THE ANALYST

A Monthly Publication dealing with all branches of Analytical Chemistry: the Journal of the Society of Public Analysts and Other Analytical Chemists

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No. 5 April, 1952

THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

BULLETIN

FORTHCOMING MEETINGS

Ordinary Meeting of the Society, May 7th, 1952

AN Ordinary Meeting of the Society will be held at 7 p.m. on Wednesday, May 7th, 1952, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

The following papers will be presented and discussed—

"A Routine Method for the Analysis of Table Jellies," by Miss E. M. Chatt, B.Sc., F.R.I.C.

"The Determination of Oxalates in Fresh Plant Material," by C. J. L. Baker, A.R.I.C.

"The Determination of Small Quantities of Ammonium Di- or Tri-ethanolamine Alginate in Rayon-Finishing Solutions and on Rayon Yarn," by E. G. Brown, A.M.C.T., F.R.I.C., and T. J. Hayes.

Meeting of the North of England Section, April 26th, 1952

AN Ordinary Meeting of the North of England Section will be held at 2 p.m. on Saturday, April 26th, 1952, at the Engineers' Club, Albert Square, Manchester.

The following paper will be presented and discussed—

"Some New Methods in Analytical Chemistry," by R. Belcher, B.Sc., F.R.I.C., F.Inst.F.

Meeting of the Scottish Section, May 2nd, 1952

AN Ordinary Meeting of the Scottish Section will be held at 7 p.m. on Friday, May 2nd, 1952, in the North British Hotel, Princes Street, Edinburgh.

At this meeting a lecture entitled "Chemistry and the Law" will be given by J. K. McLellan, M.A., B.Sc., A.R.I.C.

Joint Meeting of the Microchemistry Group with the Bristol and District Sections of the Chemical Society, the Royal Institute of Chemistry and the Society of Chemical Industry, April 23rd, 1952

A JOINT Meeting of the above bodies will be held at 7 p.m. on Wednesday, April 23rd, 1952, at Bristol University Chemical Department, Woodland Road, Bristol, 8.

The following paper will be presented and discussed—

"The Use of Cylinder Oxygen in the Organic Micro-Determination of Nitrogen," by H. Swift and E. S. Morton.

This will be followed by a discussion on "Standard Substances for Organic Micro Analysis."

Afternoon visits to the University of Bristol Agricultural and Horticultural Research Station, Long Ashton, and to Messrs. J. S. Fry & Sons, Ltd., Somerdale, have also been arranged.

Joint Meeting of the Physical Methods Group with the South Wales Section of the Royal Institute of Chemistry, May 16th, 1952

THE Thirty-seventh Ordinary Meeting of the Physical Methods Group will be held at University College, Swansea, on Friday, May 16th, 1952, at 6 p.m. The subject of this joint meeting will be "Ion Exchange Resins," and the following papers will be presented—

"The Theory of Ion Exchange," by Professor C. W. Davies, D.Sc., F.R.I.C.

"Some Newer Applications and Techniques of Cation and Anion Exchange Resins in Chemical Analysis," by G. H. Osborn, F.R.I.C., A.M.Inst.M.M.

"The Determination of Individual Rare Earths by Radioactivation Using Ion Exchange Separation," by F. W. Cornish, Ph.D., A.R.I.C.

The meeting will be preceded at 2.30 p.m. by a visit to the B.I.S.R.A. Laboratories, Sketty Hall, Swansea.

Those wishing to participate in the visit should inform Mr. R. H. Jones, F.R.I.C., The Mond Nickel Co., Ltd., Clydach, Swansea, stating whether hotel accommodation is required for Friday night.

Meeting of the Biological Methods Group, June 12th and 13th, 1952

THE Summer Meeting of the Biological Methods Group will be held on Thursday and Friday, June 12th and 13th, 1952, at Boots' Pure Drug Co., Ltd., Nottingham. In addition to a session on Friday afternoon for the reading of scientific papers, visits are being arranged to Boots' main factory at Beeston, to the Pharmacology and Physiology Division at West Bridgford and to the Veterinary Research Station at Thurgarton, where demonstrations of biological assay methods will be given.

Members of the Group will receive further details shortly; other members of the Society who would like to participate are invited to communicate with the Honorary Secretary of the Group, Mr. S. A. Price, B.Sc., Walton Oaks Experimental Station, Vitamins Ltd., Dorking Road, Tadworth, Surrey, without delay.

PAPERS ACCEPTED FOR PUBLICATION IN *THE ANALYST*

THE following papers have been accepted for publication in *The Analyst*, and are expected to appear in the near future. It is not possible to enter into any correspondence about any of them.

"The Measurement of Diffusion Current with Reference to a Pen-Recording Polarograph," by W. Furness.

> The Tinsley polarograph (model V3211) can be used with capacitance damping to record the average diffusion current during the life of successive mercury drops; alternatively it can be used with no damping to trace approximately the instantaneous values of diffusion current at maximum drop size. With capacitance damping, precision depends upon the time-constant of the pen-recorder circuit, and accuracy is limited by the quality of the electrolytic condensers. For a given drop-rate with no damping, the peak values traced by the pen-recorder depend mainly upon the viscosity of the dash-pot oil. Some limitations and advantages of the two methods in practical polarographic analysis are briefly discussed, and an oil of suitable viscosity is suggested.
>
> The relationship of the peak values, as recorded under various conditions, to the corresponding values for the average diffusion current has been investigated with the aid of a silver voltameter. For several electro-reducible substances in specified supporting electrolytes, the ratio of the average diffusion current to the instantaneous current at maximum drop size differs significantly from the fraction 6/7 derived theoretically by Ilkovič; moreover, the observed values of the ratio vary from one electro-reducible substance to another, diminishing with increasing values of the diffusion coefficient. The extent of the variation is greater than would have been predicted from the recent equation of Lingane and Loveridge, but the change is in the expected direction.

"The Determination of Total Phosphorus in Soil Material, With Particular Reference to the Control of Interference by Soluble Silica," by J. W. Muir.

A method suitable for the routine determination of total phosphorus in soil material is described. The phosphorus is released from the soil material by fusion with sodium carbonate and extracted from the melt with water. An aliquot of the solution is taken and the phosphorus determined by the formation of a molybdenum blue colour with sodium molybdate in acid solution and aqueous hydrazine sulphate. The colour is stable for at least 24 hours. Interference from silicates rendered soluble by the fusion is controlled effectively by the use of tartaric acid. The colorimetric method can determine between 0·06 mg and 0·30 mg of phosphorus pentoxide. By taking a suitable aliquot of the extract, the total phosphorus content of any soil containing more than 0·06 per cent. of phosphorus pentoxide can be determined.

"The Determination of Oxalates in Fresh Plant Material," by C. J. L. Baker.

A method is described for the determination of total oxalates in plants by extraction with hydrochloric acid, precipitation as calcium oxalate from the deproteinised extract and subsequent titration with potassium permanganate. Soluble oxalates are determined in a similar manner, from an aqueous extract. The later stages of the determination are carried out on a semi-micro scale, so a number of determinations can be made simultaneously. The method is designed for fresh green plants as oxalate is lost on drying the material.

"The Determination of Small Amounts of Bismuth in Lead," by C. J. Hall.

Existing methods for determining small amounts of bismuth in lead are criticised. A method is proposed that is based upon the separation of bismuth from lead by co-precipitating the bismuth with manganese dioxide in dilute nitric acid solution, the pH of which solution is adjusted to between 1·0 and 1·7. After dissolving the precipitate and evaporating the resulting solution to dryness, the bismuth in the residue is determined colorimetrically as the coloured complex with thiourea.

