7-diol. Saponification of the second reaction mixture gave a product whose chromatogram is shown in Fig. 4, in which peak (2) corresponds to citronellol and peak (3) is presumably produced by 3,7-dimethyloctane-1,7-diol.

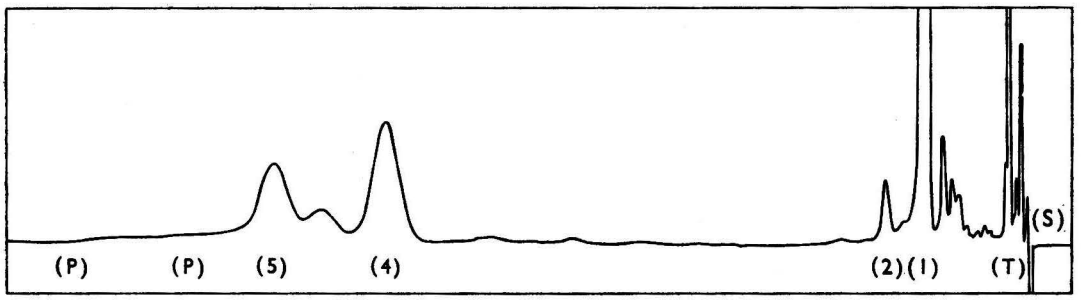

Fig. 1

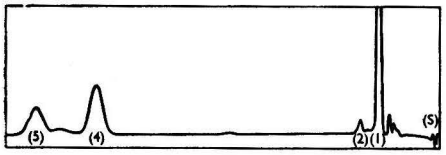

Fig. 2

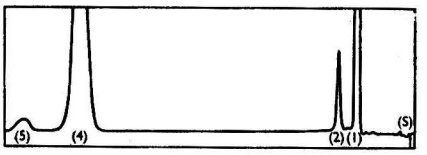

Fig. 3

Fig. 1. A mixture of equal parts of citronellol and geraniol after hot formylation
Fig. 2. Citronellol after reaction with 98 per cent. formic acid at boiling-point for 1 hour
Fig. 3. Citronellol after reaction with 75 per cent. formic acid at boiling-point for 1 hour

In Figs. 1, 2 and 3, (S) = start, (T) = a group of terpene hydrocarbons, (P) = the start of a series of compounds of very low volatility, (1) = citronellyl formate, (2) = citronellol, (4) = glycol monoformate, (5) = glycol diformate

Next, 200 g of citronellol were formylated with 99 per cent. formic acid. The product was saponified, after which it gave a chromatogram similar to Fig. 4. It was then fractionally distilled under reduced pressure, and the two major components were separated. The first collected was identified as citronellol. The other gave the chromatogram shown in Fig. 5, which indicated a purity of about 97 per cent. Its infra-red spectrum was consistent with the supposed glycol structure. Comparison with the spectrum of citronellol confirmed the increase in hydroxyl content and the loss of the isopropylidene linkage. Its chemical behaviour strongly indicated the presence of a tertiary hydroxyl group, which could hardly arise except in the 7-position.

A comparison was made between this compound and a sample (also having a purity of about 97 per cent.—see Fig. 6) of 3,7-dimethyloctane-1,7-diol prepared by hydrogenation of "hydroxycitronellal," which is known to be 7-hydroxy-3,7-dimethyloctan-1-al. Their refractive indexes were 1·459 and 1·460, respectively, their retention times with silicone oil, Apiezon L and polypropylene sebacate as stationary phases were identical and their infra-red spectra were nearly indistinguishable. The correctness of the supposed structure of the compound giving rise to peak (3) seemed to be adequately established.

Attempts to prepare the formates from the glycol led to chromatograms somewhat resembling Fig. 2, with three peaks whose retention times equalled those of peaks (1), (4) and (5). Peak (1) is produced by citronellyl formate, which suggests that addition to the isopropylidene linkage is reversible. Ester values were highest when the relative size of

peak (5) was greatest, from which it would seem that peak (5) corresponded to the diformate. The compound giving rise to peak (4) was isolated by fractionally distilling a batch of formylated citronellol under reduced pressure. The first portion of distillate consisted mainly of citronellyl formate. From later fractions, a sample whose chromatogram indicated that it contained about 95 per cent. of the compound giving rise to peak (4) was selected. It had an ester value of 282; clearly, peak (4) is produced by the monoformate (ester value 278). Too little material remained in the flask to permit distillation of the diformate without considerable decomposition. It was not considered important to identify the minor peaks on chromatograms of formylated citronellol.

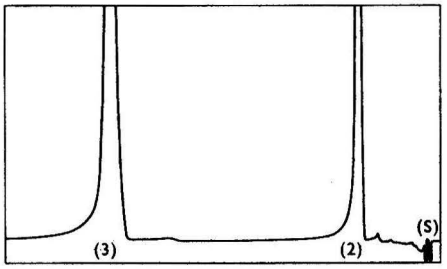

Fig. 4

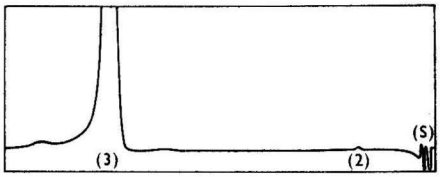

Fig. 5

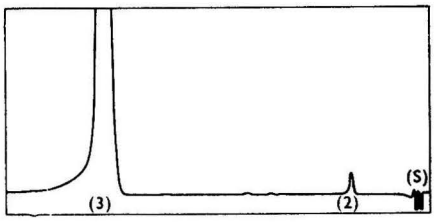

Fig. 6

Fig. 4. Citronellol after hot formylation and then saponification
Fig. 5. Glycol obtained after fractional distillation of the mixture from Fig. 4
Fig. 6. Purified 3,7-dimethyloctane-1,7-diol obtained by hydrogenation of "hydroxycitronellal"
In Figs, 4, 5 and 6, in addition to symbols used in Figs. 1, 2 and 3, (3) = 3,7-dimethyloctane-1,7-diol

EFFECTS OF REACTION CONDITIONS—

The chromatograms in Figs. 2 and 3 illustrated how greatly a change from 98 to 75 per cent. formic acid altered the composition of the reaction product, so six similar experiments were carried out in which the concentration of acid was increased in even steps from 75 to 99 per cent. In each experiment, 10 ml of citronellol and 20 ml of acid were mixed and gently boiled for 1 hour, after which the ester value was determined; the results are shown in Table I.

TABLE I

EFFECT OF CONCENTRATION OF FORMIC ACID

Experiment No.	Concentration of formic acid, % w/w	Ester value found	Equivalent, as citronellyl formate, %
1	75	287	94
2	80	299	98
3	85	311	102
4	90	323	106
5	95	342	112
6	99	362	119

Chromatograms of the reaction products of experiments Nos. 3, 4, 5 and 6 are shown in Figs. 7, 8, 9 and 10, and Fig. 3 shows a chromatogram after an experiment like No. 1. These confirm Pfau's findings that relatively more glycol diformate is produced as the concentration of acid is increased, but this occurs mainly at the expense of glycol monoformate and not citronellyl formate. Citronellyl formate is in fact the major component when the

concentration of acid used is above 80 per cent.; below this concentration, the glycol monoformate is found in greatest amount. Hydration of the 6,7 double bond would seem to occur readily when plenty of water is present, whereas subsequent formylation of the 7-hydroxy group (or, alternatively, direct addition of formic acid to citronellyl formate) proceeds best when the acid is least diluted. Some citronellol always remains unchanged, and the amount becomes greater as less concentrated acid is used. The chromatograms therefore reveal three sources of significant error in determinations of citronellol by hot formylation and show that the nearly correct results obtained in experiments Nos. 2 and 3 are only the outcome of a fortuitous balance of positive and negative errors. A true answer cannot be obtained, since no adjustment of the concentration of acid can lead to the postulated single reaction in which citronellol is completely converted to its formate.

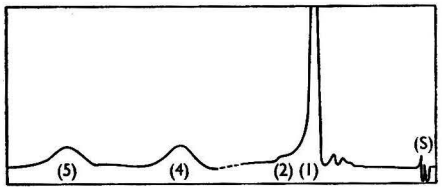

Fig. 7

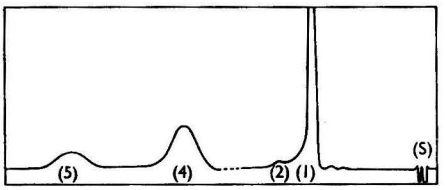

Fig. 8

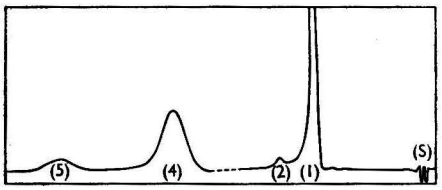

Fig. 9

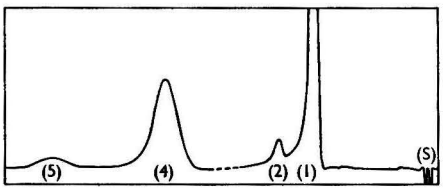

Fig. 10

Figs. 7 to 10. Effects of different concentrations of acid on the reaction between citronellol and boiling formic acid: Fig. 7, 99 per cent. formic acid; Fig. 8, 95 per cent. formic acid; Fig. 9, 90 per cent. formic acid; Fig. 10, 85 per cent. formic acid

In Figs. 7 to 10, symbols used are as for Figs. 1 to 6

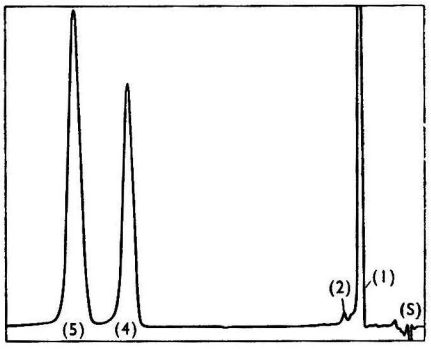

Fig. 11

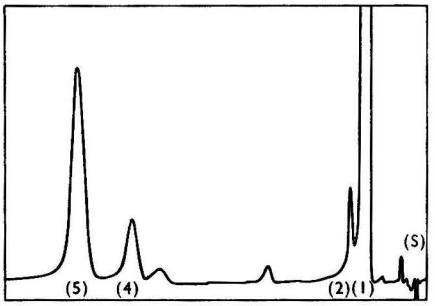

Fig. 12

Fig. 11. Citronellol *plus* 99 per cent. formic acid after 1 hour at 60° C
Fig. 12. Citronellol *plus* 99 per cent. formic acid after 1 hour at 20° C

In Figs. 11 and 12, symbols used are as for Figs. 1 to 10

Variations in temperature and reaction time were equally ineffective in eliminating these sources of error. Experiment No. 6 was repeated at 60° and 20° C; the results were—

Reaction time, minutes	10	60	120
Ester value after reaction at 60° C	356	373	388
Ester value after reaction at 20° C	247	346	375

The chromatograms of all six reaction products resembled those in Figs. 7, 8, 9 and 10 in that the chief components were citronellyl formate and the two glycol formates, although at 20° C the presence of small amounts of other unidentified materials was revealed; two of these chromatograms are shown in Figs. 11 and 12. A decrease in the amount of acid used, however, tended to decrease the ester value. An experiment similar to No. 6, but in which only 10 ml of 99 per cent. formic acid were used, gave an ester value of 340 and a chromatogram like that obtained with 20 ml of 95 per cent. acid. Presumably, this is because the water formed during the reaction more effectively dilutes the smaller amount of acid.

In practice, ideal conversion to citronellyl formate could be achieved only by slowly adding 99 per cent. formic acid to citronellol at 100° C. So long as citronellol is in excess, the acid seems to be esterified with sufficient rapidity to prevent it from catalysing hydration or additive formylation. Unfortunately, such a procedure is useless for determining citronellol in the presence of geraniol, as geraniol also gives its formate under these conditions.

The chromatograms suggested that the proportion of reaction products having low volatility increased with the concentration and amount of formic acid added. Determinations of residues after evaporation supported this view, as shown by the results in Table II. However, the residues still contained glycol formates, which cannot entirely be removed by heating on a steam-bath. The true percentages of "polymers" and their real ester values are lower than those found; nevertheless, small amounts of these compounds do occur, and they may give rise to yet another source of error inherent in hot-formylation methods.

TABLE II

EFFECT OF AMOUNT AND CONCENTRATION OF FORMIC ACID ON PROPORTION OF RESIDUE

In each experiment a 10-ml portion of citronellol was formylated for 1 hour

Formic acid		Residue	
Concentration used, %	Volume used, ml	Proportion, % w/w	Ester value
80	10	5	280
80	20	11	316
90	10	6	295
99	10	8	318

GERANIOL

REACTION PRODUCTS—

When a mixture of geraniol with excess of concentrated formic acid is heated to boiling-point, the geraniol is rapidly transformed into other compounds. The main reactions are complete in so short a time that samples withdrawn at intervals after the liquid has begun to boil give chromatograms that hardly differ from each other. Fig. 13 shows a typical example, obtained after treatment by method A.[3] Its chief feature is a group of peaks (1) that were shown to be produced by unsaturated hydrocarbons. There is a small amount of an unidentified compound, indicated by peak (2). No significant amounts of esters are shown, for similar chromatograms were obtained after the reaction mixtures had been saponified. It is also clear that little or no geraniol is present.

At first sight, the chromatographic evidence seems to confirm the supposition that geraniol is converted mainly to hydrocarbons, but closer examination reveals that the areas under the recorded peaks are far too small to account for the full volume of sample placed on the column. Further, although the chromatograms were hardly changed after saponification, the reaction mixtures had ester values greater than 30. Evidently, compounds having low volatility must be produced, some of which must presumably contain formyl groups. This was confirmed, first by running a sample on a non-polar stationary phase at a higher column temperature, when a group of large peaks having retention times corresponding to very low vapour pressures was traced (see Fig. 14), and secondly by determining the proportion of residue after evaporation (88 per cent. w/w) and the ester value (42) of this residue. There is no doubt that, under the conditions recommended for hot-formylation analysis, geraniol—like citronellol—fails to react in conformity with the theory upon which the method is based. The previously reported "polymers" are in fact the principal products.

EFFECTS OF VARIATION IN REACTION CONDITIONS—

If the reaction temperature is decreased, chromatograms of the volatile end-products are similar to Fig. 13, although the rate of reaction is slower; at 20° C the reaction is sufficiently slow to be followed in stages.

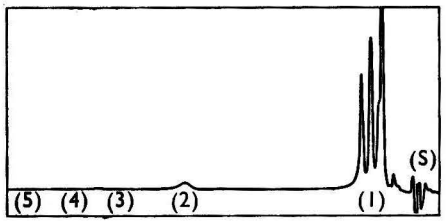

Fig. 13

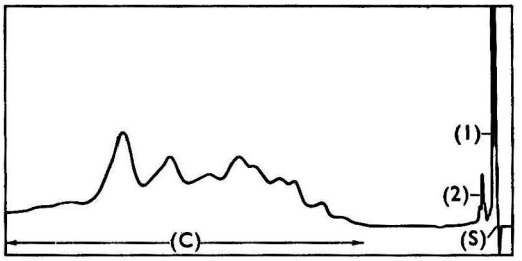

Fig. 14

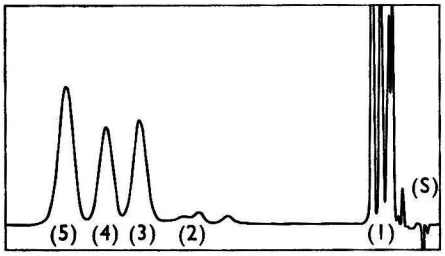

Fig. 15

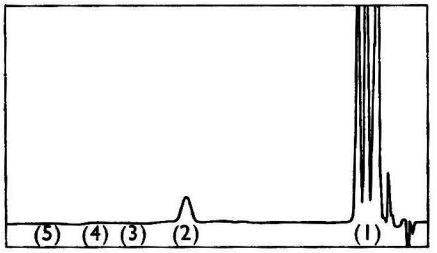

Fig. 16

Fig. 13. Geraniol treated by method A
Fig. 14. Higher temperature chromatogram of hot-formylated geraniol recorded at slow chart speed
Fig. 15. Reaction between geraniol and 99 per cent. formic acid after 10 minutes at 20° C
Fig. 16. Reaction between geraniol and 99 per cent. formic acid after 2 hours ar 20° C

In Figs. 13 to 16, (S) = start, (C) = compounds of low volatility, (1) = unsaturated hydrocarbons, (2) = unidentified material, (3) = formate of unidentified alcohol, (4) = geranyl formate, (5) = geraniol

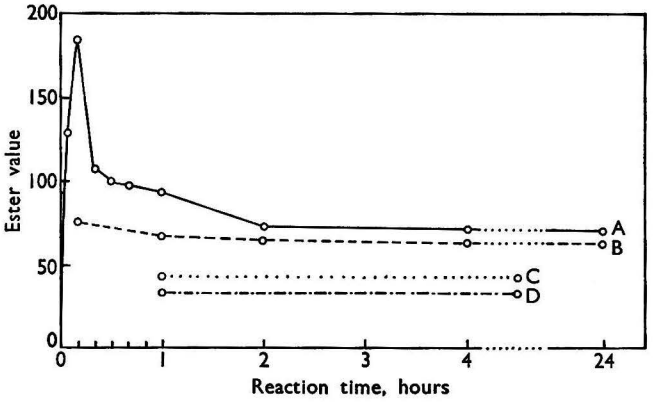

Fig. 17. Effects of time and temperature on the ester values for reaction between geraniol and 99 per cent. formic acid: curve A, 20° C; curve B, 60° C; curve C, on water bath at 100° C; curve D, on sand-bath under reflux

A 10-ml portion of geraniol and 20 ml of 99 per cent. formic acid were mixed and set aside at 20° C. Samples were withdrawn at intervals, and each was divided into two parts, one for the determination of ester value and the other for chromatography. The peaks of group (1)—the hydrocarbons—were in evidence at the start; so was that for unchanged geraniol, peak (5). Between them appeared several peaks, the largest of which were those

for geranyl formate, peak (4), and the formate of an alcohol that seems to be a cyclic isomeride of geraniol. These peaks are shown in Fig. 15, a chromatogram of a sample taken after reaction for 10 minutes, when the ester value was at its highest. From this time on, all peaks were seen to decline in size, with the exception of those in group (1) and peak (2). After a lapse of 2 hours, none but these remained of the volatile compounds, other than in trace amounts. This is seen in Fig. 16, which closely resembles chromatograms of reactions at boiling-point (*e.g.*, Fig. 13). No further changes were observed during the next 22 hours.

Fig. 17 shows graphs in which ester values are plotted against the corresponding reaction times. Curve A, derived from the experiment described above, behaves initially as would be expected from the indications given by chromatograms of the volatile products, in that the ester value increases sharply during the first 10 minutes and then decreases; it does not, however, diminish to zero, but approaches a steady value of 70. Further experiments suggested that a limiting value is approached at all temperatures between 20° C and boiling-point and that the magnitude of this limiting value varies inversely with temperature. Succesive ester values at 60° C are plotted in curve B of Fig. 17, the steady figure in this instance being 62. On a boiling-water bath it was 42, and tests under reflux over an electric heating mantle gave results between 32 and 34. Evidently, a higher proportion of "polymers" having ester groups is produced as the temperature is decreased.

The concentration of formic acid, from 75 per cent. upwards, was found to have far less influence on this ultimate ester value. Samples of geraniol heated with equal volumes or more of 75, 80, 90 or 95 per cent. formic acid at boiling-point all gave values between 32 and 36. Similarly, the concentration of acid had little effect on the volatile end-products. Even a mixture of geraniol with 75 per cent. formic acid at 20° C gave, after being set aside for 24 hours, a chromatogram essentially like those in Figs. 13 and 16.

All the experiments point to the conclusion that no adjustment of working conditions can prevent the formation of end-products having significant ester values. In the best circumstances, a pure geraniol must be expected to give an apparent-citronellol content equal to nearly 10 per cent. of its own weight.

Discussion of results

The reactions of citronellol and geraniol are far more complex than the original theory of hot-formylation analysis supposed. From citronellol, some glycol diformate is always produced, and this causes high results at high concentrations of acid. Decreases in the concentration of acid progressively decrease the content of glycol diformate and increase the proportions of monoformate and unchanged citronellol. The ester value of the glycol monoformate is slightly lower than that of citronellyl formate, and the value for citronellol is nil; a point therefore exists at which a "correct," but spurious, answer is obtained. Further decreases in concentration of acid yield results lower than the true figure. With geraniol, hydrocarbons are certainly produced in the reaction, but large amounts of several other compounds having low volatility are also formed. One or more of these compounds has an ester value, so that treatment by hot formylation invariably shows an apparent content of citronellol. This, together with the glycol diformate, could cause the high results reported from analyses of the mixed alcohols.

Fig. 1 shows that the same principal end-products occur when a mixture of geraniol and citronellol is subjected to hot formylation, although how far the presence of the one may influence the extent of each reaction undergone by the other is not clearly revealed. A check was made by applying the above findings to the figures given in the interim report of the Essential Oils Sub-Committee.[3]

The four methods tested embodied three variations in working conditions: (*a*) formic acid at 90 or 100 per cent., (*b*) the use of 10 or 20 ml of acid and (*c*) heating on a water bath or sand-bath. It was shown that neither (*a*) nor (*b*) has much influence on the ester value for geraniol, but both affect the percentages of the glycol formates from citronellol. Variation (*c*) is not important for citronellol, but it appreciably alters the result with geraniol. It is therefore deduced that—

(*i*) All four methods should give figures exceeding the true content of citronellol.

(*ii*) Method C should give the highest result, as 20 ml of 100 per cent. formic acid favours the biggest yield of glycol diformate, and heating on a water bath increases the apparent-citronellol content of geraniol.

(*iii*) Method A should show the lowest result, as 90 per cent. formic acid gives the smallest proportion of glycol diformate, and heating on a sand-bath reduces the ester value of the geraniol.

(*iv*) Methods B and D should give results intermediate between those of methods A and C. In method B (10 ml of 100 per cent. formic acid), there will be more glycol diformate than in method D or A, but less than in method C, in which 20 ml of 100 per cent. acid are used. On the other hand, the ester value due to geraniol by method D (water bath) will be greater than those by methods B and C (sand-bath). Methods B and D should consequently yield rather similar results; those of method B would tend to be higher when citronellol predominated and that of method D when geraniol predominated.

Since these deductions agreed so well with the observed trends in Table I of the interim report,[3] a quantitative comparison was made between the results for mixtures and the expected values calculated from the experiments with individual alcohols as described above. By taking 325 and 362 as the means of ester values recorded for citronellol with twice its volume of 90 and 99 per cent. formic acid, respectively, 340 as the value with an equal volume of 99 per cent. acid and 33 and 42 as the respective values for geraniol when heated on a sand-bath or water bath, the results in Table III emerged.

TABLE III

COMPARISON BETWEEN OBSERVED AND EXPECTED RESULTS

Citronellol content of mixture, % w/w	Method A		Method B		Method C		Method D	
	Citronellol found, %	Citronellol calculated, %	Citronellol found, %	Citronellol calculated, %	Citronellol found, %	Citronellol calculated, %	Citronellol found, %	Citronellol calculated, %
79	84·9	85	87·8	89	95·1	96	86·0	86
13	20·5	21	24·0	21	25·9	24	24·4	23
37	42·5	42	46·3	44	50·0	49	46·8	44
57	63·2	62	67·1	65	71·7	71	65·5	63

Within the limits of likely experimental error, these results are sufficiently concordant to strengthen the supposition that neither alcohol greatly influences the reactions of the other with formic acid and that the findings for each one taken separately may safely be extended to mixtures of both.

CONCLUSIONS

The theory of hot formylation takes no account of several important side-reactions that seem to be unavoidable whatever adjustments be made in working conditions. Every result happening to coincide with the true percentage of citronellol is the outcome of a chance combination of self-cancelling errors. For any given mixture, within a limited range of compositions, a particular concentration of acid might be selected at which a low figure for citronellol would nullify the false positive contribution from geraniol and so lead to the right answer; obviously, however, the same conditions would not yield a coincident answer with a blend of different proportions. Formylation can never lead to genuinely accurate results, nor can it even be made the basis of a generally applicable method giving superficially correct ones.

It is even less reliable as a guide to the citronellol content of essential oils. For instance, the presence of additional alcohols must further complicate the reactions, each alcohol according to its structure, and additive formylation of double bonds in compounds other than citronellol is not beyond the bounds of possibility. Such matters would demand detailed investigation were it not that the experiments described appear to be sufficiently adequate to condemn the use of formic acid in analytical procedures for determining citronellol in every type of mixture. The decision of the Essential Oils Sub-Committee not to support any method of hot formylation is, on the evidence, entirely justified.

I thank the Directors of Unilever Ltd. for permission to publish this paper and Mr. D. Welti for his help with infra-red spectra.

REFERENCES

1. Walbaum, H., and Stephan, K., *Ber.*, 1900, **33**, 2302.
2. Pfau, A. S., *J. prakt. Chem.*, 1921, **102**, 276.
3. Analytical Methods Committee, *Analyst*, 1959, **84**, 690.

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The Detection of "Additional Elements" in Plastic Materials by the Oxygen Flask Combustion Method

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A method has been devised for detecting "additional elements" in polymers, polymeric compositions and plasticisers. The sample is burnt in oxygen and the products of combustion are absorbed in sodium hydroxide solution. Some of the newer colorimetric methods have been applied to detect such elements as nitrogen, chlorine, fluorine, phosphorus and sulphur in the combustion products. The test is so designed that semi-quantitative information can be obtained about the proportions of these additional elements, particularly when present in the lower concentrations.

ONE of the essential preliminaries in the examination of polymers, polymer compositions, plasticisers, etc., in the plastics industry involves the detection of the additional elements, *i.e.*, elements other than carbon, hydrogen and oxygen. In the past this has been accomplished by means of the sodium fusion test, the aqueous extract of the fusion product being examined for (*a*) nitrogen by the ferric ferrocyanide test, (*b*) sulphur by the nitroprusside test, (*c*) phosphorus by the ammonium molybdophosphate test, (*d*) halogen (usually chlorine) by the silver nitrate test, after removal of cyanide if necessary, and (*e*) fluorine by either a colorimetric alizarin or etching test.

The sodium fusion test is hazardous and often far from satisfactory when only small proportions of the additional elements are present. It is probably useful in teaching laboratories, but, in our experience in the plastics industry, the analyst often wishes to know if relatively small amounts of additional elements are present in a material under examination. The ability to detect these small amounts of additional elements will often provide evidence of small proportions of additives and make the analysis of a given material all the more complete and informative. It has to be admitted that on many occasions the sodium fusion test has failed to provide this preliminary guidance.

Within the past year or so we have had considerable experience in opening up organic substances, such as polymers, polymer compositions and plasticisers, by combustion in oxygen,[1,2] and we have found that electrical firing gives us a simple means of initiating the combustion. The procedure adds no objectionable impurities to the combustion products, and it was thought that if it could be linked with some of the newer colorimetric methods for the detection of chlorine, phosphate, etc., then it might be possible to dispense with the older sodium fusion test in these laboratories. Further, we had in mind that a combustion process of this kind could be carried out in a fairly reproducible manner, and hence we might make the test not only qualitative but also semi-quantitative in character, particularly when the amount of each additional element present was less than 2 per cent. Moreover, we considered that, with this kind of information in his possession, it might be much easier for the analyst to fashion correct procedures for the subsequent quantitative determination of any of the additional elements.

Our first efforts were directed to finding a suitable absorbent for the combustion products. Examination of the literature indicated that, when the oxygen combustion method had

been applied in quantitative tests, different absorbents had been used for different elements, *e.g.*, sodium hydroxide and sodium sulphite for chlorine,[1,2] alkaline hydrogen peroxide for halogens other than fluorine,[3] distilled water for fluorine,[3] aqueous hydrogen peroxide for sulphur[4] and nitric acid for phosphorus.[5] For our purpose, however, we considered it desirable to use only one absorption solution so that all the tests for additional elements could be effected on the products of a single combustion, but it was manifestly impossible to combine all the desirable characteristics of the different absorbents given above in a simple single solution.

It was fairly obvious after our preliminary work that the choice of absorbent lay between water and sodium hydroxide. In the tests for chlorine, sulphur, phosphorus and fluorine, both these absorbents were of almost equal value, the products of combustion being absorbed in fairly high yield. Nitrogen, however, was a special case, and we realised that a test for this element in the combustion products of an organic compound would almost certainly have to be one for either nitrate or nitrite. We required the test to be fairly rapid, and, as we considered the test for nitrate[6] to be rather too lengthy for our purpose, we chose to apply a direct test for nitrite. It was thus necessary to choose the absorption solution giving the highest yield of this anion. Accordingly, tests were carried out on the combustion products obtained from an organic mixture containing 1 per cent. of nitrogen absorbed in (*a*) water and (*b*) N sodium hydroxide, testing for nitrite by the Griess - Ilosvay method[7] and for nitrate by Buckett, Duffield and Milton's method, which involves removal of nitrite, reaction of the nitrate with 2,4-xylenol in sulphuric acid media and final colorimetric examination of the extracted nitration products in alkaline media. Our results were—

Absorbent	Nitrogen as nitrite in absorption solution, calculated as % of total N in compound	Nitrogen as nitrate in absorption solution, calculated as % of total N in compound
Distilled water	9	12
Sodium hydroxide, N	25	5

These results indicated that with sodium hydroxide as absorbent we secured a greater proportion of nitrite and less nitrate than with water, and this helped to convince us that sodium hydroxide was the better all-round absorbent for our tests. It should be realised that in all the work described above, and indeed in all our subsequent work, no attempt has been made to secure complete absorption of the products of combustion in the sodium hydroxide solution by complicated shaking procedures. Our aim throughout has been to keep the combustion process and all subsequent operations as simple and as reproducible as possible. Although only about 25 per cent. of the total nitrogen in a sample is finally detected, we are satisfied that the whole procedure is reasonably reproducible.

Most of our work has dealt with the examination of solid and non-volatile viscous liquid samples. We have found that these materials are most satisfactorily examined by wrapping in Whatman No. 541 filter-paper for the combustion process and, moreover, that materials such as "Melinex" film, polythene film, cellophane and methylcellulose possessed no advantages over filter-papers for this purpose. B.P.C. super-quality cotton-wool manufactured by the Bardsley Wadding Company proved to be satisfactory for wick preparation. This combination of Whatman No. 541 filter-paper and cotton-wool wick has been shown to give satisfactory and complete combustion and the requisite low blank values in the tests for the individual elements.

In essence, the final procedure we have devised for the qualitative detection and semi-quantitative determination of the additional elements in plastic materials involves preliminary combustion in oxygen and absorption of the combustion products in sodium hydroxide solution. After dilution to a definite volume, the tests for the individual elements are made on suitable aliquots of this solution. Before describing the complete procedure, certain observations on the selected colorimetric tests should be made.

NITROGEN—

Much of our preliminary work on the detection of nitrogen was carried out by using the principle of the Griess - Ilosvay test.[7] Although this test could be most useful in the detection of small amounts of nitrogen in, for example, filter-paper, we considered that, for normal day-to-day work in the plastics industry, it was too sensitive. Great care was

necessary in the choice of filter-paper for holding the sample for combustion and in the preparation of reagents to avoid relatively high blank colours. The ultra-violet absorption method for determining nitrite described by Altshuller and Wartburg[8] was also considered, but was found to be too insensitive for our requirements. We finally tried Peach's method,[9] in which resorcinol is nitrosated and the product treated with ferrous salt to give a green complex proportional to the nitrite in the test solution. This method proved to be satisfactory for our purpose, and, although the recoveries of nitrite varied slightly with the functional form of the nitrogen in the sample, good positive tests were obtained with all the nitrogen compounds tested. These included amides, amines, nitroso-compounds, azo-compounds and nitro-compounds, including cellulose nitrate; a stabilised diazonium salt gave only a faint positive test.

SULPHUR—

The test that we recommend for sulphate in the combustion products is that described by Milton, Hoskins and Jackman.[10] This test depends on precipitation of the sulphate by barium chloride in the presence of a protective colloid. In the course of our work, however, information has also been accumulated about (*a*) the barium chloranilate method[11] and (*b*) the 4-amino-4'-chlorodiphenyl hydrochloride method[12] for the detection of small amounts of sulphate, and this information is also included in the paper. In our experience, the blank value with the barium chloranilate method is too high and the colour for a given amount of sulphate too low for real satisfaction. The 4-amino-4'-chlorodiphenyl hydrochloride method is the more sensitive method, and, under our conditions of test, no interference was encountered from fairly high proportions of phosphates. This method may be used when extreme sensitivity is required in the test.

PHOSPHORUS—

Fogg and Wilkinson's test[13] for phosphate is used, in which ascorbic acid is preferred to stannous chloride for the reduction of the molybdophosphate.

FLUORINE—

The method proposed here is the alizarin complexan - cerous nitrate test of Belcher, Leonard and West,[14,15] details of which were kindly supplied to one of us (J.H.) some time ago.

HALOGENS—

Chlorine is detected by the mercury thiocyanate test described by Sergeant and Thompson.[16] This test is also given by iodides and bromides, although the presence of iodine and bromine in most organic plastic materials would at present be regarded as abnormal. Nevertheless, we have included information about the fluorescein test[17] given by both iodides and bromides and the starch test for iodine. It should be noted that only a small amount of fluorescein is used in the above test in order that there shall be no difficulty whatever in distinguishing between a positive and negative test for these elements. Moreover, we take the view that if iodine and/or bromine are detected in a plastic material then this detection will almost certainly involve the analyst in subsequent quantitative determination of these constituents.

Our experience of the behaviour of the various additional elements in the combustion process, when they are present in the sample as different functional groups (*e.g.*, nitrogen as amide or nitro-groups), is obviously limited. We have, however, as a guide, given in the final methods described in full below the order of optical densities obtained under our conditions of test when (*a*) nitrogen is present as acetanilide, (*b*) sulphur as sulphonal, (*c*) fluorine as fluorobenzoic acid, (*d*) phosphorus as triphenylphosphine and (*e*) chlorine as chlorobenzoic acid. In addition, we have shown the influence of the other elements on each specific test by carrying out combustions on composite samples.

COMBUSTION OF SAMPLE AND PREPARATION OF TEST SOLUTION

APPARATUS AND REAGENTS—

Combustion unit—The electrically fired combustion unit previously described[2] was used in this work, although we see no reason why other forms of oxygen flask should not be used, with appropriate calibration.

Filter-paper—Whatman No. 541 filter-paper. When not in use, store the filter-paper in a sealed container out of contact with the laboratory atmosphere.

Cotton-wool—B.P.C. Super Quality.
Sodium hydroxide, N.

PROCEDURE—

Weigh out approximately 20 mg of sample, and transfer it to the centre of a small piece of the Whatman No. 541 filter-paper weighing approximately 0·1 g. Fold the filter-paper so that the sample is completely enclosed, and, before making the final fold, insert a small wick of cotton-wool weighing about 6 mg.

Carry out the combustion as described previously,[2] with 5 ml of N sodium hydroxide in the bottom of the flask as absorption solution. Set the flask aside for 15 minutes to allow the gases formed in the combustion to be absorbed, and then wash the contents of the flask quantitatively into a 25-ml measuring cylinder with distilled water. Dilute the solution to 25 ml, and mix well. This constitutes the test solution, aliquots of which are taken for the detection of the individual elements by the colorimetric methods detailed below. For comparison purposes in the tests, prepare a blank test solution by carrying out the combustion procedure on the filter-paper and cotton-wool only.

NITROGEN

REAGENTS—

Resorcinol—AnalaR.
Acetic acid, glacial.
Ammonium ferrous sulphate—AnalaR.

PROCEDURE—

Weigh 0·1 g of resorcinol into a clean dry 50-ml beaker, and dissolve in 0·5 ml of glacial acetic acid. Add 5 ml of the test solution, and, after mixing, add 0·1 g of ammonium ferrous sulphate. Carry out the same test on the blank test solution. The development of a green colour in the sample test solution, compared with a pale yellow in the blank, indicates the presence of nitrogen in the sample.

If a semi-quantitative estimation of the nitrogen content of the sample is required, set both sample and blank solutions aside for 20 minutes. Add 10 ml of distilled water to each, mix, and measure the optical density of the sample solution against the blank at 690 mμ in a 4-cm cell. The blank in this test is low, giving an optical density of 0·07 measured against water at 690 mμ in the 4-cm cells.

TABLE I

TYPICAL OPTICAL DENSITIES OBTAINED WITH MIXTURES CONTAINING 1 AND 2 per cent. OF NITROGEN

Mixture tested	Nitrogen in mixture, %	Optical density ($D^{4\ cm}_{690\ m\mu}$ measured against blank
Acetanilide, 1·9 mg Benzoic acid, 18·1 mg	1	0·65
Acetanilide, 3·8 mg Benzoic acid, 16·2 mg	2	1·0
Acetanilide, 1·9 mg Chlorobenzoic acid, 0·9 mg (1% Cl) Sulphonal, 0·7 mg (1% S) Triphenylphosphine, 1·7 mg (1% P) Fluorobenzoic acid, 1·5 mg (1% F) Benzoic acid, 13·3 mg	1	0·55
Acetanilide, 3·8 mg Chlorobenzoic acid, 1·8 mg (2% Cl) Sulphonal, 1·4 mg (2% S) Triphenylphosphine, 3·4 mg (2% P) Fluorobenzoic acid, 3·0 mg (2% F) Benzoic acid, 6·6 mg	2	1·0

Typical results obtained with organic mixtures containing 1 and 2 per cent. of nitrogen present as acetanilide both in the presence and absence of other additional elements are shown in Table I.

SULPHUR

Recommended barium chloride method

Reagents—

Hydrochloric acid, N.

Precipitating reagent solution A—Dissolve 0·2 g of peptone in 50 ml of 1 per cent. barium chloride ($BaCl_2.2H_2O$) solution. Buffer to a pH of 5·0 with 0·02 N hydrochloric acid, add 10 g of sodium chloride (AnalaR), and dilute to 100 ml. Heat on a water bath for 10 minutes, and add a few drops of chloroform. Filter if necessary.

Precipitating reagent solution B—Dissolve 0·4 g of gum ghatti in 200 ml of distilled water by warming slightly. When solution is complete, add 2·0 g of barium chloride ($BaCl_2.2H_2O$). Filter if necessary.

Store solutions A and B separately, and prepare the final reagent just before use by diluting 10 ml of solution A to 100 ml with solution B.

Hydrogen peroxide, 100-volume—AnalaR.

Method—

Transfer 5 ml of the test solution to a 6-inch × 1-inch test-tube, and add 2 drops of 100-volume hydrogen peroxide and then 1·2 ml of N hydrochloric acid. Mix well, and add 2·0 ml of precipitating reagent with continued shaking. A distinct turbidity will be produced in the mixed solution if sulphur is present in the sample; the blank test under the same conditions will be perfectly clear. If a semi-quantitative estimation of the sulphur content is required, add 5 ml of distilled water to both blank and test solutions, mix, and set aside for 30 minutes. Mix the solutions, and measure the optical density of the test solution in a 4-cm cell at 700 mμ with the blank solution in the comparison cell.

Other methods for sulphur

For information, we give below details of the 4-amino-4′-chlorodiphenyl hydrochloride and the barium chloranilate methods, in the form that they can be applied to the combustion test solution if required.

4-Amino-4′-chlorodiphenyl hydrochloride method

Reagents—

4-Amino-4′-chlorodiphenyl hydrochloride - peptone solution (*solution A*)—Dissolve 0·025 g of peptone (Hopkin and Williams Ltd.) and 0·125 g of 4-amino-4′-chlorodiphenyl hydrochloride (L. Light and Co. Ltd.) as completely as possible by warming with 50 ml of 0·05 N hydrochloric acid. Cool, and filter through a double Whatman No. 40 filter-paper.

4-Amino-4′-chlorodiphenyl hydrochloride - gum ghatti solution (*solution B*)—Dissolve 0·20 g of gum ghatti (Hopkin and Williams Ltd.; finely ground) and 1·00 g of 4-amino-4′-chlorodiphenyl hydrochloride as completely as possible by warming to about 70° C with 400 ml of 0·05 N hydrochloric acid. Cool, and filter through a double Whatman No. 40 filter-paper.

Final precipitation reagent—Dilute 10 ml of solution A to 100 ml with solution B. This solution should be freshly prepared.

Hydrochloric acid, N.