Results are good with bismuth concentrations of 0·001 to 0·012 per cent., the mean error being not greater than 3 per cent. if a photo-electric absorptiometer is used.

Interference from other impurities that may be present in lead is shown to be negligible.

"The Determination of Cholesterol in Serum," by P. Trinder.

A method for the determination of cholesterol in serum has been devised. The serum is heated with alcoholic potassium hydroxide to hydrolyse the cholesterol esters, and the free cholesterol is extracted with light petroleum. The residue left after evaporation of the light petroleum is dissolved in ethylene dichloride and a mixture of acetyl chloride and sulphuric acid is added. After incubation at 37° C for 15 minutes and subsequent cooling to below 20° C, the optical density is read on a photo-electric colorimeter. The optical density of the red coloration does not alter for at least 1 hour after cooling to 20° C. A single determination can be completed within 30 minutes of the receipt of the sample.

"The Colorimetric Determination of Alcohols," by V. W. Reid and R. K. Truelove.

The use of ceric ammonium nitrate as a colorimetric reagent for the precise determination of various alcohols in dilute aqueous solution is described. In particular, the method of determining alcohols when present in certain glycol esters is given. Some interferences have been investigated and methods of overcoming these are detailed.

"The Grammar of Units of Biological Activity," by N. T. Gridgeman.

NOTICE

Midlands Society for Analytical Chemistry

Symposium on Analytical Chemistry

UNDER the auspices of the Midland Society for Analytical Chemistry, a short Symposium on Analytical Chemistry, dealing with fundamental theory and original methods, will be held at the University of Birmingham on September 10th, 11th and 12th, 1952, *i.e.*, immediately following the International Congress on Analytical Chemistry at Oxford.

Arrangements are in the hands of the Honorary Secretary of the Midlands Society for Analytical Chemistry, W. T. Edwards, F.R.I.C., A.I.M., 100, Rymond Road, Ward End, Birmingham, 8.

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, February 6th, 1952, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. J. R. Nicholls, C.B.E., F.R.I.C.

At the beginning of the meeting the officers and members stood in silence as a mark of respect to the memory of the late King George VI.

This meeting was organised by the Microchemistry Group of the Society, and the following papers were presented and discussed: "Some Applications of the Conway Micro-Diffusion Technique," by N. Heron, F.R.I.C.; "The Microchemical Determination of Iron in Aluminium Alloys," by W. R. Nall; "The Microchemical Determination of Vanadium in Steels," by W. R. Nall; "The Separation of Carbides from Steel and their Analytical Examination," by R. Pemberton.

NEW MEMBERS

Geoffrey Cyril Ashton, B.Sc. (Liv.); Alfred Arnold Boucher; Thomas William Brandon, B.Sc. (Lond.), F.R.I.C.; Robert Alexander Chalmers, B.Sc. (Manc.); John William Dent, B.Sc. (Lond.), F.R.I.C.; Eric Dyke, A.I.M.; William Kenneth Ellison, B.Sc. (Durham); Louis Fletcher, F.R.I.C.; Lawrence Richard Flynn, A.M.C.T., A.R.I.C.; Albert Horsley, B.Sc. (Lond.), F.R.I.C.; Jack Lowen, B.Sc. (N.Y.); Geoffrey William Smith; Arthur Wooldridge, B.Sc. (Lond.); Peter John Woosnam, B.Sc. (Lond.), A.R.C.S.

DEATH

WE regret to record the death of

Bertram William John Warren

NORTH OF ENGLAND SECTION

THE Twenty-seventh Annual General Meeting of the Section was held at the Engineers' Club, Albert Square, Manchester, on Saturday, January 26th, 1952, at 2 p.m. The Chair was taken by Mr. A. A. D. Comrie.

The following were elected as Officers and Members of the Committee for the forthcoming year:—*Chairman*—Mr. A. A. D. Comrie. *Vice-Chairman*—Mr. T. W. Lovett. *Hon. Secretary and Treasurer*—Mr. Arnold Lees, 87, Marshside Road, Southport, Lancs. *Elected Committee Members*—Messrs. A. Alcock, W. Gordon Carey, F. Dixon, J. C. Harral, N. Heron and C. R. Louden. *Hon. Auditors*—Messrs. C. J. House and J. R. Walmsley.

The Annual General Meeting was followed by an Ordinary Meeting of the Section at which the following paper was presented and discussed: "The Use of Laboratory Animals as Analytical Reagents," by A. L. Bacharach.

SCOTTISH SECTION

THE Seventeenth Annual General Meeting of the Section was held in Glasgow on Wednesday, January 30th, 1952, at 12.30 p.m., and the following office bearers were elected for the forthcoming year:—*Chairman*—Mr. H. C. Moir. *Vice-Chairman*—Mr. R. S. Watson. *Hon. Secretary and Treasurer*—Mr. J. A. Eggleston, Boot's Pure Drug Co., Ltd., Motherwell Street, Airdrie, Lanarkshire. *Elected Committee Members*—Messrs. A. R. Campbell, F. J. Elliott, M. Pyke, S. C. Sloan, J. Sword and A. C. Wilson. *Hon. Auditors*—Messrs. J. Andrews and J. Gray.

MICROCHEMISTRY GROUP

THE Eighth Annual General Meeting of the Group was held in the Meeting Room of the Chemical Society, Burlington House, London, W.1, on Wednesday, February 6th, 1952, at 6.45 p.m. Dr. Cecil L. Wilson was in the Chair.

At the start of the meeting members present stood in silence as a mark of respect to the memory of the late King George VI.

The following Officers and Committee Members were elected for the ensuing year:—*Chairman*—Dr. Cecil L. Wilson. *Vice-Chairman*—Dr. A. M. Ward. *Hon. Secretary*—Mr. Donald F. Phillips, 101, South Promenade, St. Annes-on-Sea, Lytham St. Annes, Lancs. *Hon. Treasurer*—Mr. Gerald Ingram. *Elected Committee Members*—Messrs. W. N. Aldridge, W. T. Chambers, J. G. A. Griffiths, G. F. Hodsman, G. W. C. Milner and G. H. Osborn. *Hon. Auditors*—Messrs. L. H. N. Cooper and H. Childs.

PHYSICAL METHODS GROUP

THE Seventh Annual General Meeting of the Group was held at 6 p.m. on Tuesday, November 27th, 1951, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. Mr. B. S. Cooper, B.Sc., F.Inst.P., the Chairman of the Group, presided. The Group Officers and Elected Members of the Committee for the forthcoming year are as follows:—*Chairman*—Dr. J. Haslam. *Vice-Chairman*—Mr. A. A. Smales. *Hon. Secretary and Treasurer*—Mr. R. A. C. Isbell, Hilger & Watts Ltd., Hilger Division, 98, St. Pancras Way, London, N.W.1. *Members of Committee*—Messrs. L. Brealey, C. H. R. Gentry, J. H. Glover, R. H. Jones, G. H. Osborn and F. R. Williams. *Hon. Auditors*—Messrs. C. A. Bassett and D. C. Garratt (re-appointed).

The Annual General Meeting was followed by the Thirty-fourth Ordinary Meeting of the Group, at which Mr. B. S. Cooper delivered an address on "Light Sources in Chemical Analysis and Testing"; this was followed by a discussion.

POLAROGRAPHIC DISCUSSION PANEL

THE Annual General Meeting of the Polarographic Discussion Panel was held at 6 p.m. on Friday, November 16th, 1951, in the rooms of the Iron and Steel Institute, 4, Grosvenor Gardens, London, S.W.1. The following Officers were elected for the forthcoming year:—*Chairman*—Dr. A. J. Lindsey. *Hon. Secretary*—Mr. G. W. C. Milner, Building 148, A.E.R.E., Harwell, Nr. Didcot, Berks.

After the business meeting the following papers were presented and discussed: "The Vibrating Electrode in Polarographic Determination of Alkyl Peroxides," by E. R. Roberts and J. S. Meek; "A Recording Polarograph for Continuous Flow Operation," by K. C. Overton and J. A. Lewis; "An Improved Randles-Type Cathode-Ray Polarograph," by G. F. Reynolds and H. M. Davis.

BIOLOGICAL METHODS GROUP

THE Seventh Annual General Meeting was held at 6.15 p.m. on Thursday, December 13th, 1951, in the Anatomy Lecture Theatre, University College, Gower Street, London, W.C.1. Mr. N. T. Gridgeman was in the Chair.

The following were elected as Officers and Members of the Committee for the forthcoming year:—*Chairman*—Dr. H. O. J. Collier. *Vice-Chairman*—L. J. Harris. *Hon. Secretary*—Mr. S. A. Price, Walton Oaks Experimental Station, Vitamins, Ltd., Dorking Road, Tadworth, Surrey. *Elected Committee Members*—Messrs. A. L. Bacharach, C. H. Gray, N. T. Gridgeman, J. W. Lightbown, G. F. Somers and K. L. Smith. *Hon. Auditors*—Messrs. D. M. Freeland and J. H. Hamence.

The Annual General Meeting was followed by an Ordinary Meeting at which Mr. A. L. Bacharach, in the absence of the new Chairman, took the Chair. The following papers were presented and discussed. "A Critique of *in vitro* Estimation of Therapeutic Index (Toxicity Index)," by S. Morris and A. Jones; "Units of Biological Activity," by N. T. Gridgeman.

Obituary

HENRY EDWARD COX

HENRY EDWARD COX died in hospital on November 21st, 1951, as the result of a street accident. He was born in 1892 at Lowestoft, and was educated first at a preparatory school in Beccles and then at St. Paul's School, London. At the early age of 16 he left school and obtained a post in the firm of John Bell and Croyden, where he worked for two years. In 1911 he became an assistant to John Evans in the laboratory of Allen of Sheffield, with whom he remained for six years. In 1916 he became an Associate of the Institute of Chemistry and in 1917 left Sheffield to become assistant to George Rudd Thompson in Newport. Cox was with Rudd Thompson for five years, and during this time gained the London University degree of Doctor of Philosophy, was elected a Fellow of the Institute of Chemistry and was appointed deputy Public Analyst to Rudd Thompson for the County of Monmouth. Whilst in Newport, Cox contributed several interesting notes to *The Analyst* and in addition worked as an abstractor for this journal. As an abstractor, Cox was in close touch with the then Editor of *The Analyst*, Dr. C. A. Mitchell, who was a personal friend of Otto Hehner; knowing that Hehner wished to retire and live in South Africa, Mitchell introduced Cox to him, with the result that, in 1923, the practice of Hehner and Cox was established at 11, Billiter Square, London. This practice rapidly grew, largely through the personal energy and activity of Cox, and in 1925 he was appointed Public Analyst for the Metropolitan Borough of Hampstead, an appointment that was followed shortly after by those of Public Analyst for the County of Cornwall and for the Isles of Scilly. In 1934 he gained the D.Sc. of London University, an honour that gave him the greatest pleasure.

As an analytical chemist and consultant, Cox was without equal in matters dealing with the examination of dyed materials in connection with cases of dermatitis. He had made a special study of this field of work and contributed a most valuable paper in six parts, which was published in *The Analyst*, entitled "The Chemical Examination of Furs in Relation to Dermatitis." This paper was followed by others dealing with the chemical aspects of dermatitis, chemicals in fabrics as skin irritants, the forensic aspects of dermatitis and by one on hair dyes. The last-mentioned paper was divided into two parts, the first dealing with the chemistry and analysis of henna and the second with the functions and reactions of phenols, and was of particular interest as it was illustrated by a ciné-film in colour, prepared in great detail and with infinite care by Cox himself. Cox specialised, too, in the chemical investigations arising under patent litigation and from the discharge of trade effluents into rivers with the accompanying nuisance to riparian owners and fishing associations. Besides the papers already referred to, Cox contributed others on a variety of subjects, ranging from the occurrence of arsenic on the skins of imported apples to the available tests for identifying small quantities of war gases. He contributed also to the Encyclopedia Britannica and was the author of "The Chemical Analysis of Foods," a book that ran into four editions and is to be found in the library of practically every Public Analyst. He was also responsible for a completely re-written revision of Wynter Blyth's "Foods—Composition and Analysis."

Cox was a man of apparently unlimited energy, both mental and physical, as was shown by the many councils on which he served so loyally. To our Society he gave devoted and willing service and at the time of his death was serving as a member of Council as well as being Chairman of the Public Analysts and Official Agricultural Analysts Committee, and it was largely owing to his untiring efforts that the status and remuneration of part-time Public Analysts was so greatly improved. It was only a month before his tragic death that complete agreement was reached between the employing Authorities' representatives and those representing the part-time Public Analysts. Cox served for six periods as a Member of Council of the Society and for three as Vice-President. His knowledge was profound and his judgment so sound that there was hardly a committee formed in our Society to which Cox was not automatically proposed as a member. He was kindness itself and although obviously at times overworked and tired out, he never refused to serve on any committee if he thought by such service he was helping the Society. At the time of his death he was a member of the Committee recently constituted by the Ministry of Food to examine and make recommendations in the matter of food preservatives.

Besides his faithful and valuable service to our Society, Cox had been a Member of Council of the Chemical Society, the Society of Chemical Industry and the Royal Institute of

Chemistry, as well as being Chairman of the Library Committee of the Chemical Society and Chairman of the Food Group of the Society of Chemical Industry. In matters of policy or procedure in the various societies Cox often held very firm opinions, but he was rarely dogmatic. Indeed, his somewhat disarming smile was a characteristic accompaniment of the exposition of views that occasionally were so much against the general feeling that it undoubtedly required courage to uphold them publicly. And he rarely seemed to lose his temper, a quality that without doubt enhanced greatly his acknowledged value as a witness in Courts of Law.

Many of our members will remember Cox with gratitude as an Examiner in Branch E for the Fellowship of the Royal Institute of Chemistry for the friendly way in which he endeavoured to put the candidate at his ease and for the sympathetic manner in which he answered questions put to him.

Cox lived at Sidcup in Kent, where he was greatly interested in the local Boys' Club, of which he was Vice-Chairman, as well as being for about twelve years People's Warden of Christ Church, the church he regularly attended. It always gave him the greatest pleasure to arrange a cinematograph performance on his own projector for the boys of the Club and for church members as well. He took an active part in all social events in the locality and was a popular member of the local "Brains Trust" team, and nothing pleased him more than to address district meetings on scientific matters in a language that all could understand and enjoy.

Cox was a member of the Savage and Chemical Clubs, having assisted greatly in the founding and early growth of the latter.

Our Society and indeed all chemical societies to which he belonged have lost a trusted and devoted member, and I personally have lost someone I shall always be proud to remember as a real friend and counsellor.

He married, in 1922, Margaret Doughty, whom he met while he was in Sheffield, and leaves, besides his widow, one son and two daughters. ERIC VOELCKER

ARCHIBALD ROBERT JAMIESON

ARCHIBALD ROBERT JAMIESON, a former Vice-President of the Society, died on January 26th, 1952.

Jamieson entered the service of the City of Glasgow in 1926, became Chief Assistant to the Corporation Chemist in 1935 and was appointed Corporation Chemist and City Analyst in 1945.