Method—

Transfer 5 ml of the test solution to a 6-inch × 1-inch test-tube, and add 1·2 ml of N hydrochloric acid. Mix well, and add 10·0 ml of the prepared final precipitation reagent. A distinct turbidity will be produced in the mixed solution if sulphur is present in the sample. The blank test under the same conditions will be perfectly clear. If a semi-quantitative estimation of the sulphur content is required, set the solutions aside for 30 minutes, then mix, and measure the optical density of the test solution against the blank in 2-cm cells at 700 mμ.

Barium chloranilate method

Reagents—

Barium chloranilate—Obtained from Hopkin and Williams Ltd.
Potassium hydrogen phthalate—AnalaR.
Ethanol, absolute.

Method—

Transfer 5 ml of the test solution to a stoppered 100-ml conical flask, and add 1·0 ml of N hydrochloric acid and then 0·2 g of potassium hydrogen phthalate and 6·25 ml of absolute ethanol. Add 0·04 g of barium chloranilate, insert the stopper, and shake the flask for 15 minutes on a mechanical shaker. Filter the solution through a small Whatman No. 540 filter-paper. Treat the blank solution in a similar manner. When sulphur is present in the sample a red-purple colour will be developed in the test solution compared with the lighter coloured blank. If a semi-quantitative estimation of the sulphur present is required, measure the optical density of the sample against the blank solution at 530 mμ in a 4-cm cell, 30 minutes after beginning the filtration.

Results

Typical results for the optical densities obtained with organic mixtures containing 1 and 2 per cent. of sulphur present as sulphonal are shown in Table II for all three methods. The effect of the presence of other elements on the sulphur figure is also shown at both the 1 and 2 per cent. levels.

The blank solutions measured against water under the conditions described had optical densities of the following order: recommended barium chloride method, 0·02; 4-amino-4′-chlorodiphenyl hydrochloride method, 0·01; barium chloranilate method, 0·13.

The results in Table II show clearly the relative insensitivity of the barium chloranilate method, although it is possible to increase the sensitivity in a particular test by absorbing the combustion products from 20 mg of sample in 5 ml of 0·2 N sodium hydroxide and adding the 1·0 ml of N hydrochloric acid and 0·2 g of potassium hydrogen phthalate to the solution in the combustion flask. The 6·25 ml of ethanol is used to wash down the sides of the flask, and, when mixed, all the solution is poured into a stoppered 100-ml conical flask for treatment with the barium chloranilate and colorimetric measurement as previously described. Under these conditions, the optical densities obtained for sulphur contents of 1 and 2 per cent. are increased to 0·3 and 0·6, respectively, but no test for other elements can be made on the same combustion products.

Table II

Typical optical densities obtained with mixtures containing 1 and 2 per cent. of sulphur

Mixture tested	Sulphur in mixture, %	Optical density by recommended barium chloride method ($D^{4\ cm}_{700\ m\mu}$ measured against blank)	Optical density by 4-amino-4′-chloro-diphenyl hydrochloride method ($D^{2\ cm}_{700\ m\mu}$ measured against blank)	Optical density by barium chloranilate method ($D^{4\ cm}_{530\ m\mu}$ measured against blank)
Sulphonal, 0·7 mg Benzoic acid, 19·3 mg	1	0·2	0·35	0·06
Sulphonal, 1·4 mg Benzoic acid, 18·6 mg	2	0·4	0·70	0·13
Composite mixture as in Table I	1	0·2	0·35	0·04
Composite mixture as in Table I	2	0·45	0·70	0·09

PHOSPHORUS

Reagents—

Ammonium molybdate solution—Dissolve 10 g of AnalaR ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] in about 70 ml of water, and dilute to 100 ml. Add this solution with stirring to a cooled mixture of 150 ml of sulphuric acid and 150 ml of water.

Ascorbic acid—B.D.H. laboratory-reagent grade.

METHOD—

Transfer 2 ml of the test solution to a 100-ml beaker. Add 40 ml of distilled water and 4 ml of ammonium molybdate solution. Mix thoroughly, then add 0·1 g of ascorbic acid, and boil the solution for 1 minute. Cool in running water for 10 minutes, and dilute to 50 ml with distilled water. Treat the blank solution in a similar manner. When phosphorus is present in the sample, a blue colour will be developed in the test solution as compared with a pale yellow in the blank. If a semi-quantitative estimation of the phosphorus is required, measure the optical density of the test solution against the blank solution at 820 mμ in 2-cm cells.

TABLE III

TYPICAL OPTICAL DENSITIES OBTAINED WITH MIXTURES CONTAINING 1 AND 2 per cent. OF PHOSPHORUS

Mixture tested	Phosphorus in mixture, %	Optical density ($D^{2\text{ cm}}_{820\text{ m}\mu}$ measured against blank)
Triphenylphosphine, 1·7 mg Benzoic acid, 18·3 mg	1	0·45
Triphenylphosphine, 3·4 mg Benzoic acid, 16·6 mg	2	0·95
Composite mixture as in Table I	1	0·5
Composite mixture as in Table I	2	1·0

Typical results for the optical densities obtained with organic mixtures containing triphenylphosphine at 1 and 2 per cent. phosphorus levels are shown in Table III. The effect of the presence of other elements on the phosphorus figure is also shown at both 1 and 2 per cent. levels.

The blank solution measured against water under the conditions described had an optical density of the order of 0·01 to 0·015.

FLUORINE

REAGENTS—

Buffered alizarin complexan solution—Weigh 40·1 mg of 3-aminomethylalizarin-*NN*-diacetic acid (Hopkin and Williams Ltd.) into a beaker, and add 1 drop of N sodium hydroxide and approximately 20 ml of distilled water. Warm the solution to dissolve the reagent, cool, and dilute to 208 ml. Weigh into another beaker 4·4 g of sodium acetate ($CH_3COONa.3H_2O$), and dissolve in water. Add 4·2 ml of glacial acetic acid, and dilute to 42 ml. Pour this sodium acetate solution into the alizarin complexan solution, and mix to give the final buffered alizarin complexan solution.

Cerous nitrate, 0·0005 M—Dissolve 54·3 mg of cerous nitrate [$Ce(NO_3)_3.6H_2O$] in water, and dilute to 250 ml.

METHOD—

Transfer 20 ml of distilled water and 2·4 ml of buffered alizarin complexan solution to a 50-ml beaker. Add 1 ml of test solution, and mix by swirling the solution. Finally, add 2 ml of cerous nitrate solution, and mix again. Treat the blank solution in a similar manner. When fluorine is present in the sample a mauve colour will be developed in the test solution compared with the pink-coloured blank solution. If a semi-quantitative estimation of fluorine is required, set the solutions aside for 10 minutes, and measure the optical density of the test solution against the blank solution at 600 mμ in 1-cm cells.

Typical results for the optical densities obtained with organic mixtures containing fluorobenzoic acid at 1 and 2 per cent. fluorine levels are shown in Table IV. The effect of the presence of other elements on the fluorine figure is also shown at both 1 and 2 per cent. levels.

The blank solution measured against water under the conditions described had an optical density of about 0·08.

TABLE IV

TYPICAL OPTICAL DENSITIES OBTAINED WITH MIXTURES CONTAINING 1 AND 2 per cent. OF FLUORINE

Mixture tested	Fluorine in mixture, %	Optical density ($D^{1\text{ cm}}_{600\text{ m}\mu}$ measured against blank)
Fluorobenzoic acid, 1·5 mg Benzoic acid, 18·5 mg	1	0·07
Fluorobenzoic acid, 3·0 mg Benzoic acid, 17·0 mg	2	0·135
Composite mixture as in Table I	1	0·08
Composite mixture as in Table I	2	0·135

CHLORINE

REAGENTS—

Ammonium ferric sulphate solution—Dissolve 12 g of AnalaR ammonium ferric sulphate in water, and add 40 ml of AnalaR nitric acid. Dilute to 100 ml, and filter.

Mercuric thiocyanate solution—Dissolve 0·4 g of mercuric thiocyanate that has been recrystallised from ethanol in 100 ml of absolute ethanol.

METHOD—

Transfer 5 ml of the test solution to a 50 ml beaker, and add 1 ml of ammonium ferric sulphate solution. Mix the solution, and add 1·5 ml of mercuric thiocyanate solution. Mix, and dilute to 10 ml. Treat the blank solution in a similar manner. When chlorine is present in the sample an orange colour will be developed in the test solution compared with the yellow-coloured blank solution. If a semi-quantitative estimation of chlorine is required, set the solutions aside for 10 minutes, and measure the optical density of the test solution against the blank solution at 460 mμ in 2-cm cells.

TABLE V

TYPICAL OPTICAL DENSITIES OBTAINED WITH MIXTURES CONTAINING 1 AND 2 per cent. OF CHLORINE

Mixture tested	Chlorine in mixture, %	Optical density ($D^{2\text{ cm}}_{460\text{ m}\mu}$ measured against blank)
Chlorobenzoic acid, 0·9 mg Benzoic acid, 19·1 mg	1	0·4
Chlorobenzoic acid, 1·8 mg Benzoic acid, 18·2 mg	2	0·75
Composite mixture as in Table I	1	0·4
Composite mixture as in Table I	2	0·75

Typical results for the optical densities obtained with organic mixtures containing chlorobenzoic acid at 1 and 2 per cent. chlorine levels are shown in Table V. The effect of the presence of other elements on the chlorine figure is also shown at both 1 and 2 per cent. levels.

The blank solution measured against water under the conditions described had an optical density of 0·15.

BROMINE

REAGENTS—

Fluorescein solution—Dissolve 0·1 g of fluorescein in 25 ml of 0·1 N sodium hydroxide, and dilute to 100 ml with water.

Sodium acetate buffer solution—Mix 100 ml of N sodium acetate with 15 ml of N acetic acid.

Chloramine-T solution—Dissolve 1 g of chloramine-T in 100 ml of distilled water.

Sodium thiosulphate solution—Prepare a 0·5 per cent. w/v solution of sodium thiosulphate in 5 per cent. w/v sodium hydroxide solution.

Hydrochloric acid, N.

METHOD—

Transfer 5 ml of the test solution to a 50-ml beaker. Add 1 ml of N hydrochloric acid and then 0·5 ml of sodium acetate buffer solution and 1 drop of fluorescein solution. Mix thoroughly, and then add 1 drop of chloramine-T solution. Mix by swirling, and set aside for 30 seconds; then stop the reaction by adding 2 drops of alkaline thiosulphate reducing agent. Treat the blank solution in a similar manner. When bromine is present in the sample a rose-pink colour will be developed in the test solution compared with the yellow-green blank solution.

NOTE—This test is also given by iodine.

IODINE

REAGENTS—

Starch solution—Dissolve 0·2 g of soluble starch in 100 ml of distilled water.

Sulphuric acid, dilute—Prepare an approximately 10 per cent. v/v solution of concentrated sulphuric acid in distilled water.

METHOD—

Transfer 5 ml of test solution to a 50-ml beaker, and add a few drops of starch solution. Mix the solution, and then acidify with dilute sulphuric acid. Treat the blank in a similar manner. When iodine is present in the sample, the characteristic blue colour of starch iodide will be developed in the test solution compared with the colourless blank solution.

APPLICATION OF THE METHODS

For most purposes, application of the procedure as described provides sufficient information about the sample under test, but, if required, the sensitivity of any of the individual tests may be significantly increased. This may be accomplished by repeating the combustion, absorbing the products in 5 ml of 0·2 N sodium hydroxide and making a direct test for the particular element on this solution without dilution. An example of this has been given for the barium chloranilate test for sulphur.

The principle of the combustion procedure may be extended, if necessary, to the detection of other elements, such as boron, arsenic,[18] radioactive carbon[19] and, sometimes, metals such as zinc, cadmium, magnesium,[20] barium[21] and mercury.[22]

Although most of our work has dealt with the examination of solids and non-volatile viscous liquids, which can be wrapped in filter-paper for combustion, we see no reason why the test should not be extended to the examination of volatile liquids. In certain tests, materials such as methylcellulose or gelatin capsules could be used to hold the sample, provided careful attention is paid to the blank values obtained with these containers. Alternatively, the glass-capillary method of Bennewitz[23] might be used.

It may be useful if we indicate here some of the problems to which we have applied the procedures described.

A polymer received for analysis was suspected to contain small amounts of both iodine and fluorine. Application of the qualitative tests indicated the presence of both iodine and fluorine, and, moreover, it was shown that these qualitative tests could be readily adapted to yield immediate semi-quantitative information about the proportions of the elements present, which were of the order of 2 per cent. of fluorine and 5 per cent. of iodine. This problem might not have been solved so easily at this level by conventional procedures.

In another instance an acrylic sheet was shown to possess abnormal solubility characteristics. A portion of the sheet was submitted to the general tests outlined above, which quickly indicated that the sample contained of the order of 1 per cent. of combined nitrogen. A figure of 1·0 per cent. of nitrogen was obtained by the lengthy Kjeldahl procedure. This detection of nitrogen implied the presence in the sheet of a copolymerised nitrogen-containing compound, which was later confirmed.

A vinyl chloride copolymer was suspected from the infra-red evidence to contain a proportion of a butadiene - styrene - acrylonitrile copolymer, there being evidence of an absorption band corresponding to a $-C\equiv N$ grouping at 4·4 μ. This portion of the spectrum is particularly free from interference from other polymer groupings. Repeated sodium fusion tests indicated that nitrogen was absent from the composition, but application of our procedure indicated the definite presence of about 0·25 per cent. of nitrogen, equivalent to approximately 1 per cent. of acrylonitrile in the sample.

The more sensitive form of the test we have mentioned has proved most useful for the confirmation that certain additives have been incorporated in polymer compositions. In this connection the detection of additives equivalent to less than 0·1 per cent. of sulphur, nitrogen or phosphorus has proved possible.

We thank Miss M. Clark and Mr. J. L. Sharp for their assistance in this investigation.

References

1. Haslam, J., Hamilton, J. B., and Squirrell, D. C. M., *J. Appl. Chem.*, 1960, **10**, 97.
2. ——, ——, ——, *Analyst*, 1960, **85**, 556.
3. Schöniger, W., *Mikrochim. Acta*, 1955, 123.
4. Boos, R. N., *Analyst*, 1959, **84**, 633.
5. Fleischer, K. D., Southworth, B. C., Hodecker, J. H., and Tuckerman, M. M., *Anal. Chem.*, 1958, **30**, 152.
6. Buckett, J., Duffield, W. D., and Milton, R. F., *Analyst*, 1955, **80**, 141.
7. "The Determination of Toxic Substances in Air," I.C.I. Manual, p. 196.
8. Altshuller, A. P., and Wartburg, A. F., *Anal. Chem.*, 1960, **32**, 174.
9. Peach, S. M., *Analyst*, 1958, **83**, 644.
10. Milton, R., Hoskins, J. L., and Jackman, W. H. F., *Ibid.*, 1944, **69**, 299.
11. Bertolacini, R. J., and Barney, J. E., *Anal. Chem.*, 1957, **29**, 281.
12. Belcher, R., Nutten, A. J., Parry, E., and Stephen, W. I., *Analyst*, 1956, **81**, 4.
13. Fogg, D. N., and Wilkinson, N. T., *Ibid.*, 1958, **83**, 406.
14. Belcher, R., Leonard, M. A., and West, T. S., *Talanta*, 1959, **2**, 92.
15. ——, ——, ——, *J. Chem. Soc.*, 1959, 3577.
16. Sergeant, G. A., and Thompson, P. B., *Analyst*, 1959, **84**, 251.
17. Hardwick, P. J., *Ibid.*, 1942, **67**, 223.
18. Corner, M., *Ibid.*, 1959, **84**, 41.
19. Götte, H., Kretz, R., and Baddenhausen, H., *Angew Chem.*, 1957, **69**, 561.
20. Belcher, R., Macdonald, A. M. G., and West, T. S., *Talanta*, 1958, **1**, 408.
21. Barney, J. E., Bergmann, J. C., and Tuskan, W. G., *Anal. Chem.*, 1959, **31**, 1394.
22. Southworth, B. C., Hodecker, J. H., and Fleischer, K. D., *Ibid.*, 1958, **30**, 1152.
23. Bennewitz, R., *Mikrochim. Acta*, 1960, 54.

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The Determination of Organo-phosphorus Insecticides in Vegetables

A General Method for Insecticide Residues

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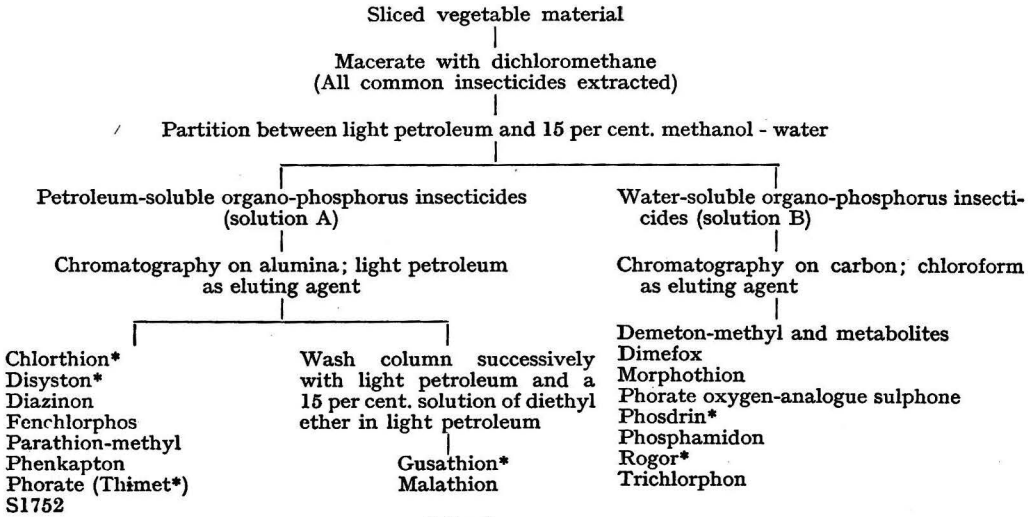
A method is described for the extraction of insecticides from treated cabbages and other vegetables and for their separation into water-soluble and petroleum-soluble groups. The further separation of members of each group from associated plant material is described for eighteen organo-phosphorus insecticides, and the quantitative separation of both groups is discussed, with particular reference to mixtures of (*a*) phorate with its oxygen-analogue sulphone and (*b*) parathion-methyl with Phosdrin.

THE method described by Laws and Webley[1] for extracting and determining demeton-methyl is applicable to a number of water-soluble insecticides. It is not applicable, as it stands, to insecticides preferentially soluble in light petroleum, since the solvent systems used fail to extract the hydrocarbon-soluble insecticides from the macerated plant material, and no attempt is made to utilise the light petroleum washings. Modifications have consequently been made to the extraction procedure, and these ensure that both types of compound are extracted from the plant tissue before they are separated by partition.

EXPERIMENTAL

EXTRACTION AND SEPARATION—

The first extraction is carried out by macerating the sliced vegetable with dichloromethane. After filtration, washing and then evaporation of the solvent, partition is effected between light petroleum and a 15 per cent. solution of methanol in water. At this stage, the insecticide is distributed as shown in the diagram below (solutions A and B)—



* Trade name.

From this point in the determination the two groups are treated separately by different methods. The petroleum-soluble portion (solution A) is chromatographed on a column of graded alumina, light petroleum being used to elute the insecticides, and the water-soluble

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portion (solution B) is examined by Laws and Webley's chromatographic method[1] involving elution with chloroform from a carbon column. The final eluates from the columns in both groups contain the insecticides substantially free from organic phosphorus of plant origin.

Table I

Recoveries of petroleum- and water-soluble insecticides

The amount of each insecticide present was 50 to 70 μg. Petroleum-soluble insecticides were eluted with light petroleum and water-soluble insecticides with chloroform

Petroleum-soluble group			Water-soluble group		
Insecticide	Recovery from alumina column, %	Recovery of insecticide added to cabbage, %	Insecticide	Recovery from carbon column, %	Recovery of insecticide added to cabbage, %
Chlorthion*	64	66	Demeton-methyl and metabolites ..	85	76
Disyston*	72	95	Dimefox	98	80
Diazinon	76	89	Morphothion	91	84
Fenchlorphos	90	89	Phorate oxygen-analogue sulphone	80	70
Gusathion*	90	79	Phosdrin*	90	65
Malathion	66	75	Phosphamidon	78	70
Parathion-methyl	95	65	Rogor*	80	65
Phenkapton	91	74	Trichlorphon	75	57
Phorate (Thimet*) ..	75	75			
S1752	100	70			

* Trade name.

Table II

Recovery of added phorate and its oxygen-analogue sulphone from cabbage

The insecticides were added to 50-g portions of cabbage (range 0·25 to 4·0 p.p.m.)

Insecticide added		Insecticide recovered		Recovery	
Phorate, μg	Oxygen-analogue sulphone, μg	Phorate, μg	Oxygen-analogue sulphone, μg	Phorate, %	Oxygen-analogue sulphone, %
209·3	200·0	146·5	128·0	70	64
111·6	106·6	83·7	64·0	75	60
111·6	55·3	74·8	19·7	67	37
55·8	106·6	27·9	90·6	50	85
55·8	53·3	47·4	53·3	85	100
55·8	26·7	55·8	21·6	100	81
27·9	53·3	24·0	38·4	86	72
27·9	26·7	17·9	17·4	64	65
13·9	13·4	11·7	8·7	84	65
Nil	53·3	Nil	33·0	Nil	62
55·8	Nil	37·4	Nil	67	Nil
			Average ..	75	69

As this procedure isolates the insecticides in a reasonably pure condition, the method used to determine the residue is a matter of choice; determination of phosphorus, cholinesterase assay, infra-red spectrography, gas chromatography or specific chemical or colorimetric methods present themselves as possibilities. The spectrophotometric determination of phosphorus as the molybdenum-blue complex was used to obtain the results reported in this paper. The solubility of the insecticides in light petroleum or water was first investigated, and then the behaviour on the appropriate chromatographic column was studied. Quantitative recovery experiments were next carried out on solutions of the commercially pure insecticide, and, finally, the compounds were added to raw cabbage and the complete method was applied.

In all the determinations reported here, the two phases of the partitition were completely analysed to ensure that the observed losses were not due to incomplete separation at this stage. In considering the recoveries of the various insecticides, the difficulties of residue work must be borne in mind. There are systematic losses at various stages of the procedure, and these are well known to practitioners in this field. Extraction from the plant rarely exceeds 95 per cent., and recovery from the chromatographic column varies with the purity of the insecticide; commercially pure insecticides may contain from 90 to 100 per cent. of pure active ingredient. Further, some insecticides are volatile, and, although the proposed method includes precautions to avoid losses due to volatility, it is a factor to be reckoned with at all stages. It is generally accepted that, for this type of analysis, an over-all recovery of 70 to 80 per cent. is satisfactory. The results of the experiments are shown in Table I.

APPLICATIONS OF METHOD—

To illustrate the application of the method to mixtures of insecticides, two series of recovery experiments were carried out on cabbage; two insecticides were used in each series, one component of each pair being petroleum-soluble and the other water-soluble. The chosen mixtures were (*a*) phorate (Thimet) with its oxygen-analogue sulphone and (*b*) parathion-methyl with Phosdrin.

TABLE III

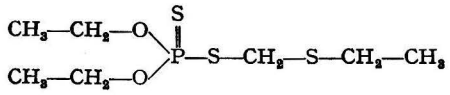
RECOVERY OF ADDED PARATHION-METHYL AND PHOSDRIN FROM CABBAGE

The insecticides were added to 50-g portions of cabbage (range 0·5 to 2·0 p.p.m.)

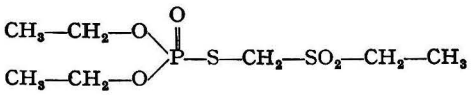
Insecticide added		Insecticide recovered		Recovery	
Parathion-methyl, μg	Phosdrin, μg	Parathion-methyl, μg	Phosdrin, μg	Parathion-methyl, %	Phosdrin, %
97·5	112·0	84·8	75·0	87	67
97·5	56·0	60·5	38·1	62	68
58·5	112·0	49·3	80·6	84	72
58·5	56·0	37·4	42·6	64	76
29·3	56·0	17·9	26·9	61	48
58·5	33·6	33·9	21·5	58	64
29·3	33·6	25·5	20·5	87	61
97·5	Nil	59·5	Nil	60	Nil
Nil	112·0	Nil	62·7	Nil	56
97·5	112·0	—*	73·9	—*	66
58·5	Nil	30·4	Nil	52	Nil
Nil	56·0	Nil	37·0	Nil	66
			Average ..	65	65

* Solution lost.

The compound *OO*-diethyl *S*-(ethylthiomethyl) phosphorothiolothionate, **I**, is known as phorate (Thimet). It undergoes a series of oxidative changes, either by chemical or enzyme systems, the main product of which is the oxygen-analogue sulphone, **II**.



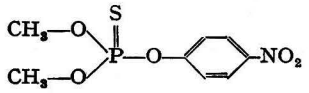
(I)



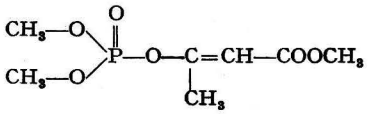
(II)

Samples of compounds **I** and **II**, provided by Cyanamid of Great Britain Ltd., had a purity of 98 per cent., based on phosphorus content. Suitable standard solutions were prepared, each containing approximately 15 μg of insecticide per ml of the final dilution, and the amounts of insecticide added and recovered are shown in Table II. Compound **I** is typical of the petroleum-soluble group and compound **II** of the water-soluble group.

The compounds *OO*-dimethyl *O*-*p*-nitrophenyl phosphorothionate, **III**, and 2-methoxycarbonyl-1-methylvinyl dimethyl phosphate, **IV**, are commercially known as parathion-methyl and Phosdrin, respectively; the former is petroleum-soluble and the latter water-soluble.



(III)



(IV)

A sample of compound **III** having a purity of 100 per cent. (by phosphorus content) was provided by Fisons Pest Control Ltd., and one of compound **IV** (purity of 95 per cent. by phosphorus content and 88 per cent. by extraction with chloroform) by Shell Chemicals Ltd. Two standard solutions, one containing 19 μg of compound **III** per ml and the other 11 μg of compound **IV** per ml were prepared, and the results of recovery experiments with these solutions are shown in Table III.

To show that the method was capable of wider application, some experiments were carried out on other vegetables and some fruits; the blank values found are shown in Table IV.

TABLE IV

BLANK VALUES FOUND FOR 50-g SAMPLES OF VARIOUS VEGETABLES AND FRUITS

Sample	Elution with light petroleum from alumina column		Elution with chloroform from carbon column	
	Optical-density blank value	Equivalent amount of phosphorus, μg	Optical-density blank value	Equivalent amount of phosphorus, μg
Cabbage	0·040	0·64	0·045	0·72
Peas..	0·070	1·12	0·032	0·50
Apples	0·010	0·15	0·015	0·23
Plums	0·015	0·23	0·013	0·22
Cherries	0·017	0·26	0·018	0·29
Lettuce	0·025	0·42	0·015	0·23
Tomatoes	0·055	0·89	0·015	0·23
Potatoes (tubers)	0·017	0·26	0·043	0·69
Brussels sprouts	0·018	0·27	0·040	0·64
Carrots	0·044	0·68	0·040	0·64

METHOD

The method consists in the extraction of the insecticides from the plant material, their separation into petroleum- and water-soluble groups, chromatography of the groups on alumina and activated carbon, respectively, and then determination of the phosphorus by spectrophotometric measurement of the molybdenum-blue complex.

APPARATUS—

Unicam SP500 absorption spectrophotometer.

Glass tubes for chromatography—Tubes 1·5 cm in diameter and 14 cm long, fitted with a tap at the lower end and a B19 ground-glass joint at the upper end for attachment to a reservoir of solvent.

M.S.E. homogeniser, with 100-ml Vortex beakers—Obtainable from Measuring and Scientific Equipment Ltd., London, S.W.1.

REAGENTS—

All reagents must be of recognised analytical grade and phosphate-free.

Chloroform.
Dichloromethane.
Diethyl ether.
Light petroleum, boiling range 40° to 60° C.
Methanol, absolute.

Alumina—Heat chromatographic aluminium oxide at 500° C for 2 hours to ensure conversion to the γ-form. Adjust the activity to Brockmann grade V by adding 15 per cent. w/w of water.

Active carbon—Heat 14- to 22-mesh carbon (grade 207, type B, obtainable from Messrs. Sutcliffe Speakman, Leigh, Lancashire) at 600° C in closed crucibles for 2 hours to remove organic impurities, boil twice with concentrated hydrochloric acid for 30 minutes on each occasion, wash free from acid with water, and dry in an oven at 100° to 110° C.

Perchloric acid, N.

Sulphuric acid, 10 *and* 1 N.

Nitric acid, sp.gr. 1·420.

Hydrochloric acid, sp.gr. 1·180.

Ammonia solution, sp.gr. 0·880.

Ammonium molybdate solution—Dissolve 50 g of ammonium molybdate in 400 ml of 10 N sulphuric acid, and dilute to 1 litre with distilled water.

Stannous chloride solution, concentrated—Dissolve 10 g of stannous chloride dihydrate in 25 ml of hydrochloric acid, sp.gr. 1·180.

Stannous chloride solution, dilute—Dilute the concentrated solution 200-fold with 1 N sulphuric acid. Prepare a fresh solution daily.

Ethanolic sulphuric acid—Mix 5 ml of concentrated sulphuric acid with 245 ml of absolute ethanol.

Isobutyl alcohol - benzene mixture, (1 + 1, *v/v*).

Standard insecticide solutions—Prepare from the pure active ingredients, and suitably dilute.

Potassium dihydrogen orthophosphate.

EXTRACTION AND SEPARATION—

Shred a representative sample of the plant in a slicing-and-grating machine, and transfer 50 g of the shredded material to the homogeniser beaker. If an addition of insecticide is required, add an aliquot of standard insecticide solution, and set aside for 30 minutes. Add 100 ml of dichloromethane, macerate for 15 minutes, filter the mixture through a Buchner funnel, and wash the solid on the filter-pad with 60 ml of dichloromethane. Transfer the combined filtrate and washings to a separating funnel, and run the lower organic layer into a 250-ml conical flask. Wash the small aqueous layer with two 15-ml portions of dichloromethane, and add the washings to the main extract. Reject the aqueous layer, and heat the dichloromethane solution on a hot-plate in a current of air until all the solvent has evaporated, taking care not to heat the flask overmuch in removing the last traces. Immediately add 5 ml of methanol to the residue, pour the methanol solution into a 100-ml separating funnel, wash the flask with 30 ml of light petroleum and 25 ml of water, and add the washings to the contents of the separating funnel. Shake the funnel vigorously, allow to settle, and run the lower aqueous layer back into the 250-ml conical flask. Re-extract the light petroleum with 5 to 10 ml of water, run the aqueous layer into the conical flask, and pour the light petroleum layer from the top of the funnel into a 100-ml flask. Return the aqueous layer to the funnel, rinsing the flask with 30 ml of light petroleum and 10 ml of water. Shake vigorously, and separate as before. Rinse the funnel with a little light petroleum, and add the rinsings to the petroleum extract. Again return the aqueous portion to the funnel, extract it with four 20-ml portions of chloroform, and combine the chloroform extracts.

CHROMATOGRAPHY—

Petroleum-soluble insecticides—Evaporate the light petroleum extract to about 10 ml by heating on a water bath in a current of air. Prepare a column 1·5 cm in diameter from 8 g of the Brockmann grade V alumina; use light petroleum as liquid phase. Transfer the extract to the column, and elute with 150 ml of light petroleum at about 1·5 ml per minute. To recover Gusathion and malathion, elute with 150 ml of light petroleum - diethyl ether mixture (85 + 15 v/v).

Water-soluble insecticides—Evaporate the chloroform extract to about 10 ml by heating on a hot-plate; blow a current of air across the surface of the solvent to cool the liquid and so minimise loss of volatile insecticides. With chloroform as liquid phase, prepare a column

from 4 g of active carbon in a tube similar to that used for the alumina. Transfer the extract to the column, and elute with 100 ml of chloroform at 1·5 ml per minute.

TREATMENT OF ELUATES—

Evaporate the light petroleum, the light petroleum - diethyl ether and the chloroform eluates from the columns in separate conical flasks fitted with ground-glass joints; heat the first two eluates on a water bath and the third on a hot-plate, and assist evaporation with a stream of air to avoid loss of volatile insecticides. The chloroform solution need not be taken completely to dryness, as the last traces of chloroform will boil away from the mixed acids and water in the final stages of the determination.

Wet combustion of separated insecticides—To each evaporated eluate add 10 ml of water, 4 ml of N perchloric acid, 5 ml of nitric acid, sp.gr. 1·420, and 1 ml of hydrochloric acid, sp.gr. 1·180, and heat the solution under reflux beneath a Liebig condenser for 40 minutes. Drain the cooling water from the condenser, and leave until brown fumes begin to appear. At this point, remove the condenser, and evaporate the solution until fumes of perchloric acid are evolved. Rinse the condenser with 6 to 7 ml of water, add the rinsings to the contents of the flask, and again evaporate until white fumes are evolved. Add 3 ml of water and 4 ml of ammonia solution, sp.gr. 0·880, and remove the excess of ammonia by boiling. If it is known that volatile insecticides are not present, the heating under reflux may be omitted; the solution is heated to fumes with the acids as described above and then with 5 ml of water, with 2 ml of nitric acid and again with 5 ml of water before the ammonia solution is added.

Place the test solution containing the phosphorus in a 100-ml separating funnel, and bring the volume to 10 ml, including any washings. Add 3·5 ml of ammonium molybdate solution, and mix after adjusting the total volume to 14 ml. If the molybdate solution is added before transfer to the separating funnel, the final volume should still be 14 ml.

Add 10 ml of isobutyl alcohol - benzene mixture, and shake the funnel vigorously. Allow the layers to separate, and discard the lower layer. Wash the remaining organic layer with 5 to 10 ml of N sulphuric acid, and again discard the lower layer. Add 15 ml of the dilute stannous chloride solution, shake for 5 to 10 seconds, and allow the layers to separate. Discard the lower aqueous layer, and allow the organic layer to fill the bore of the tap, but do not allow any of this layer to run to waste. Remove any of the aqueous layer left in the stem of the separating funnel with a piece of cotton-wool, and run the organic layer, which is blue if phosphorus is present, into a 10-ml measuring cylinder fitted with a stopper. The volume at this stage is about 8 ml. Wash the separating funnel with 1 to 2 ml of the ethanolic sulphuric acid, add the washings to the contents of the cylinder, and adjust the volume to 10 ml with ethanolic sulphuric acid. Insert the stopper, and mix thoroughly. Measure the optical density of the solution in 1-cm cells at 730 mμ with a spectrophotometer; use as blank solution a mixture of 4 parts of isobutyl alcohol - benzene mixture and 1 part of ethanolic sulphuric acid.

NOTES—

For successful production of molybdenum blue from orthophosphate, the acidity should be between 0·5 and 1·5 N and the amount of neutral salts other than ammonium molybdate should not exceed 0·5 g in 10 ml of test solution. To fulfil these conditions, the initial phosphate solution, whether derived from a wet combustion during a determination or from the standard solution in the preparation of a calibration graph, is made up to 14 ml, including 3·5 ml of the ammonium molybdate solution, which is in 4 N sulphuric acid.

The time of shaking with the stannous chloride solution is restricted to 10 seconds, because a longer time results in a decrease in the intensity of the final colour. It is important that the procedure should be carried out as rapidly as possible from the addition of the stannous chloride solution to the production of the final solution. The final colour is stabilised by the addition of the ethanolic sulphuric acid and remains unchanged for a considerable time.

The method is essentially that of Berenblum and Chain[2] and was more recently described by Field and Laws.[3] Minor changes in procedure have been included in the above.

Discussion of results

A general picture of the application of the method to eighteen different insecticides is shown in the diagram on p. 249 and in Table I. Recoveries of mixtures of phorate with its oxygen-analogue sulphone at various levels are reported in Table II and those of mixtures of parathion-methyl with Phosdrin in Table III. Blank values obtained on vegetables and fruit are summarised in Table IV.

From Table I it can be seen that recovery of insecticide is sometimes higher in the presence of the vegetable matter than in its absence. We believe that this is caused by displacement of the adsorbed insecticide by some part of the extracted plant material, which results in the insecticide leaving the column earlier than it would if no such substance were present. In general, the results are reasonable for the type of system studied. The plant material contains phosphorus many hundreds of times in excess of the amount contributed by the insecticide, and the greater part of this is removed by the column. It is very important that the stated rates of elution are not exceeded, as experiment has shown that the amount of interfering plant substance passing through the column grows rapidly as the rate of elution is increased. Most of the experiments were carried out on cabbage, as this material is readily available and is typical of the crops likely to be examined. Since fruit is also likely to be sprayed with insecticide and is often eaten without preparation, some results were obtained to illustrate the wider application of the method (Table IV).

Gusathion and malathion are unlike the other petroleum-soluble organo-phosphorus insecticides studied in that they are not eluted from the alumina column by light petroleum alone. They are, however, eluted by a mixture of light petroleum and diethyl ether (85 + 15 v/v). Some of the malathion is sometimes eluted by the light petroleum before the application of the mixture, but the mixture removes it all. The "plant blank" is not excessive when the mixed solvent is used.

Preliminary examination of the petroleum-soluble insecticides on the alumina column demonstrated that the various compounds were eluted by different amounts of solvent. The amount chosen (150 ml) is the minimum that will effectively elute all the insecticides studied except malathion and Gusathion. It is possible that separation of the individual insecticides could be achieved by using a second column of alumina and a fraction collector, with the purified eluate from the first column as starting material.

The general utility of the method lies in the fact that it divides the insecticides into two distinct groups and is applicable as a general method of analysis to at least eighteen well known insecticides; it is potentially useful for a far greater number of compounds. Although it is accepted that methods are available for determining chlorinated insecticides, such as DDT, BHC and dieldrin, it should be mentioned that these compounds will be found in the light petroleum solution obtained by the proposed method and that this solution could in fact be used as a starting point in the determination of such residues. With one exception, optical-density blank values obtained on the vegetables so far tested have not exceeded 0·050, which corresponds to about 0·10 p.p.m. of insecticide. It would be possible to determine residues of the order of 0·10 p.p.m. in a 50-g sample.

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References

1. Laws, E. Q., and Webley, D. J., *Analyst*, 1959, **84**, 28.
2. Berenblum, J., and Chain, E., *Biochem. J.*, 1938, **32**, 295.
3. Field, K., and Laws, E. Q., *Analyst*, 1957, **82**, 667.

ห้องสมุด กรมวิทยาศาสตร์

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Characterisation of Organic Acids by means of their Benzylamine Salts

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A simple procedure is described for characterising organic acids by formation of benzylamine salts. The method is shown to be generally applicable, and melting-points are reported for the benzylamine salts of thirty-three acids, including carboxylic and sulphonic acids and nitrophenols. The infra-red spectra of these salts are briefly discussed, with special reference to the absorptions associated with the NH_3^+ group.

The preparation of crystalline derivatives of organic acids is conveniently achieved by salt formation, and *S*-benzylthiouronium salts have been favoured. These salts are readily prepared by treating an aqueous solution of the sodium salt of the acid with an *S*-benzylthiouronium halide, but careful control of pH is necessary to avoid alkaline hydrolysis of the reagent to benzyl mercaptan. The method also suffers from the disadvantage that the melting-points of the salts lie within a relatively narrow range of temperatures.

Characterisation of acids by conversion to their benzylamine salts has been proposed,[1,2] although Boudet's paper[1] contains no analytical results. Some text-books of qualitative organic analysis quote the melting-points of a limited number of these derivatives,[3,4] but the method has not come into general use. In an investigation of the scope of this method, thirty-three benzylamine salts have been prepared, and a simple general procedure has been devised. Salts of a wide variety of organic acids, including sulphonic acids and nitrophenols, have been examined and their melting-points and infra-red spectra recorded.

Method

Reagent—

Benzylamine solution, 10 per cent. v/v in benzene—This solution is stable and can be kept.

Procedure—

The acid (1·0 g) is dissolved in the minimum volume of hot absolute ethanol or benzene, and 10 ml of the benzylamine solution are added. The salt is rapidly precipitated, and only rarely is refrigeration or evaporation required. If necessary, the salt is recrystallised, usually from ethyl acetate or a mixture of ethyl acetate with ethanol or benzene.

Discussion of results

The melting-points of the benzylamine salts are shown in Table I; results for carbon and hydrogen contents are quoted for new compounds, for compounds not previously analysed and when our melting-points differed from those in the literature.