A native of Glasgow, he was educated at Renfrew High School and graduated in Science at Glasgow University in 1924. He was admitted as an Associate of the Institute of Chemistry in 1924, became a Fellow of the Institute by examination in Branch E in 1930, and was elected a member of the Institute of Sewage Purification in 1934.

He was keenly interested in his profession, the extent of his interest being marked by the attention he gave to the affairs of its associated societies. A member of this Society since 1930, he was a founder member of the Scottish Section and was, in turn, Member of Committee, Vice-Chairman (1941–42) and Chairman (1943–44).

Always closely associated with the affairs of the Royal Institute of Chemistry, he was for a number of years Honorary Secretary of the Glasgow and West of Scotland Section, again being in turn Member of Committee, Chairman and District Member of Council. As the field of his professional duties included the Chemistry of Sewage Purification, he was closely identified also with this Institute from 1934, latterly as Chairman of the Scottish Section, and Member of Council. He was a member of the Society of Chemical Industry, and also of the Chemical Society.

Jamieson was well-known and respected as a lecturer on technical matters, particularly in the realms of Public Health, Sewage Purification and Civil Defence.

With all his attention to professional interests he yet found time for relaxation. A great lover of the open, his activities here included both walking and climbing and, with the day's exertions over, he was ever ready to regale his fellows with quip and song. He was a member of the Rotary Club (Glasgow), and also of the Trades House (Bonnetmakers).

He leaves a widow and two young children, and his death at the early age of 53 removes from our midst a respected official, a considerate and helpful colleague, and a most likeable personality. R. S. WATSON

Analytical Methods Committee

REPORT PREPARED BY THE CAROTENE PANEL OF THE SUB-COMMITTEE ON VITAMIN ESTIMATIONS

The Determination of Carotene in Green-Leaf Material

Part II. Green-Leaf Materials other than Grass

THE terms of reference and the constitution of this Panel are given in Part I (*Analyst*, 1950, **75**, 568), since when Dr. F. E. Moon has resigned and his place has been taken by Miss M. Olliver.

INTRODUCTION

The extension to other green-leaf material of the analytical method for determining carotene in fresh grass (Part I of this Report) was thought, when that method was put forward, to be unlikely to encounter any obstacles other than sampling difficulties. It was, however, plainly desirable to investigate both its extensibility and the emergent sampling problems; this has now been done. The choice of material for trial was governed mainly by the wish to cover a wide botanical range, but partly by availability in fresh condition. As a preliminary, some tests were made with canned spinach purée, spinach being taken as a typical dicotyledon and the form presenting no sampling difficulties.

SPINACH PURÉE

The method recommended for carotene in fresh grass has been applied successfully to spinach purée, with two minor modifications. First, maceration of a purée is superfluous; secondly, it has been found that the mixture of equal parts of acetone and light petroleum used for the extraction of carotene from fresh grass is not ideal for a spinach purée nor, by implication, for any material whose ratio of moisture to carotene is appreciably greater than the 6000 or 7000 to 1 characteristic of fresh grass. The greater the moisture, the richer in acetone should the solvent be. A mixture of acetone and light petroleum in the ratio of 4 to 1 is recommended for this particular material.

Cans representative of two specially prepared batches, A and M, of purée were assayed for carotene in eight independent laboratories, with the mean results shown in Table I.

TABLE I

CAROTENE CONTENTS OF TWO PREPARATIONS OF SPINACH PURÉE

Laboratory	Preparation A, p.p.m.	Preparation M, p.p.m.
I	17·9 (2)	19·2 (2)
II	18·7 (3)	19·8 (12)
III	14·7 (2)	19·3 (2)
IV	19·4 (6)	20·7 (6)
V	18·8 (6)	19·9 (6)
VI	19·8 (3)	21·0 (1)
VII	18·3 (4)	19·5 (3)
VIII	17·0 (4)	20·9 (4)

NOTE—Figures in brackets give the numbers of tests contributing to the means.

The mean standard deviation of one estimate in any one laboratory is 0·744 p.p.m., corresponding to a coefficient of variation of about 4. If, however, calculation is based on the interaction term "laboratories × samples" in the analysis of variance, a coefficient nearer 10 is obtained. It can be argued that the latter is the more meaningful value; it often happens in analytical work that reproducibility tests within laboratories on sets of sub-samples yield a more optimistic estimate of precision than that obtained when the same laboratories compare results on shared samples. A coefficient of 10 is not unsatisfactory for estimates of the order of 20 p.p.m., and it can be seen that, with the exception of one rogue result, the present estimates are as concordant as can reasonably be expected. All laboratories, it may be noted, agreed that preparation M is slightly richer in carotene than preparation A.

Fresh greens

The fresh green-leaf materials examined, and a synopsis of the results obtained (mostly by at least two different laboratories), are given in Table II.

Table II

Carotene contents of various green-leaf materials

	Number of estimates	Average carotene content, p.p.m.	Coefficient of variation
Brussels sprouts (*Brassica oleracea gemmifera*)	41	3 to 8	15
Cabbage (*Brassica oleracea capitata*)	20	1 to 4	50
Carrot leaf (*Daucus carota*)	36	130	1
Chive (*Allium schoenoprasum*)	2	40	—
Clover leaf (*Trifolium pratense*)	14	120	5
Dandelion (*Taraxacum officinalis*)	4	50	1
Leek (*Allium porrum*)	6	22	—
Nettle (*Urtica dioica*)	6	110	1
Parsley (*Petroselinum hortense*)	26	70	3
Sage (*Salvia officinalis*)	3	70	—
Sugar-beet leaf (*Beta vulgaris*)	6	50	5
Watercress (*Nasturtium officinalis*)	8	70	6

In nearly all respects, including sample size, these analyses were satisfactorily carried out by the method established for fresh grass. It should be noted that materials such as brassica and leek, having a high ratio of moisture to carotene (see previous section), are more efficiently extracted with solvent mixtures containing over 50 per cent. of acetone, up to 80 per cent. being recommended for the initial extractions, with progressively smaller proportions in subsequent extractions.

The coefficient of variation, based on replications within laboratories, is, for most of the items in Table II, substantially the same as that found in the analysis of fresh grass, namely, about 4, but if the carotene content is below about 40 p.p.m. the coefficient is, understandably, higher. For materials containing less than 10 p.p.m. of carotene, such as brassica, the error of estimation is best expressed as a standard deviation of about 1 p.p.m. The variation is also positively correlated with stalkiness, as is to be expected from the observation that stalk usually contains little or no carotene.

Brussels sprouts and cabbage, because of their non-uniform pigmentation, present special sampling and preparative problems. It has been found that the variation between sprouts in any nominally homogenous batch (same variety, same picking) is not very great, and four sprouts will form a reasonable sample. Two methods of sub-sampling are recommended, namely, (*i*) the cutting of thin semi-circular sectors or wedges from each sprout, followed by chopping and mixing, or (*ii*) the chopping and mixing of the four whole sprouts. Wedges may be similarly cut from cabbage. The importance of proper sampling of cabbage and sprouts is demonstrated by the results obtained from various leaf layers in the course of this work. Integration of these results makes possible an expression of carotene distribution between three concentric shells and the heart of a typical mature cabbage or sprout, the four parts being so cut as to be of equal weight. The outer quarter was found to contain 75 per cent. of the total carotene, the next inner contained 15 per cent., the next 7 per cent. and the heart 3 per cent. (One Sells sprout, after removal of the outside damaged leaves, was found to contain about 0·2 mg of carotene, and a large Winningstad cabbage, similarly trimmed, about 5 mg of carotene.)

The necessarily heavy sampling of plant material with a very small or very heterogeneously distributed carotene content can alternatively be handled with a mechanical macerator,* but the operator must satisfy himself that full recovery (*e.g.*, no losses of carotene by splashing) is being effected and that no chemical changes occur; this is best—although even then not certainly—secured by replacement of the air with an inert gas, such as carbon dioxide, hydrogen or oxygen-free nitrogen.

* On page 569, line 20, of Part I of this report (*Analyst*, 1950, **75**, 568–573) mechanical homogenisation was, by a misprint, described as "more efficient" instead of "no more efficient" than the hand grinding technique. The error has been corrected in the reprints.

Supplementary Bibliography on Carotene Estimation, with Special Reference to Green-Leaf Material

PREPARED BY N. T. GRIDGEMAN FOR THE CAROTENE PANEL OF THE SUB-COMMITTEE ON VITAMIN ESTIMATIONS OF THE ANALYTICAL METHODS COMMITTEE

THE following list supplements the Bibliography published with Part I of the Carotene Panel's Report on the Determination of Carotene in Green-Leaf Materials in *The Analyst*, **1950, 75**, 574–576—

98. Berkhout, H. W., Hekking, J. W. H., and Geerling, H., "A Fast and Reliable Carotene Determination in Dried Grass," *Chem. Weekblad*, 1948, **44**, 546.
99. Bickoff, E. M., and Thompson, C. R., "Determination of β-Carotene Stereoisomers in Alfalfa," *J. Assoc. Off. Agric. Chem.*, 1949, **32**, 766, 775.
100. Bukatsch, F., "Field Method for the Estimation of Carotene in Edible Plants," *Münch. med. Woch.*, 1950, **92**, 402.
101. Clowes, G. J., and Hallsworth, E. G., "The Preservation of Pasture Samples for Carotene Estimation," *Chem. and Ind.*, 1949, 624.
102. Cooley, M. L., "Stabilisation of Standard Carotene Solutions," *J. Assoc. Off. Agric. Chem.*, 1949, **32**, 706.
103. Fulop, L., "Determination of Carotene," *Bull. Fac. Hort. Viticult., Budapest*, 1944, **10**, 152; through *Chem. Abs.*, 1950, **44**, 5494.
104. Heyrovský, J., and Husselbach, H., "Polarographic Determination of Carotene," *Z. Pflanzenzücht.*, 1943, **25**, 443.
105. Pumpelly, C. T., Mitchell, H. L., and Silker, R. E., "Determination of Carotene in Alfalfa Meal," *J. Assoc. Off. Agric. Chem.*, 1951, **34**, 821.
106. Stitt, F., Bickoff, E. M., Bailey, G. F., Thompson, C. R., and Friedlander, S., "Spectrophotometric Determination of β-Carotene Stereoisomers in Alfalfa," *Ibid.*, 1951, **34**, 460.
107. Thompson, C. R., and Bickoff, E. M., "A Proposed Modification of the A.O.A.C. Method for Carotene in Alfalfa," *Ibid.*, 1951, **34**, 219.
108. Wall, M. E., and Kelley, E. G., "Note on Carotene Paper by Derby and De Witt," *Ibid.*, 1949, **32**, 804.
109. Zscheile, F. P., and Whitmore, R. A., "Determination of Carotene in Alfalfa," *Anal. Chem.*, 1947, **19**, 170.

The Evaluation of Amoebicidal Substances *in vivo*

BY L. G. GOODWIN

(*Presented at the meeting of the Biological Methods Group on Saturday, October 27th*, 1951)

The occurrence of *Entamoeba histolytica* in man and other hosts is described. The effect of drugs on this infection in man and animals, and particularly of emetine, diodoquin or carbarsone on rats, is detailed.

The interference of *E. muris* in the treatment of amoebiasis is also mentioned.

I SHOULD like first to remind you of the nature of amoebic infection in man. *Entamoeba histolytica* is a curious parasite. In the majority of people who harbour it in their guts it lives quite happily, feeding upon bacteria, and produces, at the most, only minute areas of damage to the wall of the large bowel. In such conditions, the infection is usually symptomless, the amoeba quietly completes its life history and the host passes cysts in his faeces. Occasionally, for reasons that we do not understand, but that may depend upon particular strains of the amoeba or the presence of certain bacteria, it acquires a thirst for blood and plays havoc with the gut wall. It produces deep and extensive ulceration, and blood and mucus are passed in the stools. The amoebae feed themselves with red blood cells and are swept out of the gut to their death before they can form cysts. They occasionally enter the portal vessels and are carried to the liver, where they give rise to amoebic hepatitis or liver abscess.

In the design of a laboratory test for new remedies for a disease of this kind, we must be careful to choose a suitable host and a suitable strain of amoeba. Monkeys are easy to

infect experimentally and frequently carry natural symptomless *E. histolytica*. The action of drugs in this infection has been investigated by Anderson in California, who has obtained very useful information. Monkeys, however, are expensive and difficult to handle, and we need something smaller. Kittens, when inoculated *per rectum* with amoebae from a human patient or from cultures, suffer from very severe ulceration, leading to a bacterial septicaemia that kills them in a few days. However, the infection in kittens does not respond to treatment with emetine or with any of the other drugs known to have activity in human amoebiasis, so that we cannot really expect to discover new amoebicides with an infection of that type. Rats have been known for many years to be susceptible to infection with *E. histolytica*. They can be infected experimentally by giving cysts by mouth in the natural way, although infection takes some time to develop and is not always established. A more certain way is to inoculate a culture of the amoebae directly into the caecum, exposed under anaesthesia through a small incision in the abdominal wall. This produces ulceration in all of the animals if the strain of amoeba is a suitable one and if the rats are very young. This method, with weanling rats, was first described by W. R. Jones in 1946.

It was believed that the infection cured itself spontaneously in a few weeks; however, my colleague Dr. Neal has shown that with some strains of amoeba the infection persists for many months and the animals pass cysts in their faeces. Moreover, different strains of amoeba from acute, sub-acute and chronic human infections usually produce similar types of infection in the rat. Differences in the virulence of strains may be associated with differences in the accompanying bacterial flora, but this we do not yet know for certain.

You will see, therefore, that the infection in the rat is closely similar to the infection in man.

When infected rats are treated, for one week after inoculation, with drugs such as emetine, diodoquin or carbarsone, they show less severe lesions of the caecum than do untreated control animals. With sufficiently large doses the rats are cured, and then no amoebae can be found in their caecal contents. The severity of the lesions, or the proportion of a group of rats freed from amoebae by a dose of a drug, can be used as quantitative measures of effect and form the basis for an assay of potency.

This method, then, agrees well with the conditions required for a good biological test. Untreated control animals always become infected, and emetine and other drugs have a recognisable action. The infecting organism is derived from man and produces lesions similar to those in man. A simple test upon the acute infection is completed in one week.

I must, however, mention a difficulty met early in the course of our work with the infection, one that I know has also given Dr. Fulton a good deal of trouble. A harmless commensal amoeba, *E. muris*, is often found in the caeca of rodents. With experience it is not difficult to distinguish this organism from *E. histolytica* under the microscope, but difficulties arise if there is a mixed infection and both species are present. *E. muris* does not respond to treatment with emetine, so that its presence in an experiment of the kind described is a disaster. The infection is transmitted to young rats in foodstuffs and bedding that have been soiled by the excreta of rodents carrying the cysts of *E. muris*. There is a simple way of avoiding the trouble. The rats must be bred from a stock free from *E. muris*, and all cages, wood-wool and sawdust used for the experiment must be sterilised before use. Food stocks must be protected from contamination. Hamsters, which often carry very heavy infections, should not be kept in the same room as the rats.

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(After the presentation of the above paper a film made by Dr. Goodwin to illustrate the method of carrying out the test was shown.)