This method of characterising organic acids seems to be generally applicable and gives derivatives having melting-points distributed over a wide range of temperatures.

When the standard procedure was used, nearly all the dicarboxylic acids formed acid salts, the neutral salts being obtained by using excess of benzylamine. The neutral salt of adipic acid, however, was unstable and, on attempted purification, decomposed to give the acid salt. Fumaric and phthalic acids were exceptional in that the standard procedure led to the isolation of the neutral salts. By using exactly one equivalent of benzylamine it was possible to isolate the acid phthalate, but the acid fumarate was unstable and could not be obtained in a pure condition. Citric acid formed a dibenzylamine salt under the standard conditions; the neutral salt, obtained by using excess of reagent, decomposed during recrystallisation to give the more stable dibenzylamine salt.

The sulphonic acids examined formed stable non-deliquescent salts (the salt of benzenesulphonic acid was obtained by adding the reagent to a concentrated aqueous solution of the acid).

Picric acid and *o*- and *p*-nitrophenol formed salts, but attempts to form a salt with the more weakly acidic *m*-nitrophenol were unsuccessful.

TABLE I

MELTING-POINTS OF BENZYLAMINE SALTS

For the salts of benzoic and picric acids, other melting-points intermediate between those shown in column 4 have been reported in the literature

Acid	Type of salt (when applicable)	Melting-point found, °C	Melting-point reported in literature, °C	Reference[a]	Elemental analysis: Carbon found, %	Hydrogen found, %	Theoretical carbon content, %	Theoretical hydrogen content, %
Adipic	Acid	171 to 172	162·5 to 163·5 (corr.)	1	61·7	7·3	61·65	7·6
p-Aminobenzoic		196 (decomp.)	197·6 to 198·6 (corr.)	2	—	—	—	—
Anisic		145·5 to 146·5	143·5 to 144·5 (corr.); 144·8 to 145·6 (corr.)	1; 2	—	—	—	—
Anthranilic		115 to 117[b]	113 to 114 (corr.)	1	69·0	6·1	68·8	6·6
Benzenesulphonic		191 to 193	186; 190; 193	5; 6; 7	—	—	—	—
Benzoic		128 to 129	127·2 to 128·4 (corr.); 132 to 134	2; 8	—	—	—	—
Benzylic		149 to 150	—	—	75·4	6·3	75·2	6·3
Cinnamic		138 to 139	131 to 131·5 (corr.); 135·9 to 136·3 (corr.)	1; 2	75·5	6·6	75·3	6·7
Citric		164 to 165[c]	—	—	59·3	6·5	59·1[d]	6·5[d]
Fumaric	Neutral	179 to 180[c]	171 to 172 (corr.) or 181 to 182 (corr.)	1	65·4	6·7	65·4	6·7
Gallic		200 (decomp.)[e]	188 to 190 (corr.)	1	60·7	5·5	60·6	5·45
Hippuric		134 to 135[f]	—	—	67·05	6·2	67·0	6·3
Lactic		159 to 159·5	—	—	61·3	7·6	60·9	7·7
Maleic	Acid	147 to 148	—	—	59·6	6·4	59·2	5·9
	Neutral	186 to 187 (decomp.)	—	—	65·6	6·7	65·4	6·7
Malonic	Acid	116 to 116·5 (decomp.)[g]	—	—	56·9	6·2	56·9	6·2
	Neutral	144 to 145 (decomp.)	142·5 to 143 (corr.)	1	64·2	7·1	64·1	7·0
Mandelic		190·5 to 191[c]	—	—	70·0	6·6	69·5	6·6
o-Nitrophenol		123 to 124[b]	121 to 121·5 (corr.)	1	63·3	5·75	63·4	5·7
p-Nitrophenol		102 to 103	103 to 103·5 (corr.)	1	63·55	5·5		
Oxalic	Acid	176 (decomp.)	175; 176	9; 10	52·6[i]	5·6	54·8	5·6
	Neutral	207 to 208 (decomp.)[h]	195; 200 to 202 (decomp.)	8; 11	63·2	6·75	63·1	6·6
Phthalic	Acid	105 to 106	—	—	61·6	5·9	61·8[j]	5·9[j]
	Neutral	180 (decomp.)[c]	185; 184·5 to 185 (corr.)	12; 1	69·3	6·45	69·45	6·4
Picric		200 to 202 (decomp.)	194; 200 to 201	13; 14	—	—	—	—
Salicylic		98 to 99	94·3 to 95·5 (corr.)	2	68·8	6·2	68·6	6·2
Succinic	Acid	134·5 to 135·5	116 to 117	15	59·1	6·7	58·65	6·7
	Neutral	147 to 149[c]	144 to 145; 147·5 to 148 (corr.)	15; 1	—	—	—	—
Sulphanilic		237 to 238 (decomp.)[e]	—	—	55·9	6·1	55·7	5·7
(+)-Tartaric	Acid	170[c]	141	16	50·9	6·2	51·4	5·9
	Neutral	148 to 150[c]	112; 147·5 to 148 (corr.); 148	17; 1; 16	—	—	—	—
p-Toluenesulphonic		186 to 187·5	184·5 to 185·5 (corr.); 185·5	18; 19	—	—	—	—
p-Toluic		166	164 to 164·5 (corr.)	1	74·4	7·0	74·0	7·0

[a] See reference list, p. 259.
[b] Crystallised from benzene.
[c] Crystallised from 96 per cent. ethanol.
[d] Value for dibenzylamine salt.
[e] Crystallised from butan-1-ol.
[f] Crystallised from nitromethane.
[g] Crystallised from ethyl methyl ketone.
[h] Crystallised from aqueous ethanol.
[i] No satisfactory result could be obtained.
[j] For the monohydrate, this compound gave a satisfactory result for nitrogen content.

Infra-red spectra

It was thought that the infra-red spectra of the benzylamine salts would be a useful supplement to the melting-point data. The spectra obtained would also be of interest, as little information is available about the absorptions associated with the NH_3^+ group in simple amine salts,[20] although many studies are recorded of the spectra of amino acids,[21] in which the same group occurs. In the series of benzylamine salts studied by us, the NH_3^+ group is associated with widely different anions, and it was of interest to measure the infra-red spectra of these compounds and to attempt to identify the NH_3^+ absorption peaks.

The spectra of all the benzylamine salts mentioned, together with that of benzylamine hydrochloride, were measured in potassium bromide discs with an Infracord spectrophotometer (model 137). For comparison, the spectrum of benzylamine itself, as a liquid film, was also measured.

Examination of the spectra showed, in appropriate instances, the presence of the characteristic absorptions associated with the CO_2^- and SO_3^- groups. Absorptions attributed to the NH_3^+ group occur in several regions of the spectrum, and, although the spectra of the benzylamine salts generally show peaks in these regions, often only tentative assignations are possible, owing to the complexity of the spectra. For these reasons, discussion is restricted here to the three spectral regions indicated in Table II.

Table II

Infra-red absorptions of benzylamine salts

The results for citric acid refer to the dibenzylamine salt

Acid	Type of salt (when applicable)	Absorptions[a] in the region— 3150 to 2830 cm^{-1}	2200 to 1990 cm^{-1}	825 to 775 cm^{-1}
Adipic		2900(s)	2170(w)	898(w), 796(w)[b]
p-Aminobenzoic		2950(m)	2150 to 2070(w)[c]	823(w), 798(m)
Anisic		2920(s), 2880(s)[b]	2150(w)	825(w), 797(s), 782(w)
Anthranilic		2880(s)	2200(m)	822(w), 803(m)
Benzenesulphonic		3090(s), 3010(s), 2900(m)	2070(w)	782(w)
Benzoic		2880(s)	2190(m)	815(w),[b] 803(m), 792(w)
Benzylic		3010(s)	2100(w)	817(w),[b] 803(m), 783(m)
Cinnamic		2900(s),[b] 2850(s)	2180(m)	783(s)
Citric		3150(m),[b] 3010(s)	2030(w)	815(w),[b] 794(w)
Fumaric	Neutral	3010(s), 2830(s)	2170(m)	810(m), 794(w)
Gallic		3040(m),[b] 3000(m), 2860(m)	1990(w)	796(m)
Hippuric		3050(s), 2980(s)[b]	2090(w)	812(w), 808(w), 797(w)
Lactic		2840(s)	2180(w)	776(w)
Maleic	Acid	2990(s)	2010(w)	794(w)
	Neutral	3030(s)	2170(w)	798(m), 778(m)
Malonic	Acid	2990(s)	2080(w), 2000(w)	793(m)
	Neutral	2980(s), 2880(s)[b]	2190(m), 2020(w)	788(m)
Mandelic		2970(s), 2880(s)	2160(w)	810(w),[b] 800(w), 786(m)
o-Nitrophenol		2950(s),[b] 2880(s)	2170(m), 2140(m)	820(w), 798(w), 784(m)
p-Nitrophenol		3100(m), 2950(s),[b] 2830(s)	2200(m)	822(m), 806(w),[b] 793(w)[b]
Oxalic	Acid	3000(s)	2010(m)	813(w), 794(w)
	Neutral	3000(s), 2880(s)	2180(w)	798(w),[b] 783(s)
Phthalic	Acid	3000(s), 2880(s)	2000(w)	818(s), 796(m)
	Neutral	3000 to 2860(s)[c]	2130(m)	808(w), 793(w)
Picric		2990(s), 2880(m)	2070 to 1990(w)[c]	800(w), 794(m)
Salicylic		2970 to 2830(s)[c]	2080(m)	815(m), 807(m)
Succinic	Acid	2980(s)	2000(w)	815(m), 795(m)
	Neutral	3100(m), 3000(m), 2790(s)	2180(m)	812(m), 796(m)
Sulphanilic		3000(s), 2900(s)	2050(w)	793(w)
(+)-Tartaric	Acid	2980(s), 2870(s)	2000(m)[b]	795(m)
	Neutral	3000(s)	2190(w), 2020(w)	794(w)
p-Toluenesulphonic		3100(s), 3020(s), 2920(m)	2070(w)	819(s), 808(w), 782(w
p-Toluic		2980 to 2850(s)[c]	2190(m)	803(w), 788(w)
Benzylamine hydrochloride		2980(s)	2040(w)	795(w)
Benzylamine		3000(s), 2890(s), 2850(s)	—	785(s)

[a] Weak intensity = w; medium intensity = m; strong intensity = s.
[b] Point of inflexion.
[c] Broad absorption band.

THE 3150 TO 2830 cm^{-1} REGION—

With the exception of the salts of the amino acids, *i.e.*, those of anthranilic, *p*-aminobenzoic and sulphanilic acids, the two medium-intensity bands associated with the N–H stretching frequency in the NH_2 group are absent from the spectra of the benzylamine salts. These bands occur in the 3500 to 3300 cm^{-1} region[22] for measurements made on solutions, but would be displaced to lower frequencies in solid-state spectra. The figures in column 3 of Table II indicate that these bands are displaced to lower frequencies similar to the N–H stretching frequencies in methylamine hydrochloride, which absorbs[20] at 3075 and 2972 cm^{-1}, and are superimposed on the C–H stretching frequencies.

THE 2200 TO 1990 cm^{-1} REGION—

The figures in column 4 of Table II show that all the benzylamine salts exhibit absorption of weak to medium intensity in the range 2200 to 1990 cm^{-1}, benzylamine itself being transparent in this region. Weak-intensity absorption at about 2100 cm^{-1} has been reported in the spectra of amino acids and tentatively associated with the NH_3^+ group.[23] This peak, although weak in intensity, could be important in the identification of the NH_3^+ group, as it occurs in a spectral region where interference from other groups is minimal.

THE 825 TO 775 cm^{-1} REGION—

The NH_3^+ rocking frequency occurs as a weak-intensity absorption[20] near 800 cm^{-1}, and all the spectra of the benzylamine salts indicated absorption in the range 825 to 775 cm^{-1} (column 5 of Table II). The strong-intensity absorptions exhibited by some of the salts and benzylamine itself are almost certainly produced by aromatic C–H "out-of-plane" vibrations. Of the thirty-four salts studied, twenty-nine show only weak- to medium-intensity absorption in this region, and nineteen of these have two or more peaks. The range for the ten salts exhibiting single peaks of weak to medium intensity is 796 to 776 cm^{-1}.

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REFERENCES

1. Boudet, R., *Bull. Soc. Chim. France*, 1948, **15**, 390.
2. Buehler, C. A., Carson, L., and Edds, R., *J. Amer. Chem. Soc.*, 1935, **57**, 2181.
3. Linstead, R. P., and Weedon, B. C. L., "A Guide to Qualitative Organic Chemical Analysis," Butterworths Scientific Publications, London, 1956, pp. 110 *et seq.*
4. Wild, F., "Characterisation of Organic Compounds," Cambridge University Press, Cambridge, 1947, pp. 172 and 174.
5. Neber, P. W., and Uber, A., *Annalen*, 1928, **467**, 60.
6. Schlegl, F., *Ber.*, 1931, **64**, 1743.
7. Oxley, P., and Short, W. F., *J. Chem. Soc.*, 1948, 1514.
8. Dhommée, R., *Compt. Rend.*, 1902, **134**, 1314.
9. Holleman, A. F., *Rec. Trav. Chim. Pays-Bas*, 1894, **13**, 411.
10. Strain, H. H., *J. Amer. Chem. Soc.*, 1927, **49**, 1562.
11. Mazurewitsch, H., *Bull. Soc. Chim. France*, 1925, **37**, 1164.
12. Tingle, J., and Brenton, B. F., *J. Amer. Chem. Soc.*, 1909, **31**, 1161.
13. Moureu, C., and Lazennec, I., *Bull. Soc. Chim. France*, 1906, **35**, 1183.
14. Jones, L. W., and Fleck, E. E., *J. Amer. Chem. Soc.*, 1928, **50**, 2026.
15. Werner, E. A., *J. Chem. Soc.*, 1889, **55**, 628.
16. Anselmino, O., *Chem. Zentr.*, 1903, II, 566.
17. Hilditch, T. P., *J. Chem. Soc.*, 1911, **99**, 237.
18. Noller, C. R., and Poe Liang, *J. Amer. Chem. Soc.*, 1932, **54**, 670.
19. Oxley, P., and Short, W. F., *J. Chem. Soc.*, 1951, 1252.
20. Bellamy, L. J., "The Infra-red Spectra of Complex Molecules," Second Edition, Methuen & Co. Ltd., London, 1958, p. 259.
21. ——, *op. cit.*, p. 234.
22. ——, *op. cit.*, p. 249.
23. ——, *op. cit.*, p. 243.

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Silica Gel Chromatography of Organic Acids from Plant Tissue

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An improved method for determining organic acids from plant tissues by silica gel chromatography is described. The acids are extracted by aqueous ethanol, purified by elution from silica gel and then determined by gradient elution from a second column of silica gel. A special method for volatile acids is described. As an example of the method an analysis of the acids from green peas is quoted.

A method for separating and determining organic acids of plant origin by chromatography on silica gel was first proposed by Isherwood[1] in 1946; since then, several modifications to the original method have been introduced.[2 to 7] However, none of these was satisfactory for the routine analysis of small samples of vegetable materials, when it was necessary to transfer all the extracted acids, including the volatile ones, to the analysing column of silica gel.

Further modifications to the original method were required, and the procedure finally developed is described below. It has been used successfully in the routine determination of acids from peas and should have a much wider application in the analysis of organic acids from other plant tissues.

Method

The procedure described is for peas, and it must be emphasised that conditions would probably have to be varied to some extent for other biological material. The extracts, especially the concentrated ones, must be kept at a low temperature. All concentrations were carried out at less than 20° to 25° C, large volumes being concentrated in a rotary film evaporator[8] and small ones over sulphuric acid.[9]

Reagents—

*Silica gel**—This was prepared by Isherwood's method.[1] It was purified by repeated treatment with hydrochloric acid and washing with water until, in a test experiment, the recovery of malic and citric acids from a standard analysing column of the purified gel (see below) was quantitative; the recovery of oxalic acid was slightly lower, about 95 per cent.

Solvents—All solvents used were of analytical-reagent grade except for tetrachloroethylene, which was technical. Ethanol was redistilled from solid sodium hydroxide; chloroform and tetrachloroethylene were shaken with solid sodium carbonate and filtered before use.

Sodium hydroxide, 0·01 N, in 80 per cent. ethanol—A mixture of 400 ml of ethanol and 50 ml water was made slightly alkaline (pale pink to phenolphthalein) with 0·1 N aqueous sodium hydroxide, and 50 ml of 0·1 N aqueous sodium hydroxide were added. This solution was stored in an atmosphere free from carbon dioxide in an aspirator attached to the burette used for titrating the acid fractions.

Silica gel - 10 N sulphuric acid mixture—Silica gel was mixed with an equal weight of 10 N sulphuric acid, and the mixture was made into a thin suspension by adding chloroform.

Ethanolic phenolphthalein solution—A 0·01 per cent. w/v solution of phenolphthalein in ethanol was made slightly alkaline by adding 0·1 N sodium hydroxide until the solution was a very pale pink.

Extraction of acids—

The peas in a 20-g (fresh weight) sample containing 1 to 3 milli-equivalents of all organic acids were killed by dropping them into 100 ml of boiling ethanol, and boiling was continued for 5 minutes. The supernatant ethanol was decanted, and the killed peas were added

* Recently it has been found much easier to acid wash Mallinckrodt's "A.R. silicic acid." The product has less water-holding capacity than that prepared as described, so that the weight of gel to aqueous phase has to be increased to 2 to 1.

to 50 ml of 70 per cent. v/v aqueous ethanol in the glass container of a high-speed blender and were disintegrated for 5 minutes. The mixture was filtered, and the residue was repeatedly extracted by setting aside for 1 to 2 hours with 100-ml portions of the 70 per cent. ethanol until the last extract contained a negligible amount of citric acid (see below); six extractions were usually sufficient. The combined ethanolic extracts were concentrated to a syrup, about 50 mg of magnesium carbonate having been added to prevent loss of volatile acids. The syrup was repeatedly shaken with light petroleum (boiling range 80° to 100° C) to remove chlorophyll, carotenoids and much of the lipid material and was then diluted with water and transferred to a 50-ml beaker. The solution was evaporated to a paste and re-dissolved in 4·5 ml of water to give a final volume of 5 ml. The light petroleum extracts were combined and spun in a centrifuge, and the aqueous droplets so separated were added to the main solution in the beaker before the evaporation; the fine droplets still remaining were removed by filtration through a short column of silica gel (0·5 g of silica gel *plus* 0·5 ml of water).

PURIFICATION OF EXTRACTED ACIDS—

A 5-g portion of dry silica gel was added to the main solution, with thorough mixing. At this stage, the mixture was normally a "dry," but slightly coherent, powder; if it was at all sticky, as occurred with solutions containing much sugar, a little more of the dry gel was added. The gel through which the light petroleum extracts had been filtered was added, the mixture was made into a slurry with chloroform, a few drops of a 1 per cent. w/v solution of thymol blue in butanol were added, and the mixture was transferred to a tube 2 cm in diameter having a B19 socket at one end and a sintered plate and tap at the other. The slurry was acidified by cautiously adding silica gel - 10 N sulphuric acid mixture, with vigorous shaking, until the indicator was bright red (about pH 1). The solvent was then allowed to drain from the column into a separating funnel, and the gel was lightly tamped down. A 250-ml portion of a 50 per cent. v/v solution of butanol in chloroform, which had been equilibrated with 0·5 N sulphuric acid, was allowed to flow through the column over a period of at least 6 hours and was collected in the separating funnel containing the previous drainings from the column. About 10 ml of water were added to the contents of the separating funnel, which were then neutralised to phenolphthalein with 0·1 N sodium hydroxide. The aqueous layer was removed, and the organic layer was washed twice with water to which 0·1 N sodium hydroxide had been added to bring the pH to 8·5. The aqueous fractions were combined, concentrated to about 5 ml, transferred, with washing, to a small beaker and evaporated to dryness.

TEST FOR COMPLETENESS OF EXTRACTION AND ELUTION—

Citric acid was the most difficult of the major constituents to extract and was therefore used as a test substance to check the efficiency of each stage in the purification. To check the completeness of the extraction with ethanol, the sixth extract was evaporated to dryness, the residue was dissolved in a little water, and the solution was passed through a small column (0·5 g) of Zeo-Karb 225 to remove cations. The effluent was concentrated to 0·1 ml and examined by paper chromatography. A 10-cm chromatogram was obtained by the ascending-solvent technique, and the citric acid spot was compared with spots prepared from known concentrations of this acid; n-butanol - acetic acid - water (30 : 5 : 10) was used as solvent.

A similar check was made on the final 25 ml of eluate from the purifying column, the organic acids being isolated from the butanol - chloroform mixture by two extractions with dilute aqueous ammonia and concentration of the aqueous phase to 0·1 ml.

ANALYSING-COLUMN SYSTEM—

A diagram of the complete column system is shown in Fig. 1. Two small aspirators served to apply a low pressure (up to 4 to 5 feet of water) to either or both solvent reservoirs. A 500-ml portion of the 50 per cent. solution of butanol in chloroform was placed in reservoir 1 and 340 ml of chloroform in reservoir 2, which contained the bar of a magnetic stirrer. A capillary tube terminating in a very fine nozzle led to the top of column A. This column was packed by running into it a slurry (in chloroform) of 2·5 g of silica gel mixed with 2·5 ml of a 0·75 M solution of sodium sulphate in 0·5 N sulphuric acid (see p. 264). The slurry was allowed to drain, and the gel was lightly tamped down and then covered with a layer of

chloroform; on this was floated a layer (30 cm deep) of the 0·75 M sodium sulphate in 0·5 N sulphuric acid. In operation, the mixed solvent from reservoir 2 was sprayed as fine droplets into the top of the aqueous layer, through which the droplets fell to join the layer of chloroform at the lower end of the column and finally passed through the pad of silica gel in the base of the column.

Column B contained the unknown acids and was prepared as described below.

Column C, the analysing column, was packed with 5 g of silica gel mixed with 5 ml of 0·5 N sulphuric acid. This mixture was made into a slurry with chloroform and poured into the column in small amounts, the chloroform was allowed to drain away, and the gel was lightly tamped down. A 0·5- to 1-cm layer of gel was added each time. The exit from this column was located above a photoelectric drop counter coupled to an automatic fraction collector,[10] and approximately 3-ml fractions were collected in conical 10-ml centrifuge tubes.

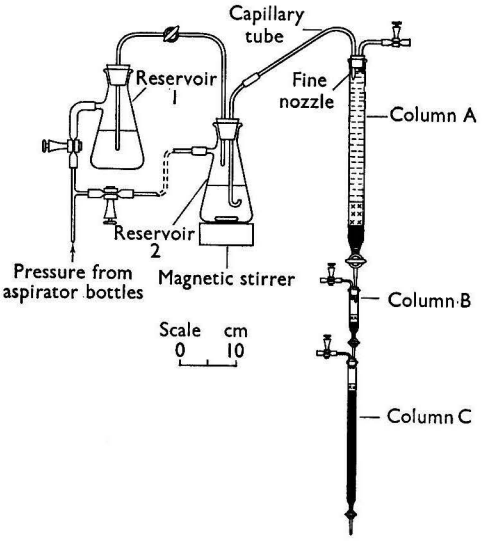

Fig. 1. Diagram of column system for determination of organic acids. Column fillings: A, a slurry of silica gel in 0·5 N sulphuric acid containing 0·75 M sodium sulphate covered with a layer of chloroform supporting a column of 0·5 N sulphuric acid containing 0·75 M sodium sulphate; B, silica gel in 0·5 N sulphuric acid *plus* unknown acids; C, silica gel in 0·5 N sulphuric acid

TRANSFER OF ACIDS TO ANALYSING COLUMN—

Two procedures for transferring the acids to the analysing-column system are described. The first and simpler one is used when exact determination of volatile acids is not required; this procedure will probably be suitable for most biological extracts (see Table I and "Addition of Acids to Column," p. 263). The alternative procedure permits accurate determination of both volatile and non-volatile acids, but it is less convenient.

1. *Procedure for mainly non-volatile acids*—

The dried sodium salts of the unknown acids were dissolved in 0·5 ml of water, 0·5 g of silica gel was added, and the whole was thoroughly mixed as rapidly as possible. The powder, which should be slightly coherent (but not damp), was transferred to column B (see Fig. 1) and made into a slurry with 2 to 3 ml of chloroform. The mixture was acidified by cautiously adding silica gel - 10 N sulphuric acid mixture, with vigorous stirring, until the thymol blue was bright red.

2. *Procedure for volatile and non-volatile acids—*

For the quantitative transfer of volatile as well as non-volatile acids, 0·5 g of dry gel was placed in column B, the sodium salts of the unknown acids were dissolved in 0·6 ml of water, and exactly 0·5 ml of this solution was transferred to the top of the gel. The column was then rapidly and vigorously stirred with a stainless-steel rod, and, as soon as a uniform dry powder had been produced, it was covered with tetrachloroethylene. The mixture was then rapidly acidified by adding silica gel - 10 N sulphuric acid mixture as described above, and the column was closed with a rubber stopper.

The subsequent procedure was similar to that described below for non-volatile acids, except that chloroform was replaced throughout by tetrachloroethylene and titration of the initially eluted fractions containing the volatile acids was carried out without undue delay. Only five-sixths of the amount of acids present in the original material was determined, and an appropriate correction was applied to the results.

OPERATION OF ANALYSING COLUMN—

For an analysis, columns A and C were joined together, and about 40 ml of pure chloroform, under slight pressure from the aspirators, were passed through, collected and titrated as a measure of the blank. Column B was meanwhile prepared and then inserted between columns A and C, the pressure was adjusted to give a flow of about 30 ml of solvent per hour through the apparatus, and the screw-clip in the pressure lead to reservoir 2 was then closed. The 50 per cent. solution of butanol in chloroform then flowed from reservoir 1 into reservoir 2 and caused a progressive increase in the butanol content of the eluting solution. About 150 fractions were collected during 24 to 30 hours from the time that column B was inserted.

TITRATION OF FRACTIONS—

To each of the fractions was added 1 ml of the ethanolic solution of phenolphthalein, and a stream of air, free from carbon dioxide, was bubbled through the liquid. The fractions were then titrated under conditions free from carbon dioxide with the 0·01 N sodium hydroxide in 80 per cent. ethanol, the titre being measured to the nearest 0·01 ml. The addition of ethanol to the titration solution prevented the formation of aqueous droplets, which, when present, interfered with the end-point.

DISCUSSION OF THE METHOD

EXTRACTION WITH LIGHT PETROLEUM—

The ethanolic extract of the peas contained considerable amounts of lipoidal material, chlorophyll and carotenoids. These were removed from the concentrated ethanolic extract by repeated extraction with light petroleum, as the presence of the pigments interfered with the end-points of indicators used for the acid - base titrations of the organic-acid fractions and the lipids sometimes interfered with the separation of the organic acids on the analysing column; the most noticeable effect of the presence of lipids was that the initially eluted acids were liable to give rise to irregular peaks in variable positions.

ACIDIFICATION WITH SILICA GEL - SULPHURIC ACID—

In the method proposed by Isherwood,[1] the acids on the column had to be un-ionised, and this was achieved by eluting them from an aqueous phase of 0·5 N sulphuric acid. Solution of the sodium salts of the unknown acids in 0·5 N sulphuric acid before transfer to the sample column was satisfactory only when the acids were present in low concentration. In the presence of larger amounts of the acids the pH increased and the stronger acids were partly ionised, thereby hindering elution. To overcome this effect, the gel containing the unknown acids was titrated to a pH of about 1·0 with silica gel - 10 N sulphuric acid mixture.

ADDITION OF ACIDS TO COLUMN—

Ideally, the acids should be applied directly to the top of the analysing column, but there are many objections to this in practice; no satisfactory method for completely quantitative transfer of volatile acids could be devised, and, if the acids were transferred in a solvent rich in t-pentanol,[1] the operation of the analysing column was liable to be disturbed. The use of a separate column for the sample proved to be a better method and did not lead to broadened peaks unless a large amount of solvent was allowed to collect on the top of the gel in the analysing column.

A selection of results obtained when known amounts of acids were added to the analysing column by Isherwood's original method[1] and to the sample columns by the two proposed procedures is shown in Table I.

The recovery of non-volatile acids is clearly satisfactory (experiments Nos. 1 to 4); however, when volatile acids, typified by acetic acid, were transferred by Isherwood's method or by the procedure proposed for non-volatile acids, recoveries were only 85 to 95 per cent. (experiments Nos. 5 and 6). This loss was traced to two main causes.

The first arose from the fact that acetic acid was eluted when the solvent was almost pure chloroform, and both the acetic acid and the chloroform evaporated rapidly from the drops of eluate at the tip of the analysing column and from the collecting tubes (compare the results of experiments Nos. 5 and 7). Some 5 to 10 per cent. of the acetic acid was lost in this fashion, depending on the rate of flow of the eluting solution and the lapse of time before titration of the fraction. This difficulty was obviated by using an eluting solvent having a higher boiling-point and titrating the fraction without undue delay (compare the results of experiments Nos. 5 and 8). Tetrachloroethylene, boiling-point 121° C, was found to be a satisfactory solvent in place of the chloroform, but the rate of elution was a little slower.

TABLE I

RECOVERY OF PURE ORGANIC ACIDS

Experiment No.	Method of adding acid to column*	Recovery of added— acetic acid, %	fumaric acid, %	oxalic acid, %	succinic acid, %	malic acid, %	citric acid, %
With n-*butanol - chloroform as eluting agent*—							
1	A	—	—	99	—	—	—
2	B	—	—	—	—	99	100
3	B	—	99	—	99	99·5	98·5
4	B	—	—	95	101·5	97	—
5	A	94	—	—	—	—	—
6	B	85, 89	—	—	—	—	—
7	A	100†	—	—	—	—	—
With n-*butanol - tetrachloroethylene as eluting agent*—							
8	A	98, 101, 99	—	—	—	—	—
9	B	90, 92	—	—	—	—	—
10	C	98, 98·5, 100	—	—	—	—	—

* A—Isherwood's original method[1] (solvent rich in t-pentanol).
B—Proposed method for non-volatile acids (procedure No. 1, p. 262).
C—Proposed method for volatile acids (procedure No. 2, p. 263).
† Evaporation prevented by collecting fractions in enclosed vessels.

The second main loss occurred during mixing the gel with the aqueous solution of sodium acetate. The gel adsorbed the cation present and liberated free acid, so that acetic acid was rapidly lost by evaporation from the large surface area of the gel during mixing and transfer to the sample column. This loss was avoided by using the procedure described for volatile acids (p. 263, procedure No. 2), provided that the entire operation from the addition of sodium acetate solution to the insertion of column B between columns A and C was carried out rapidly (compare the results of experiments Nos. 9 and 10).

EQUILIBRATION OF ELUTING SOLUTION WITH AQUEOUS PHASE—

A continuous change in the composition of the eluting solution (gradient elution) was preferable to the abrupt changes used in Isherwood's method,[1] because no spurious peaks were formed as a result of sudden changes in composition of the solvent. However, the need to keep the continuously changing solvent in equilibrium with the aqueous phase posed a problem. If the solvent rich in butanol and that poor in butanol (usually pure chloroform) were both equilibrated with 0·5 N sulphuric acid and used in reservoirs 1 and 2, respectively, aqueous droplets were precipitated during the early stages of mixing. These droplets tended to block the capillary tube leading from reservoir 2 and also caused the gel in the sample column to become too wet for satisfactory elution of the acids. To avoid this situation, the dry solvents were mixed, and the dry mixture was continuously equilibrated with the aqueous

phase in column A, first by falling, as droplets, through a 30-cm aqueous layer and finally by passage through a shallow layer of silica gel wetted with the same aqueous solution.

Equilibration of the eluting solution with 0·5 N sulphuric acid was satisfactory only when the unknown acids were present in low concentration and were relatively free from other solutes. In the analysis of samples of tissue, the concentration of the acids, when dissolved in 0·5 ml of water before addition to the column, was about 1 M; further, the presence of sugars, sulphate and chloride increased the water-attracting properties. For such samples, water was withdrawn from an eluting solution in equilibrium with 0·5 N sulphuric acid by the aqueous phase of the sample column, which then became too wet for satisfactory elution. This was prevented by adding osmotically active material to the aqueous phase in column A; sodium sulphate was suitable, and a concentration of 0·75 M was used with extracts of immature peas, but this concentration has to be adjusted to suit the material being analysed. The upper layers of the analysing column slowly dried out during an elution, but the performance of the column did not seem to be affected.

SEQUENCE OF ELUTION OF ACIDS—

The sequence in which sixteen to eighteen organic acids were eluted from a column of silica gel wetted with 0·5 N sulphuric acid by a mixture of chloroform and butanol has been described,[2,3] and we have confirmed this sequence. In general, the chromatographic behaviour of a column of silica gel is similar to that of paper at the same pH and with similar solvents. In practice, only sulphurous acid is suitable for use on paper,[11] and the relative position in which an acid will be eluted from a column of silica gel can hence be predicted with reasonable certainty by chromatography on paper, with a n-butanol - chloroform mixture equilibrated against water saturated with sulphur dioxide as solvent; the optimum proportion of butanol depends, as with the column, on the particular acids being treated. For instance, the positions of closely related acids, *e.g.*, citric and isocitric, may be shown by developing paper chromatograms for 2 days with the system described above, 35 per cent. of butanol being used, when the citric acid spot runs slightly in front of that for isocitric acid.

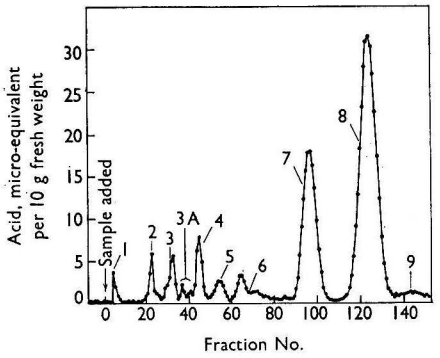

Fig. 2. Histogram of the distribution of acids in fresh peas (for details of individual peaks, see p. 266)

RESULTS

The proposed method has been in routine use for the analysis of maturing seeds of the pea (*Pisum sativum*) for some years.[12] A typical histogram constructed from the titration readings obtained from an extract of 26 g of fresh peas is shown in Fig. 2.

As a test of the reproducibility of the method, peas were extracted with a 70 per cent. solution of ethanol in water, and aliquots corresponding to about 20 g of peas were purified and analysed. The content of acids in each of the main peaks is shown in Table II; reproducibility is good for all peaks except the rather indeterminate peak No. 6.

The compositions of the peaks were examined by paper chromatography. Standard chromatograms were prepared by two-dimensional paper chromatography in n-butanol - acetic acid - water (30:5:10) and n-propanol - ammonia solution, sp.gr. 0·880 (3:2). The

chromatograms were sprayed with B.D.H. universal indicator (made slightly alkaline with 0·1 N sodium hydroxide). Other solvent systems and sprays were used for individual acids.

All the peaks contained small amounts of sulphate and chloride, and these probably constituted the acids giving the blank-titration value (about 0·02 to 0·05 ml of 0·01 N sodium hydroxide for each 3-ml fraction in the early part of an elution and increasing to 0·1 ml by the end). The organic acids provisionally identified are listed in Table II; most of the acids constituting peak No. 1 are normally removed in the light petroleum fraction (see Fig. 2, in which peak No. 1 is small).

TABLE II

CONTENT OF ACIDS FOUND IN PEAS

Peak No.	Acid content of peak (as titre of 0·01 N sodium hydroxide), ml			Acids provisionally identified
1	10·2	10·6	10·3	Propionic, butyric, valeric and some unknown
2	6·0	6·0	6·3	Acetic only
3	6·1	5·0	5·4	Three unknown, probably all dicarboxylic
4	3·8	4·1	3·7	Succinic and lactic only
5	2·2	2·0	2·3	More than four unknowns
6	5·1	3·7	3·2	Oxalic, *cis*-aconitic and more than four unknown
7	22·5	22·5	21·2	Malic only
8	49·7	47·0	49·9	Citric only

Fumarate should be eluted[2] in about the position of peak No. 3, but paper chromatography of the fractions containing peaks Nos. 3 and 3A in a phenol - formic acid - water solvent[13] showed that none of the five acids giving defined spots was fumaric acid.

The two acids constituting peak No. 4, succinic and lactic, were separated and determined by chromatography on a further column, as described by Neish.[14] The small peak No. 9 (see Fig. 2) appeared to consist mainly of isocitric acid.

In all, about twenty-five acids can be separated from a single 25-g sample of peas by using the proposed column system and then paper chromatography. There is little doubt that many other acids, appearing only as faint indications on these chromatograms, would be separated from a larger initial sample.

REFERENCES

1. Isherwood, F. A., *Biochem. J.*, 1946, **40**, 688.
2. Bulen, W. A., Varner, J. E., and Burrell, R. C., *Anal. Chem.*, 1952, **24**, 187.
3. Phares, E. F., Mosbach, E. H., Denison, F. W., jun., and Carson, S. F., *Ibid.*, 1952, **24**, 660.
4. Zbinovsky, V., and Burris, R. H., *Ibid.*, 1954, **26**, 209.
5. Resnik, F. E., Lee, L. A., and Powell, W. A., *Ibid.*, 1955, **27**, 928.
6. Clements, R. L., and Deatherage, F. E., *Food Res.*, 1957, **22**, 222.
7. DeKock, P. C., and Morrison, R. I., *Biochem. J.*, 1958, **70**, 272.
8. Partridge, S. M., *J. Sci. Instrum.*, 1951, **28**, 28.
9. Wager, H. G., *Analyst*, 1961, **86**, 266.
10. Snow, A., Porter, F. A. E., and Tomalin, A. W., *J. Sci. Instrum.*, 1958, **35**, 243.
11. Howe, J. R., *J. Chromatography*, 1960, **3**, 389.
12. Wager, H. G., *J. Exp. Bot.*, 1960, in the press.
13. Stark, J. B., Goodban, A. E., and Owens, H. S., *Anal. Chem.*, 1951, **23**, 413.
14. Neish, C. J., *Canad. J. Res., B.*, 1949, **27**, 6.

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Notes

A METHOD FOR DRYING SMALL LIQUID SAMPLES

THE normal method of drying small aqueous samples over a desiccant at a reduced pressure suffers from the disadvantages that bumping is liable to occur while the pressure is being reduced and that transfer of heat, and hence also drying, is slow.

An alternative method used for many years in the analysis of plant tissues for organic acids[1] is to stand the samples in rapidly moving dry air at room temperature. This can be conveniently accomplished by placing the samples on the floor of a large desiccator, alternating their containers with crystallising dishes containing a desiccant (usually concentrated sulphuric acid) and vigorously stirring the air by means of a paddle mounted in the lid of the desiccator (see Fig. 1). In our

model, a variable-speed fan motor (1/25 horse-power) is mounted above the desiccator on a suitable staging and coupled to the axle of the paddle by means of a short length of thick-walled rubber tubing; the base of the desiccator is slid forwards in order to insert samples. Alternatively, a flexible drive to a motor mounted on the bench could be used to actuate the paddle.

The only disadvantage so far found is that volatile compounds contained in one sample contaminate the other samples in the desiccator and may be "fixed," *e.g.*, free formic acid in one sample may lead to the presence of formates in other samples, depending on their pH values.

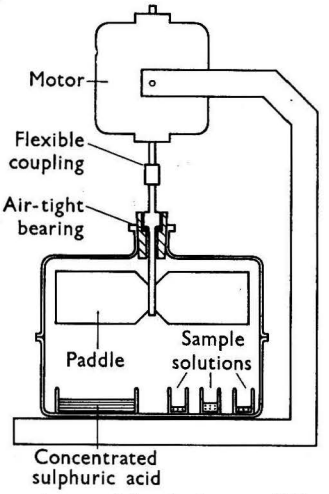

Fig. 1. Apparatus used for drying small liquid samples

Samples of up to 20 ml in small beakers are evaporated to dryness overnight. The rate at which water is lost depends to some extent on (*a*) the number of samples in relation to the exposed area of desiccant and (*b*) the rate at which the paddle revolves. Samples containing ethanol can also be dried with sulphuric acid as desiccant.

Reference

1. Wager, H. G., and Isherwood, F. A., *Analyst*, 1961, **86**, 260.

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THE SPECTROGRAPHIC DETERMINATION OF TRACE IMPURITIES IN GALLIUM

In the chemical - spectrographic procedure previously described[1] for the analysis of high-purity acids, the impurities were concentrated by evaporation in the presence of a small amount of sulphuric acid and therefore spectrographically analysed in a standard form. The method is equally applicable to the analysis of other substances, provided that the base material can be separated from a mineral acid solution, so leaving the impurities to be concentrated in sulphuric acid; such a procedure has now been successfully applied to the analysis of gallium. The metal is dissolved in hydrochloric acid, and the gallium chloride is extracted by di-isopropyl ether. The impurities remaining in the acid phase, after the addition of sulphuric acid and copper sulphate, are then concentrated by evaporation, dried on a graphite electrode and excited in a d.c. arc.