DISCUSSION

DR. J. D. FULTON said he was still troubled with *E. muris* in his rats, and asked if Dr. Goodwin was still able to keep his rats free from it. He said that his difficulty was limited space, and that he had to keep rats, mice, hamsters and cotton rats all in the same room; *E. muris* was always found in the guts of the last two species.

DR. GOODWIN replied that he was still able to keep his rats free from *E. muris*.

PROFESSOR BUTTLE enquired about the effects of antibiotics on rats.

DR. GOODWIN replied that aureomycin, streptomycin, terramycin and penicillin all had some effect on rat amoebiasis, but that, as in human cases, the amoebae were not eradicated.

DR. G. M. FINDLAY asked if it was proved that the bacterial flora was responsible for the dying-out of amoebic infections in people who had returned to Britain from the tropics.

DR. GOODWIN said that it was probable that this was so, but that nobody had proved it.

DR. F. HAWKING said that in general, as soon as possible after definite activity has been found in a new chemical series in the search for new chemotherapeutic substances, a preliminary clinical trial should be arranged, as—

(i) the relation between activity in the laboratory and therapeutic activity in man was not at all close. Often substances that were very active on laboratory infections had no action in man. Occasionally substances that were only mediocre in the laboratory were highly successful in man,

(ii) the substance might cause peculiar toxic effects in man, *e.g.*, peripheral neuritis or toxic action on the finger nails, which would not be revealed by animal experiments.

The results of this preliminary clinical trial were very valuable in planning the further development of the chemical series involved.

The Evaluation of Chemotherapeutic Agents Directed Against Trypanosome Infections

BY E. M. LOURIE

(*Presented at the meeting of the Biological Methods Group on Saturday, October 27th*, 1951)

Refined statistical methods have been essential for the development of techniques of biological standardisation, but in the search for new compounds of clinical value they are useless or deceptive unless appropriate experimental procedures are used.

In the control of sleeping sickness in Africa the tendency is for "mass-treatment" to be replaced by "mass-chemoprophylaxis." The compounds now used for this purpose are highly effective, but are not necessarily the best that might be devised. There is accordingly need for a simple and rapid method for screening compounds as to their prophylactic activity.

There is good reason to believe that the composition of certain trypanocides of the melaminyl series, already fairly extensively used in the field, cannot be guaranteed by chemical or physical means. It is therefore necessary that standard preparations of these substances should be established and that batches intended for use in man should be biologically compared with those standards.

THE history of the systematic evaluation of drugs against infective disease really begins with trypanosomiasis, because it was with trypanosome infections that Ehrlich, some fifty years ago, began his pioneer investigations of new chemotherapeutic agents. The measures of toxicity and of efficacy he used, namely the "minimal lethal dose" and the "minimal curative dose," have since been much criticised as being either impossible to determine within reasonable limits of error, or indeed as having no meaning at all, in face of the notorious variability of biological reactions. The later introduction of the concept of the LD50 and the ED50 by Trevan[1] and the elaboration of methods of determining the fiducial limits to these end-points and of the direction and variance of the dose - response curve have been of inestimable value towards clear thinking and towards the evolution of techniques of biological standardisation. Yet there is no reason to believe that Ehrlich's search for chemotherapeutic agents of clinical usefulness would have met with any greater success if he had striven to improve on his own crude statistical methods.

LIMITATIONS OF SCREENING TESTS IN MOUSE INFECTIONS

It is easy to justify the statement that a more refined statistical technique would not necessarily have led Ehrlich to select better chemotherapeutic agents than he in fact chose. In so-called screening tests in laboratory infections, the species of animal used is neither man

himself nor the large and expensive domestic animal for whose eventual benefit the tests are performed; the circumstances and the type of infection are usually so different in the laboratory animal from those in man or the domestic animal that the result in the laboratory can often at best give no more than a slight hint at the possible clinical effect. A series of drugs arranged in the order of their efficacy in mice cannot be expected to fall into the same order when arranged according to their usefulness in man or the domestic animal. For the moment one example alone will suffice. On the basis of the chemotherapeutic index in mice, suramin would be placed far ahead of tryparsamide,[2] yet in man tryparsamide is much the more valuable of the two. If the relative efficacy of two compounds can be so spectacularly reversed when tested first in mice and then in man, it is clearly futile merely to arrange drugs in a close order of their efficacy in mouse infections, with the aid of refined statistical procedures, where the object is limited to the selection of those that are most likely to be useful in man.

In recent tests of cinnoline compounds in mice infected with the cattle trypanosome, *T. congolense*, one member of this chemical series was carefully compared with antrycide methylsulphate.[3] Where the chemotherapeutic index was defined as the LD50/CD50, antrycide was found to be slightly the more effective compound of the two, but where the index was defined as the LD10/CD90, which approximates more closely than the LD50/CD50 to the requirements of actual clinical practice, there was no significant difference between the two compounds. A statistical exercise of this nature may serve the useful purpose of encouraging the field worker to test a compound he might otherwise reject without trial, but it does practically nothing to increase the likelihood that the trial will in fact show the drug to be of great clinical value.

Let us examine more closely the limitations imposed by a dependence on infections in small laboratory animals for the initial screening tests of anti-trypanosomal drugs. One essential and obvious difficulty is that absorption, excretion and degradation of a drug may be so completely different in two species of animal that there can be no assurance that trypanosomes will be equally exposed to the drug in the laboratory animal, on the one hand, and in man (or the domestic animal), on the other. Then there are the differences between the type of infection in the laboratory animal and in the natural host. The infection usually used for screening tests in mice is of the acute fulminating septicaemic type, and in man or domestic animals it is of a chronic, or relatively chronic, tissue-invading type, in which contamination of the circulating blood is relatively slight and easily controlled. The problem is to reach the trypanosome in the more or less inaccessible spaces to which it retreats and in which at least some species of trypanosome may perhaps assume an increased degree of resistance even if the drug were capable of reaching it there.

Preliminary investigations of potential trypanocides in laboratory animals other than mice

The work that led to the introduction of tryparsamide for human trypanosomiasis by American workers[4,5] in 1919 took full account of the fact that tests in the septicaemic infection of mice cannot be a reliable guide to the results that might be obtained in the type of infection that occurs in man. For this reason much reliance was placed on trials in rabbits rather than in mice, since trypanosome infections in rabbits are normally of a chronic, tissue-invading type and, to that extent, approximate more nearly than the septicaemic infections of mice to the type that occurs in man. However, the mere device of testing the drugs in rabbit infections is not enough. Pentamidine was found to be more effective than tryparsamide in rabbits,[6] yet it proved to be far inferior to tryparsamide in the late stage of human trypanosomiasis.[7,8] It is for this stage, characterised by involvement of the brain and cerebrospinal fluid, that the need for new drugs is greatest. A measure is therefore needed of the drug's efficacy in dealing with trypanosomes in tissue spaces other than those invaded in the rabbit, namely, in the brain and cerebrospinal fluid. Tests of new drugs for human trypanosomiasis should accordingly include attempts at determining their power of penetrating the blood - brain barrier or of entering the cerebrospinal fluid. These two properties are not necessarily synonymous,[9] but simple routine methods of measuring the power of penetrating the blood - brain barrier are not available for many types of drug, and there are good reasons for accepting a drug's powers of entering the cerebrospinal fluid in trypanocidal form as some indication of its potential usefulness in late human trypanosomiasis.[10,11] Such tests can easily be made in rabbits, and there is at least some suggestion

of their potential value in the facts that Rollo, Williamson and Lourie[12] found that Friedheim's melaminyl arsenical, melarsen, entered the cerebrospinal fluid in trypanocidal form to much greater effect than any other compound tested, and that the latest report from Nigeria[13] has shown this drug to be at least as effective as tryparsamide in advanced *T. gambiense* infections in man.

In trypanosomiasis of cattle, unlike human trypanosomiasis, the main problem is not invasion of the central nervous system with consequent failure of the drug to reach the trypanosome in that particular site. The trypanosome (*T. congolense*) may, however, remain hidden for long periods of time in some unknown site, and Fiennes[14] believes that such cryptic infections do much damage and constitute one of the main difficulties in the chemotherapy of trypanosomiasis in cattle. He has produced evidence that cardiac muscle may be one of the sites of such cryptic infections. Here also, therefore, tests on the septicaemic infection of mice cannot afford any precise forecast of eventualities in the host for which the drug is finally intended.

The species or strain of trypanosome used in screening tests

Complications in interpreting the results of mouse screening tests in terms of the infection in man or domestic animals arise not only because of differences between the species of host and between their reactions to the parasite, but also because the species or strain of parasite used in the mouse may be very different from those of natural infections in man or domestic animals. When a strain of *T. rhodesiense* was used for screening tests in mouse infections during the work which led to the use of pentamidine,[6] some of the more important characteristics of the strain were very different from those it had exhibited when it was originally isolated from man many years earlier; the anomalous situation arose where a group of drugs, the diamidines, was introduced into clinical practice for the treatment of *T. gambiense* infections although it has found little or no place in the treatment of natural infections by the very species, *T. rhodesiense*, against which it was originally screened.[15] In screening tests of drugs intended for the treatment of cattle trypanosomiasis, one difficulty is that, although the most important species in cattle, *T. congolense*, is inoculable into mice, another dangerous and widely-prevalent pathogen, *T. vivax*, cannot be readily maintained in small laboratory animals and so cannot be used for simple and rapid screening tests.

Chemoprophylactic activity

The laboratory investigation of new drugs intended for trypanosomiasis must take special account not only of the pathology and parasitology of natural infections, but also of the actual practice of trypanosomiasis control in the field. This is at present undergoing a revolutionary change. For more than 40 years an important feature in campaigns against sleeping sickness has been the practice known as "mass-treatment," involving examination of all the inhabitants of a large area and the administration of a full course of curative treatment to all who are found to be infected, whether as invalids or carriers. Few are aware of the prodigious scale on which such work has been carried out by the branches of the medical services dealing with sleeping sickness in tropical Africa. Every year, for many years, millions of people have been examined in the course of this work,[16] treatment being given where necessary. The tendency now is for "mass-treatment" to be replaced in many territories, especially French and Belgian, by "mass-prophylaxis," which involves the administration of a drug with prophylactic activity to every uninfected person in the selected area; pentamidine has become very widely used for this purpose.[17,18,19,20] Valuable though pentamidine is as a prophylactic agent, it is not necessarily the best that might be devised, and there is, accordingly, need for a simple rapid technique in screening compounds for their prophylactic activity. The ordinary tests for therapeutic properties are useless for this special purpose. For example, the polymer of Friedheim's antimonial analogue of melarsen, known as MSb, far outstrips pentamidine as a prophylactic agent in mouse infections, while the monomer is practically devoid of prophylactic action.[21,12] Yet the difference between the polymer and the monomer in therapeutic activity is relatively slight,[21] so that the ordinary tests of therapeutic activity would fail completely to reveal the particular difference between these two preparations that is of greatest significance to the worker in the field. The kind of screening test for prophylactic activity that might perhaps serve as a routine in mice would involve the inoculation of a standard number of trypanosomes at a fixed time (say

2 weeks) after administering the drug, and examining the blood after a further fixed period (say a further 2 weeks).

Biological standardisation of compounds directed against trypanosomiasis

I have so far discussed drug assay only from the point of view of discovering new chemotherapeutic agents. The other, and entirely different, purpose for which drugs are assayed against laboratory infections is to confirm the freedom from undue toxicity and the potency of individual batches of established chemotherapeutic agents whose constitution cannot be guaranteed by chemical or physical means. Members of the arsphenamine group and suramin come into the category of substances for which such biological standardisation is still regarded as necessary. The methods used for this purpose need not be described here, for they have recently been well summarised by Goodwin.[22]

It was the alarming and unpredictable incidence of severe toxic reactions or of therapeutic inefficacy (against syphilis), in a type of compound that could not be produced to a uniform physico-chemical pattern, that led to international agreements in 1925 on biological standards for the arsphenamines, and on methods of comparing batches intended for clinical use with the agreed standards. The time seems to have arrived, if indeed it is not already overdue, for biological standards to be provided for some of the newer trypanocides, which also surely come into the category of substances that cannot be accurately characterised by chemical or physical means. These are the compounds of Friedheim's melaminyl series, especially "Mel B," which is a condensation product of melarsen and BAL (used for therapy) and MSb, mentioned above (used for prophylaxis). Mel B, in particular, has already been fairly extensively tested in man, even before the publication of any results of tests on laboratory infections. The clinical reports on this substance have almost inevitably been highly conflicting, both as to toxicity and to efficacy.[13,23,24,25,26] It is certain that some of the batches used in man have been extremely toxic. MSb has also been tested as a prophylactic agent under field conditions, in trials involving at least seven hundred subjects, with inconclusive but encouraging results.[27]

In order that a proper judgment should at last be formed of these melaminyl substances, and in the interests of the patients themselves, it is necessary that agreed standard preparations should now be established and that batches intended for use in man should satisfy the requirements of biological tests, of which the details remain to be decided in the light of a careful study of the standard preparations. From the chemical nature of melarsen it is likely that reliable methods of preparation and chemical characterisation will soon render a biological standard and biological tests unnecessary for this particular member of the group, but for Mel B and MSb standards will probably continue to be necessary for a much longer time.

References

1. Trevan, J. W., *Proc. Roy. Soc., B*, 1927, **101**, 483.
2. Yorke, W., Murgatroyd, F., and Hawking, F., *Ann. Trop. Med. Parasitol.*, 1931, **25**, 313.
3. Lourie, E. M., Morley, J. S., Simpson, J. C. E., and Walker, J. M., *Brit. J. Pharmacol.*, 1951, **6**, 643.
4. Jacobs, W. A., and Heidelberger, M., *J. Exp. Med.*, 1919, **30**, 411.
5. Brown, W. H., and Pearce, L., *J. Amer. Med. Ass.*, 1924, **82**, 5.
6. Lourie, E. M., and Yorke, W., *Ann. Trop. Med. Parasitol.*, 1939, **33**, 289.
7. Saunders, G. F. T., *Ibid.*, 1941, **35**, 169.
8. Lourie, E. M., *Ibid.*, 1942, **36**, 113.
9. ——, *Trans. Faraday Soc.*, 1943, **39**, 340.
10. Voegtlin, C., Smith, M. I., Dyer, H., and Thompson, J. W., *Publ. Hlth. Rep. Wash.*, 1923, **38**, 1003.
11. Hawking, F., Hennelley, T. J., and Quastel, J. H., *J. Pharmacol.*, 1937, **59**, 157.
12. Rollo, I. M., Williamson, J., and Lourie, E. M., *Ann. Trop. Med. Parasitol.*, 1949, **43**, 194.
13. Duggan, A. J., and Hutchinson, M. P., *Trans. R. Soc. Trop. Med. Hyg.*, 1951, **44**, 535.
14. Fiennes, R. N. T.-W.-, *Ibid.*, 1951, **44**, 605.
15. Lourie, E. M., Comment on Proceedings of a Mulago Staff Clinical Meeting, *Trop. Dis. Bull.*, 1950, **47**, 526.
16. Wilcocks, C., Corson, J. F., and Sheppard, R. L., "A Survey of Recent Work on Trypanosomiasis and Tsetse Flies," Bureau Hyg. Trop. Dis. Review Monograph No. 1, London, 1946.
17. Harding, R. D., and Hutchinson, M. P., *Trans. R. Soc. Trop. Med. Hyg.*, 1950, **43**, 503.
18. Scaillet, L., and Haddad, E., *Ann. Soc. Belge Méd. Trop.*, 1950, **30**, 1525.
19. Jonchère, H., *Bull. Soc. Path. Exot.*, 1951, **44**, 83.
20. Kernevez, H., and Chassain, J., *Ibid.*, 1951, **44**, 337.