Method

Reagents—

Hydrochloric acid—Prepared by isothermal diffusion[2] in polythene apparatus.

Di-isopropyl ether—A 300-ml portion of the ether was heated under reflux with 60 ml of hydrochloric acid for 30 minutes. The mixture was boiled vigorously and then cooled, the acid layer was discarded, and the ether was heated under reflux with 100 ml of de-ionised water until it had become clear. After a second heating under reflux with de-ionised water, six to eight pellets of sodium hydroxide were added to the separated ether layer, and this was then distilled. The first 15 to 20 ml of distillate were discarded; care was taken not to distil to less than 30 ml and to adjust the water bath so as to keep the level of water below that of the ether.

PROCEDURE—

A 1-g sample of gallium was weighed into a small silica crucible, and surface contamination was removed by treatment with cold 10 N hydrochloric acid, cold diluted hydrochloric acid (1 + 1) and finally de-ionised water. The gallium was dried at room temperature, re-weighed, transferred to a polytetrafluoroethylene beaker and dissolved in 10 ml of hydrochloric acid, 1 ml of 0·1 N sulphuric acid being added; solution was facilitated by heating on a silica hot-plate. When the gallium had dissolved, the solution was evaporated to about 5 ml, and 5·0 ml of 10 N hydrochloric acid were added. The normality of the solution was determined by diluting 0·1 ml with 5 ml of water and titrating with 0·1 N sodium hydroxide, 4·3 ml being deducted from the titre on the assumption that $Ga(OH)_3$ was precipitated.[3] The acidity was adjusted to 8 N, the final volume being between 10 and 15 ml.

This solution was transferred to a stoppered 60-ml polythene bottle, 15 ml of di-isopropyl ether were added, and the mixture was cooled to 20° C in a water bath and shaken vigorously for 5 minutes. It was then spun in a centrifuge for 1 minute at 3000 r.p.m. and 16-cm radius. The ether layer was removed by means of a polythene pipette, and the acidity of the aqueous layer was again determined; if necessary, it was adjusted to 7·5 N (unpublished work by Messrs. W. T. Rees, D. L. Mack and K. G. Rye). A second extraction was made with 10 ml of di-isopropyl ether, and the ether was removed by pipette as before. To ensure complete removal of the ether, the acid solution was filtered through a pad of quartz-wool (work by Rees, Mack and Rye referred to above), and the filtrate was collected in a platinum crucible. After the addition of 1 ml of 0·1 N sulphuric acid, the solution was evaporated on the hot-plate until fumes were evolved. A 1-ml portion of copper sulphate solution (1 mg of copper per ml of 0·1 N sulphuric acid) was added, the solution was warmed, and the sides of the crucible were washed down with de-ionised water. The solution was then evaporated to incipient fuming, and the residue was dissolved in 0·1 ml of de-ionised water. This solution was transferred to a graphite electrode and excited as previously described.[1]

DISCUSSION OF THE METHOD

EFFECT OF GALLIUM—

Since not all the gallium was removed by the solvent extraction, the effect of residual gallium was determined by adding different amounts of the metal to a standard containing 0·5 μg of each impurity. It was found that up to 200 μg of gallium per 0·1 ml had no effect; this was well in excess of the amount remaining after extraction (about 100 μg per 0·1 ml). Concentrations of gallium greater than 200 μg per 0·1 ml increased the over-all density of the spectrograms, including background, but did not affect the intensity ratio of most line pairs.

CONCENTRATION OF ACID—

When analysing high-purity acids[1] it was not difficult to reproduce the final concentration of acid. There is less certainty, however, when an organic solvent is used, as there may be a reaction between the sulphuric acid and any residual organic matter. The effect of concentration of acid was determined by examining solutions containing 1·0 μg of impurities in 0·1 ml of sulphuric acid of concentration between 0·2 and 3 N.

In general, the spectrograms showed little variation for concentrations of acid between 0·8 and 2 N. However, since increases in acidity reduce the line intensity, an excess of acid should be avoided. The variation in intensity ratios was more pronounced when the concentration of acid was less than 0·8 N, and the optimum acidity was between 1 and 1·5 N. The reproducibility of the density of the copper spectrum in the routine exposures indicated that, provided the final evaporation was taken only to incipient fuming, the normality was maintained within the prescribed limits.

RECOVERY OF IMPURITIES AND APPLICATION OF THE METHOD—

The recovery of impurities after extraction with di-isopropyl ether and concentration was determined by dividing a solution containing 2 g of gallium in hydrochloric acid into two equal portions, to one of which was added a series of impurities, each at a concentration of 1 p.p.m. The amount of each impurity recovered was calculated by deducting from the results for the prepared sample the amounts found in the untreated sample. The results of these recovery tests, together with those for three samples of gallium and their associated blanks, are shown in Table I; sample A consisted of high-purity gallium, and samples B and C were "transistor-grade" material. The recoveries of aluminium and magnesium could not be precisely determined because of the high blanks, but the evidence was sufficient to show that the percentage recovery was reasonably high.

TABLE I

RESULTS OF RECOVERY TESTS AND IMPURITY DETERMINATIONS

Impurity	Recovery of 1 p.p.m. of added impurity, p.p.m.	Sample A		Sample B		Sample C	
		Impurity found, p.p.m.	Blank value, p.p.m.	Impurity found, p.p.m.	Blank value, p.p.m.	Impurity found, p.p.m.	Blank value, p.p.m.
Manganese ..	0·9	0·05	<0·01	<0·05	<0·05	0·10	<0·05
Chromium ..	0·9	0·3	<0·02	<0·1	0·05	<0·1	0·05
Bismuth ..	1·3	0·1	<0·02	0·04	<0·02	<0·02	<0·02
Nickel ..	1·0	0·05	0·05	<0·1	0·08	<0·1	0·1
Molybdenum	0·7	<0·03	<0·03	<0·03	<0·03	<0·03	<0·03
Beryllium ..	0·8	<0·005	<0·005	<0·005	<0·005	<0·005	<0·005
Titanium ..	0·9	1·5	<0·05	<0·2	0·15	<0·05	<0·05
Zirconium ..	1·1	0·3	<0·05	<0·05	<0·05	<0·05	<0·05
Cadmium ..	1·0	<0·2	<0·2	<0·2	<0·2	<0·2	<0·2
Vanadium ..	1·3	<0·03	<0·03	<0·03	<0·03	<0·03	<0·03
Zinc	0·7	1·0	0·3	<0·5	0·5	<0·5	0·5
Cobalt ..	1·0	<0·03	<0·03	<0·03	<0·03	<0·03	<0·03
Magnesium ..	—	1·5	<0·5	<1·0	<0·5	<1·0	0·5
Aluminium ..	—	>2·0	<0·5	<1·0	1·0	<0·5	0·5

The reagent blanks for chromium, nickel, zinc, magnesium and aluminium are sometimes greater than the spectrographic limit of detection and therefore limit the ultimate sensitivity attainable.

We thank the Superintendent, Admiralty Materials Laboratory, for permission to publish this Note.

REFERENCES

1. Oldfield, J. H., and Bridge, E. P., *Analyst*, 1960, **85**, 97.
2. Irving, H., and Cox, J. J., *Ibid.*, 1958, **83**, 526.
3. Nachtrieb, J. N., and Fryxell, R. E., *J. Amer. Chem. Soc.*, 1949, **71**, 4035.

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A RAPID METHOD FOR DETERMINING MAGNESIUM IN PLANT MATERIAL

IN a recent method[1] involving use of Titan yellow for the accurate determination of magnesium in plant material, phosphate was first removed by passing the solution through an ion-exchange column. This was necessary because it was found that phosphate produced a decrease in the absorption of the magnesium hydroxide - Titan yellow adsorption compound. Further, precipitation of calcium phosphate occurred with some materials analysed, particularly those having high contents of phosphorus.

The ion-exchange process made the method more lengthy than most procedures involving use of Titan yellow, but the accuracy achieved was similar to that of a volumetric method.[2] Further work has been carried out in an attempt to decrease the time required for the determination without any loss of accuracy, and the method described below is now proposed.

METHOD

REAGENTS—

Titan yellow solution, 0·2 per cent.—Dissolve 0·1 g of Titan yellow in water, and dilute to 50 ml.

Polyvinyl alcohol - glycerol - Titan yellow reagent solution—Dissolve 0·1 g of polyvinyl alcohol in water, with warming, add 100 ml of glycerol and x ml of the 0·2 per cent. solution of Titan yellow, and dilute to 200 ml. (Determine the value of x as described under "Standardisation of Titan Yellow Solution.")

Sodium hydroxide solution, 14 per cent.—Dissolve 28 g of analytical-reagent grade sodium hydroxide in water, and dilute to 200 ml.

Compensating solution—Dissolve 5 g of ethyleneglycol bis-(β-aminoethyl ether)-*NN'*-tetra-acetic acid (EGTA, obtained from J. R. Geigy A.G., Basel, Switzerland) in the minimum volume of 10 per cent. sodium hydroxide solution, and adjust the pH of the solution to approximately 7

by adding dilute hydrochloric acid (check with a wide-range test paper). Add 0·045 g of aluminium chloride hexahydrate dissolved in a small amount of water, then add 20 ml of triethanolamine, and dilute to 200 ml.

STANDARDISATION OF TITAN YELLOW SOLUTION—

Prepare standards containing 0·001, 0·002, 0·003, 0·004 and 0·005 per cent. of Titan yellow in a solution containing 0·05 per cent. of polyvinyl alcohol and 50 per cent. of glycerol. In pairs of tubes containing 0 and 25 μg of magnesium and 2 ml of compensating solution in a volume of 12 ml place 4 ml each of dye and sodium hydroxide solutions. After 30 minutes in a water bath at 25° C, measure the optical densities of the solutions in 4-cm cells at 550 mμ with a Unicam SP600 spectrophotometer. Plot a graph of the difference in optical density between the solutions containing 25 and 0 μg of magnesium (ΔA) against the concentration of dye present. The concentration of Titan yellow at which ΔA reaches a maximum is selected for use, and the amount of a 0·2 per cent. colution (x ml) needed to prepare a solution having this concentration can be calculated.

PROCEDURE FOR DETERMINING MAGNESIUM—

Ash 0·1 g of ground plant material, previously dried for 1 hour at 105° C, in a muffle furnace at 500° C for 2 hours. Dissolve the ash in 5 ml of 0·5 N hydrochloric acid, filter, if necessary, and dilute to 50 ml. By pipette, place an aliquot of the solution containing between 5 and 25 μg of magnesium in a 25-ml graduated and stoppered tube, and dilute to 10 ml. Add 2 ml of compensating solution, place the tube in a water bath at 25° C, and then add 4 ml of polyvinyl alcohol - glycerol - Titan yellow reagent solution. Mix, immediately add 4 ml of sodium hydroxide solution, and mix again. Leave in the water bath for 30 minutes, and then measure the optical density of the solution in 4-cm cells at 550 mμ with a Unicam SP600 spectrophotometer. Calculate the magnesium content of the sample from a calibration graph covering the range 5 to 25 μg of magnesium; prepare the graph by the procedure described above, and include the compensating solution.

EFFECTS OF OTHER IONS

PHOSPHATE—

It has been found that phosphate has no effect on the reaction between magnesium hydroxide and Titan yellow, provided that the concentration of magnesium in the final solution is below 1·25 p.p.m. A phosphorus-to-magnesium ratio of 10 to 1 can be tolerated without interference.

CALCIUM—

EGTA forms a much stronger complex with calcium than with magnesium and has been used for titrating calcium in the presence of magnesium.[3] In this work, it is effectively used to form a chelate with calcium in alkaline solution without preventing the formation of magnesium hydroxide. Calcium, under these conditions, has no effect on the magnesium hydroxide - Titan yellow adsorption compound; further, calcium phosphate is not precipitated from solutions high in phosphate.

IRON—

Triethanolamine overcomes interference from iron up to an iron-to-magnesium ratio of 2 to 1.

ALUMINIUM—

In the presence of triethanolamine, the effect of aluminium is constant and is overcome by including 50 μg of aluminium in the compensating solution. Aluminium-to-magnesium ratios of 2 to 1 can be tolerated.

MANGANESE—

Interference from manganese is the least easily controlled. In the presence of the com pensating solution, manganese-to-magnesium ratios of 0·5 to 1 can be tolerated.

ZINC—

Triethanolamine overcomes interference from zinc up to a zinc-to-magnesium ratio of 4 to 1.

COPPER—

In the presence of glycerol, copper does not interfere up to a copper-to-magnesium ratio of 2 to 1.

Results

In Table I, results by the proposed method are compared with those obtained by a volumetric method involving use of 8-hydroxyquinoline.[2]

Table I

Comparison of results for magnesium by proposed and volumetric methods

Sample	Magnesium content of dry matter found by— volumetric method, %	proposed method, %
Oat grain	0·120, 0·115, 0·118 (mean 0·118)	0·120, 0·117, 0·119 (mean 0·119)
Pasture herbage ..	0·212, 0·192 (mean 0·202)	0·216, 0·210, 0·214 (mean 0·213)
Kale	0·120, 0·118 (mean 0·119)	0·121, 0·122, 0·119 (mean 0·121)
Cabbage	0·123, 0·126, 0·121 (mean 0·123)	0·127, 0·123, 0·124 (mean 0·125)
Apple leaf	0·148, 0·148, 0·148 (mean 0·148)	0·145, 0·149, 0·146 (mean 0·147)
Strawberry leaf ..	0·250, 0·245, 0·254 (mean 0·250)	0·245, 0·245, 0·251 (mean 0·247)
Blackcurrant leaf ..	0·275, 0·277, 0·281 (mean 0·278)	0·267, 0·272, 0·284 (mean 0·274)

References

1. Bradfield, E. G., *Analyst*, 1960, **85**, 666.
2. Metson, A. J., *N.Z. J. Sci. Tech.*, 1940, **22**, 125.
3. Schmid, R. W., and Reilley, C. N., *Anal. Chem.*, 1957, **29**, 264.

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THE DETERMINATION OF ZINC IN METALLURGICAL MATERIALS BY ATOMIC-ABSORPTION SPECTROPHOTOMETRY

The development of a method for determining zinc in metallurgical materials by atomic-absorption spectrophotometry was described previously.[1] It was reported that solutions of hydrochloric acid caused considerable absorption at the wavelength of the zinc resonance line (2138 A). Absorption produced by hydrochloric acid was also observed at discrete wavelengths between 2000 and 2200 A, the source of light being unidentified hollow-cathode emission lines.

After the paper had been published, further work was carried out in our laboratories in an attempt to establish the cause of this interference, and two further facts were ascertained.

(*i*) When this acid was neutralised with sodium hydroxide the interference was overcome, but neutralisation with ammonia caused a slight increase in interference.

(*ii*) The hollow-cathode emission lines used for absorption measurements in the region 2000 to 2200 A were identified as ground-state copper lines, 2024, 2165, 2182 A, etc. (we were using a brass hollow-cathode lamp).

Our interpretation of these observations now is that the surface-oxide layers of the brass burner were attacked by hydrochloric acid or ammonium chloride, and reaction products were carried into the flame. This would cause absorption due to zinc and copper to be observed, an explanation fitting the recorded facts and in keeping with the experience of other workers (personal communication from Mr. D. J. David, Division of Plant Industry, C.S.I.R.O., Canberra, Australia), who have detected no interference when stainless-steel or aluminium burners were used.

Reference

1. Gidley, J. A. F., and Jones, J. T., *Analyst*, 1960, **85**, 249.

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Received *November 22nd*, 1960

THE PRECISION AND LIMIT OF DETECTION OF ANALYTICAL METHODS

THE method of defining the limit of detection, which was suggested by the author in his Note,[1] has been suggested previously in two papers[2,3] dealing with spectrographic analysis. The author would like to make an acknowledgment to the authors of these papers, which were brought to his notice after publication of the Note.

REFERENCES

1. Wilson, A. L., *Analyst*, 1961, **86**, 72.
2. Kaiser, H., *Spectrochim. Acta*, 1947, **3**, 40.
3. Garton, F. W. J., Ramsden, W., Taylor, R., and Webb, R. J., *Ibid.*, 1956, **8**, 94.

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Book Reviews

DEVELOPMENT OF BREWING ANALYSIS: A HISTORICAL REVIEW. By J. R. HUDSON, B.Sc., Ph.D., F.R.I.C. Pp. vi + 102. London: The Institute of Brewing. 1960. Price 42s.

The appearance of this monograph is timely, for, although analytical chemistry was first tentatively used in the malting and brewing industries a century ago, there has been a great extension in the choice and application of methods during the last fifteen years. This means that the chemist wishing to carry out an investigation in this field of work is faced with a large number of proposed methods for his necessary analyses and will probably need to spend considerable time in examining a number of them for their accuracy and suitability. Several brief surveys have appeared in recent years, notably in the pages of *The Analyst*, with the aim of guiding the chemist in his choice of method and procedure, but they have generally been confined to a specific form of analysis, and none, so far as I am aware, has attempted to review the spread of analysis throughout an entire industry as Dr. Hudson has done here.

The subject is handled under four main headings: Examination of Barley and Malt, Analysis of Hops, Water and Wort and Beer. References to the literature, of which there are nearly a thousand, are grouped at the end of each of the four sections, and the methods of analysis are considered under sub-headings, which are sufficiently detailed in the list of contents as to make an index unnecessary. An idea of the scope of the text may be gained by quoting at random from this list: determination of nitrogen, germinative properties and potential extractives of barley; methods of assessing "modification" in malt; analysis of hops for the α-acid and β-fraction of the soft resins, for the tannins and for the essential oils; carbohydrates, nitrogenous constituents and hop bitter substances in wort and beer; and the organisation of tasting trials. Dr. Hudson does not hesitate to criticise a method or mode of approach to a problem and to make an appraisal based on his own experience. It is interesting, for example, in view of recent recommendations of the molybdenum-blue method of determining arsenic, to have his opinion that measurement of the colour produced by interaction with silver diethyldithiocarbamate in pyridine is preferable.

Few errors or omissions have been noticed. One might suggest at the outset that an explanation of what is meant by "brewer's pounds" and their relationship to other units of measurement would have been a great help to the young chemist entering the industry or to the analyst looking in from without. Harris and Ricketts' method for determining anthocyanogens in beer, quoted on page 92, requires hydrochloric and not acetic acid for dissolving the nylon and producing the pigments, and in the description on page 9 of Decker's method of following moisture removal during malt kilning the expression $(g' - g'')/g'$ should be $100(g' - g'')/g'$. Another arithmetical error, which is not so obvious, occurs in the formula for calculating the original extract of wort (page 64), which should read—

$$O = \frac{(A \times 2{\cdot}0665) + E}{100 + (A \times 1{\cdot}0665)} \times 100.$$

The reference given to Lundin and Schröderheim's work on nitrogen fractionation (No. 256, p. 72) is incomplete, since it deals only with the "A" fraction of wort nitrogen and not, as stated, with the "B" and "C" fractions also.

These are minor details. The skill and care with which the author has carried through his exacting task and the time he must have given to it become increasingly obvious as one turns the pages. The result is a reference book that will be of outstanding value to anyone concerned in any way with the chemistry of malting and brewing. It reminds the analyst of what has been attempted and with what degree of success or failure the attempts have been attended. It will not only give him a great deal of help, it will stimulate him to further experiments. Such a compilation deserves, and will certainly require, something more durable than the paper covers with which it is furnished.

A. A. D. COMRIE

CHEMICAL INSTRUMENTATION: A SYSTEMATIC APPROACH TO INSTRUMENTAL ANALYSIS. By HOWARD A. STROBEL. Pp. xviii + 653. Reading, Mass., U.S.A., and London: Addison-Wesley Publishing Co. Inc. 1960. Price $9.75; 74s.

Many books about instrumental methods of analysis consist of a series of monographs on individual techniques, and great emphasis is often placed on the use of particular commercial equipment. Dr. Strobel has avoided this approach and treats the subject of chemical instrumentation as a study in its own right. The book is based on a course given to advanced undergraduates at Duke University.

After two introductory chapters the book is divided into two sections. The first deals with optical methods and begins with chapters on the interaction of electromagnetic radiation and matter, and optics. This is followed by sections on emission spectroscopy, absorption photometry, light-scattering photometry, refractometry and polarimetry. The second section is entitled "Electrometric Methods" and, after chapters on electrical phenomena and electronics, deals with conductimetric methods, potentiometry, polarography, coulometry and radioisotope methods. At the end of each chapter a list of well chosen exercises and a number of references to books for further reading are given. The book has an appendix of twenty-four laboratory experiments.

The different sections of the book are treated uniformly well, and the chapters on simple electronics, comprising 90 pages, are especially welcomed. All through, the emphasis is placed on fundamental theory and design rather than on the use of specific items of equipment. It is inevitable that components and those instruments that are quoted should be exclusively of North American origin, but this does not reduce significantly the usefulness of the book in other countries, and it is one that can be recommended to students, to those just starting on a career in analytical chemistry and to all those who have only limited experience of instrumental techniques and wish to gain a more fundamental understanding of the equipment than one will find in the instrument-makers' instruction books.

L. BREALEY

THE USE OF ORGANIC REAGENTS IN INORGANIC ANALYSIS. By A. I. BUSEV and N. G. POLIANSKII. Translated from the Russian by J. T. GREAVES, M.A. Pp. viii + 76. Oxford, London, New York and Paris: Pergamon Press Ltd. 1960. Price 21s.

According to the publisher's cover-note, this slim volume of 60 pages of text reproduced by photo-lithography "surveys and summarises the present state of knowledge in this currently active field." This is incorrect and entirely misleading. Apart from the first six references, which form a most unrepresentative introduction to the literature of the subject, the remaining 464 references deal entirely with papers published during 1953, 1954 and 1955. This is stated explicitly by the author on page 59! In short, this book forms a sort of uncritical "Triennial Report on the Progress of Analytical Chemistry with the Aid of Organic Reagents." It makes no reference to any work carried out in the last five years, and the only attempt at organisation is the grouping of papers according to the elements as they occur in the eight groups of Mendeleeff's Periodic Table.

This book certainly reflects the great activity of Russian workers in the field of organic reagents, and its greatest value must be to call attention to papers published in the U.S.S.R., often in proceedings of University societies or in other journals not normally accessible to the Western world and often not even abstracted.

Difficulties in transliteration add to the problems of the reader. Yatsimirskii and Iatsimirskii, Bobtelsky and Bobtel'skii, Erdey and Erdei, appear interchangeably. Other mis-spellings include Buttler, Sendel, and Prshibil. The translator has doubtless done his best with the stilted style

of the original, but he has not always been happy in his choice of nomenclature (*e.g.*, p. 12, the ethylenediamine sulphonate of ferrous iron; p. 23 *et seq.*, tetraphenyl boro-salts; p. 24, solution of dipricylaminate) and it is a pity that no attempt has been made to clarify the clumsy formulae or the original, *e.g.*, $(C_2H_5)_2NCSS$; CSN_2H_4: $ZrO(COO)_2C_6Cl_4$: $OC(C_6H_5)=C(C_6H_5)CH=O$ . . . $Fe_{1/3}$, or to assist the reader in interpreting the composition of N-(N-bromo-C-tetradecylbetainyl)-C-tetradecylbetaine (p. 28).

It is difficult to see why this particular book was selected for translation and publication. In a foreword, the publisher gives reasons for its high cost.

H. IRVING

VIII. COLLOQUIUM SPECTROSCOPICUM INTERNATIONALE. Edited by Dr. H. GUYER. Pp. 319. Aarau, Switzerland: Verlag H. R. Sauerlander & Co. 1960. Price: 68 NF; DM 68.

This publication contains the complete proceedings of the eighth international spectroscopy colloquium held in Lucerne in September, 1959. The contents include the five principal lectures, over sixty papers and the brief addresses of welcome and valediction. Contributions in English, French and German appear in about equal proportions, but every paper includes short summaries in all three tongues.

The principal interest of those attending the C.S.I. series of conferences is optical-emission spectroscopy (49 papers), although mass spectrometry (6 papers) and X-ray fluorescence spectroscopy (8 papers) are also included. The papers are, in general, of a good standard; both the research worker and the practising analyst will find several contributions of significant value. Theory, new methods and trends in instrumentation all receive their share of attention.

The book is well printed, with excellent illustrations, some of which are in colour; an adequate list of contents is also included. Paper covers are a marked departure from an otherwise excellent standard of production.

It is no doubt fitting that each host country should, in turn, decide on the method to be adopted in publishing the proceedings of this series of colloquia. Such independence of action can, however, bring confusion in its train, particularly to literature searchers and librarians. How many of us are aware that the proceedings of the seventh meeting were published in a special issue of the *Revue Universelle des Mines* (Liège) or that the sixth formed Volume Eleven of *Spectrochimica Acta*? A list giving publication details of all earlier proceedings in this series could well be given prominence in each succeeding volume. This course is commended to those who will be responsible for the ninth colloquium.

B. S. COOPER

RADIOACTIVE ISOTOPES IN BIOCHEMISTRY. By ENGELBERT BRODA. Pp. x + 376. Amsterdam, London, New York and Princeton: Elsevier Publishing Co.; London: D. Van Nostrand Co. Ltd. 1960. Price 57s.

This volume is a direct translation into English by Dr. P. Oesper (Hahnemann Medical College, Philadelphia) of *Radioaktive Isotope in der Biochemie*, which has already been reviewed in *The Analyst* (1959, **84**, 71). The text, except for the incorporation of a few references to work published in 1958, is the same as that of the original German volume.

The book is concisely written and provides an excellent, well documented introduction to the principles of and experimental techniques for biochemical procedures involving radioactive isotopes. The way in which isotopes are used to study the absorption and excretion of elements and drugs and to investigate the mechanism of biological procedures, such as photosynthesis and the intermediary metabolism of carbohydrates, fats, proteins and nucleic acids, is described.

The presentation and printing of the book are good; there are few printing errors.

J. E. PAGE

TITRATION IN NON-AQUEOUS SOLVENTS. By A. H. BECKETT and E. TINLEY. Third Edition. Pp. iv + 56. Poole, Dorset: The British Drug Houses Ltd. 1960. Gratis.

This is a handy booklet on non-aqueous titrimetry. Some preliminary observations are made on the theoretical considerations underlying the procedures and the scope and limitations of the various tests.

The titration of basic substances is dealt with at some length, particular attention being paid to apparatus, solvents, titrants and colour indicators, as well as to potentiometric titrations. Applications of these procedures are described. The titration of acidic substances is described on similar lines, and some attention is paid to high-frequency titrimetry.

There is a very useful list of references to original papers and review articles, and the book concludes with lists of those substances in the British Pharmacopoeia, 1958, the British Pharmaceutical Codex, 1959, the United States Pharmacopoeia, Fifteenth Revision, and the International Pharmacopoeia that are assayed by non-aqueous titration.

All this is accomplished in 55 pages of readable text in a useful little handbook that analytical chemists will welcome. J. HASLAM

Publications Received

THE IDENTIFICATION OF ORGANIC COMPOUNDS: A MANUAL OF QUALITATIVE AND QUANTITATIVE METHODS. By STIG VEIBEL, Dr.Phil. Fifth Edition. (Second English Edition.) Pp. xvi + 426. Copenhagen: G. E. C. Gad Publisher. 1961.

THE RADIOCHEMISTRY OF ZINC. By HARRY G. HICKS. Pp. vi + 58. Washington, D.C.: National Academy of Sciences—National Research Council. 1960. Price 75 cents.
Nuclear Science Series: NAS—NS-3015.

THE RADIOCHEMISTRY OF PROTACTINIUM. By H. W. KIRBY. Pp. vi + 80. Washington, D.C.: National Academy of Sciences—National Research Council. 1959. Price $1.00.
Nuclear Science Series: NAS—NS-3016.

THE RADIOCHEMISTRY OF IRON. By J. M. NIELSEN. Pp. vi + 42. Washington, D.C.: National Academy of Sciences—National Research Council. 1960. Price 50 cents.
Nuclear Science Series: NAS—NS-3017.

TREATISE ON ANALYTICAL CHEMISTRY. Edited by I. M. KOLTHOFF and PHILIP J. ELVING, with the assistance of ERNEST B. SANDELL. Part I. Theory and Practice, Vol. 2. Pp. xx + 811–1308. New York and London: Interscience Publishers Inc. 1961. Price (single volume) $16.00; 120s.: (subscribers to whole series) $14.00; 105s.

TOXICOLOGY AND BIOCHEMISTRY OF AROMATIC HYDROCARBONS. By HORACE W. GERARDE, M.D., Ph.D. Pp. xiv + 329. Amsterdam, London, New York and Princeton: Elsevier Publishing Company; London: D. Van Nostrand Company Ltd. 1960. Price 30s.

SCIENTIFIC RUSSIAN: A TEXTBOOK FOR CLASSES AND SELF-STUDY. By JAMES W. PERRY. Second Edition. Pp. xxviii + 565. New York and London: Interscience Publishers Inc. 1961. Price $9.50; 72s.

QUINONES IN ELECTRON TRANSPORT: A CIBA FOUNDATION SYMPOSIUM. Edited by G. E. W. WOLSTENHOLME, O.B.E., M.A., M.B., M.R.C.P., and CECILIA M. O'CONNOR, B.Sc. Pp. xii + 453. London: J. & A. Churchill Ltd. Price 60s.

CONTRIBUTI TEORICI E SPERIMENTALI DI POLAROGRAFIA. Volume V. Supplemento a "La Ricerca Scientifica." Pp. 315. Padova, Italy: Centro di Polarografia. 1960. Price 2500 Lire.

ELEMENTARY CHEMICAL THERMODYNAMICS. By G. HARGREAVES, M.Sc., A.R.I.C. Pp. viii + 120. London: Butterworths Publications Ltd. 1961. Price 10s. 6d.

REPRINTS OF "BIOLOGICAL STANDARDISATION AND THE ANALYST" BY J. W. LIGHTBOWN

REPRINTS of the Review Paper, "Biological Standardisation and the Analyst," by J. W. Lightbown, published in this issue of *The Analyst* (pp. 216–230), will be available shortly from the Assistant Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 5s. per copy, post free. A remittance for the correct amount, made out to The Society for Analytical Chemistry, MUST accompany the order; these reprints are not obtainable through Trade Agents.

International Atomic Weights, 1959

(*Reprinted from the Report of the Commission on Atomic Weights in the* Comptes rendus *of the 20th Conference of the International Union of Pure and Applied Chemistry*, 1959.)

Name	Symbol	Atomic No.	Atomic weight
Actinium	Ac	89	—
Aluminium	Al	13	26·98
Americium	Am	95	—
Antimony	Sb	51	121·76
Argon	Ar	18	39·944
Arsenic	As	33	74·91
Astatine	At	85	—
Barium	Ba	56	137·36
Berkelium	Bk	97	—
Beryllium	Be	4	9·013
Bismuth	Bi	83	209·00
Boron	B	5	10·82
Bromine	Br	35	79·916
Cadmium	Cd	48	112·41
Cæsium	Cs	55	132·91
Calcium	Ca	20	40·08
Californium	Cf	98	—
Carbon	C	6	12·011
Cerium	Ce	58	140·13
Chlorine	Cl	17	35·457
Chromium	Cr	24	52·01
Cobalt	Co	27	58·94
Copper	Cu	29	63·54
Curium	Cm	96	—
Dysprosium	Dy	66	162·51
Einsteinium	Es	99	—
Erbium	Er	68	167·27
Europium	Eu	63	152·0
Fermium	Fm	100	—
Fluorine	F	9	19·00
Francium	Fr	87	—
Gadolinium	Gd	64	157·26
Gallium	Ga	31	69·72
Germanium	Ge	32	72·60
Gold	Au	79	197·0
Hafnium	Hf	72	178·50
Helium	He	2	4·003
Holmium	Ho	67	164·94
Hydrogen	H	1	1·0080
Indium	In	49	114·82
Iodine	I	53	126·91
Iridium	Ir	77	192·2
Iron	Fe	26	55·85
Krypton	Kr	36	83·80
Lanthanum	La	57	138·92
Lead	Pb	82	207·21
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Mendelevium	Md	101	—
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Nickel	Ni	28	58·71
Niobium	Nb	41	92·91
Nitrogen	N	7	14·008
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Osmium	Os	76	190·2
Oxygen	O	8	**16**
Palladium	Pd	46	106·4
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Potassium	K	19	39·100
Praseodymium	Pr	59	140·92
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* Because of natural variations in the relative abundance of the isotopes of sulphur the atomic weight of this element has a range of ± 0·003.

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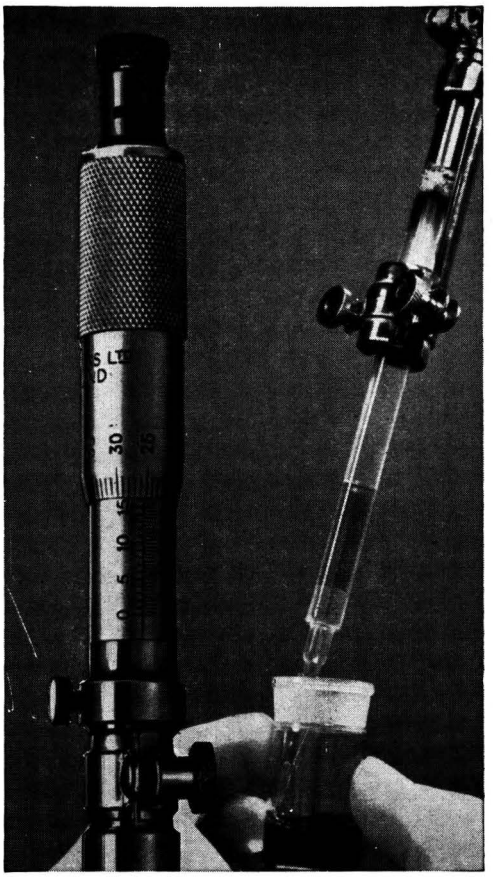

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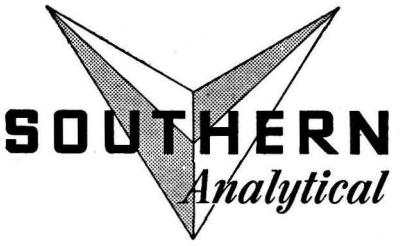

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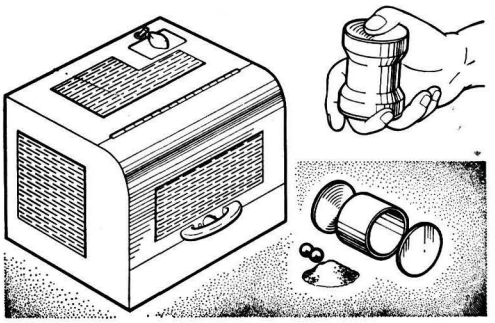

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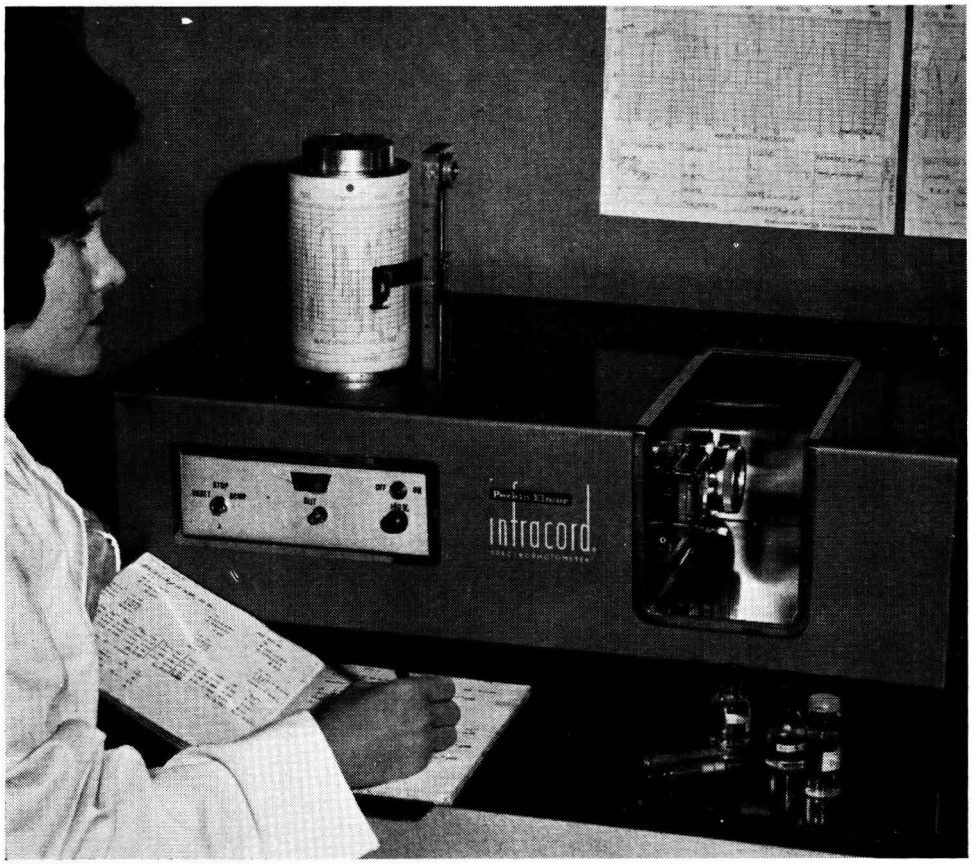

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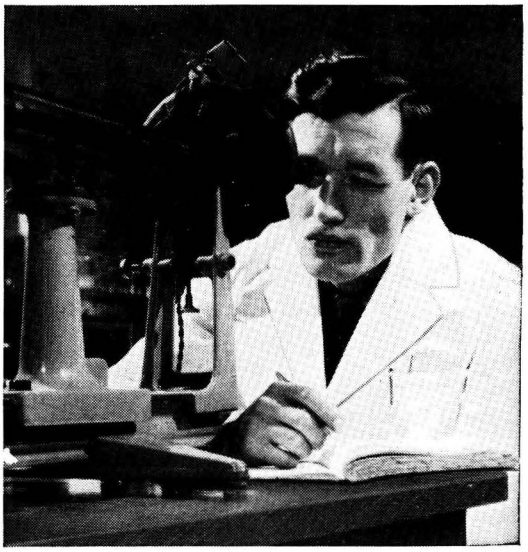

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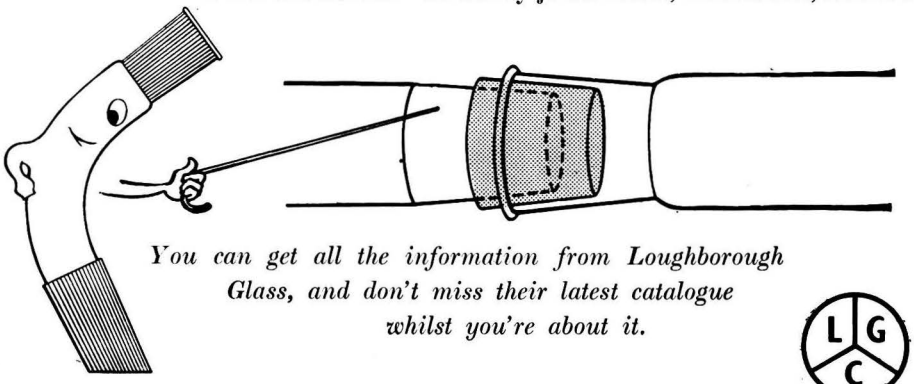

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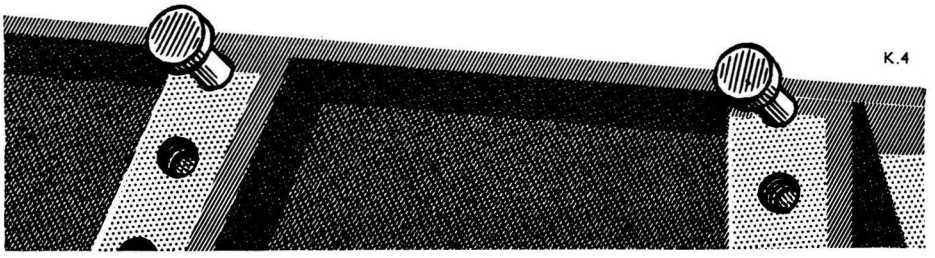

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