21. Friedheim, E. A. H., Vogel, H. J., and Berman, R. L., *J. Amer. Chem. Soc.*, 1947, **69**, 560.
22. Burn, J. H., Finney, D. J., and Goodwin, L. G., "Biological Standardisation," Second Edition, Oxford University Press, 1950, Chapter XXII.
23. Friedheim, E. A. H., *Amer. J. Trop. Med.*, 1949, **29**, 173.
24. Le Rouzic, *Bull. Méd. de l'Afrique Occident. Franç.*, 1949, No. Spéc., 63; *Trop. Dis. Bull.*, 1950, **47**, 449.
25. Commission du Mel B, *Ibid.*, 1949, No. Spéc., 73; *Trop. Dis. Bull.*, 1950, **47**, 449.
26. Ceccaldi, J., Trinquier, E., Arnoult, H., and Menu, P., *Bull. Soc. Path. Exot.*, 1950, **43**, 83.
27. Le Rouzic, *Bull. Méd. de l'Afrique Occident. Franç.*, 1949, No. Spéc., 53; *Trop. Dis. Bull.*, 1950, **47**, 451.

DEPARTMENT OF PHARMACOLOGY
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Rapid X-Ray Quantitative Analysis of Crystalline Powders

With Particular Reference to Titanium Dioxide Pigments

BY W. HUGHES AND H. SMITH

(*Presented at the meeting of the Physical Methods Group on Friday, October 19th*, 1951)

A simple X-ray diffraction camera is described, the action of which is based on the flat powder layer method. The camera can be constructed easily and it gives a tenfold reduction in exposure times compared with a normal 9-cm powder camera. Specimen preparation is simplified, and where large numbers of specimens are involved there is considerable saving of time in processing the film. The instrument has proved useful for very rapid qualitative and quantitative examinations of crystalline powders. The accuracy with rutile - anatase mixtures is about $\pm 2{\cdot}5$ per cent.

A second method of quantitative analysis by means of a Geiger-counter X-ray spectrometer is described. No photographic processing is required and the accuracy with rutile - anatase mixtures is probably better than ± 1 per cent.

THE method of analysis of crystalline powder mixtures by X-ray diffraction techniques has been used for many years and is adequately described in the literature. Briefly, a powder mixture irradiated by X-rays gives a composite diffraction pattern, consisting of the superimposed unique patterns of the individual components. A comparison of the intensity of suitably chosen lines in the constituent patterns gives an estimate of the amounts of the various substances present in the mixture. The comparison can be made visually or photometrically from a photographed record of the pattern as in the first method described below, or by direct intensity measuring devices such as the ionisation chamber or Geiger-counter tube, as described in the second method.

X-ray diffraction methods are not generally regarded as particularly sensitive for detecting small quantities of materials, nor is indiscriminate application possible; but by ingenious adaptation or careful development, the methods can be applied to many analyses to give a high degree of accuracy. Rooksby[1] for example, showed that 0·1 per cent. of calcium oxide in magnesium oxide and 0·2 per cent. of zinc oxide in zinc sulphide could be detected.

The following methods were developed specifically for the rapid analysis of mixtures of the anatase and rutile forms of titanium dioxide pigments. They may be of more general interest however, as they should be adaptable to the analysis or examination of other powder mixtures.

METHOD

THE FLAT POWDER LAYER CAMERA METHOD—

In general, in the past, the analysis of crystalline powders by X-ray diffraction techniques has involved the use of Debye-Scherrer cameras, which require that the specimen be in the form of an accurately centred thin cylinder. The major defects of the Debye-Scherrer

camera compared with those of the flat powder layer camera, are the relatively long exposures and the necessity for standardisation of specimen preparation.

The need for a method that involved considerably reduced exposure times and a simplified technique of specimen preparation arose when many hundreds of anatase - rutile mixtures were to be analysed in such a short period of time that conventional methods were out of the question. This need was met admirably by adopting the flat powder layer method developed by Brentano.[2] The principle is illustrated in Fig. 1, in which A represents the

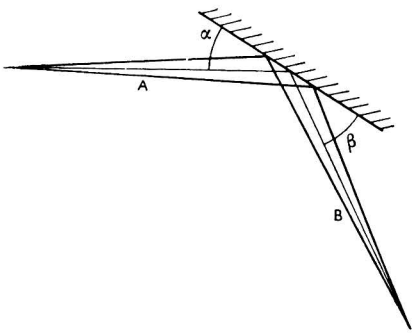

Fig. 1. The principle of the flat powder layer camera method

distance of the X-ray source from the point of intersection of the incident beam with the powder layer, and B, the distance of the point of observation from the same incidence point. The glancing angle of incidence is α and the glancing angle of reflection is β. The Bragg angle is θ, where $\alpha + \beta = 2\theta$. The powder layer is sufficiently thick to absorb fully the incident radiation and is normal to the plane containing the incident and reflected beams.

It can be shown easily that when

$$\frac{\sin \alpha}{\sin \beta} = \frac{A}{B}$$

the reflected beam is partially focused round the point of observation and that when this focusing condition is satisfied the absorption by the specimen is proportional to $\mu(1 + A/B)$, *i.e.*, it is independent of θ, where μ is the linear absorption coefficient of the powder. The advantages of this method are, (*a*) the reduced exposure times which follow from the para-focusing property of the flat specimen, (*b*) rapid preparation from the simple nature of the specimen, and (*c*) the elimination of the necessity for special care in specimen preparation because the absorption is independent of θ.

The flat powder layer camera as developed by Brentano is not of particularly simple construction and in this respect suffers in comparison with the conventional Debye-Scherrer camera. However, during routine examination of powder mixtures of two crystalline substances only, it is possible to introduce a major simplification. Inspection of the Debye-Scherrer photographs (Fig. 2, *a*, *b* and *c*) of titanium dioxide pigments shows that convenient lines for comparison lie in the angular range $\theta = 17°$ to $\theta = 34°$. This range contains well resolved lines of such relative intensity that small quantities of anatase are more readily detected, and it corresponds to the compositions important in the pigment industry. A flat powder layer camera was designed and constructed that would take photographs over this restricted angular range and in which maximum focusing was attained in the middle of the range. Fig. 3 is a diagram and Fig. 4 a photograph of the camera.

The instrument consists essentially of a slit system and collimator, a specimen holder and its mount, and a film holder. Exposures are short by virtue of the short target-specimen-film distances, the relatively wide slit-system giving an appreciably divergent beam from a line focus tube, and because of some degree of focusing by the relative positions of beam, specimen and film. The camera, Figs. 3 and 4, is built on a flat metal plate that is fixed on a camera stand by the conventional hole, slot and plane arrangement. The source of X-rays, S, used, was the vertical line focus of a Metropolitan Vickers Raymax unit and B is a single slit 2·5 mm high and 0·8 mm wide; the distance SB is 6·5 cm. A removable Perspex specimen holder, C, whose vertical plane is set at an angle of 28° to the beam, has

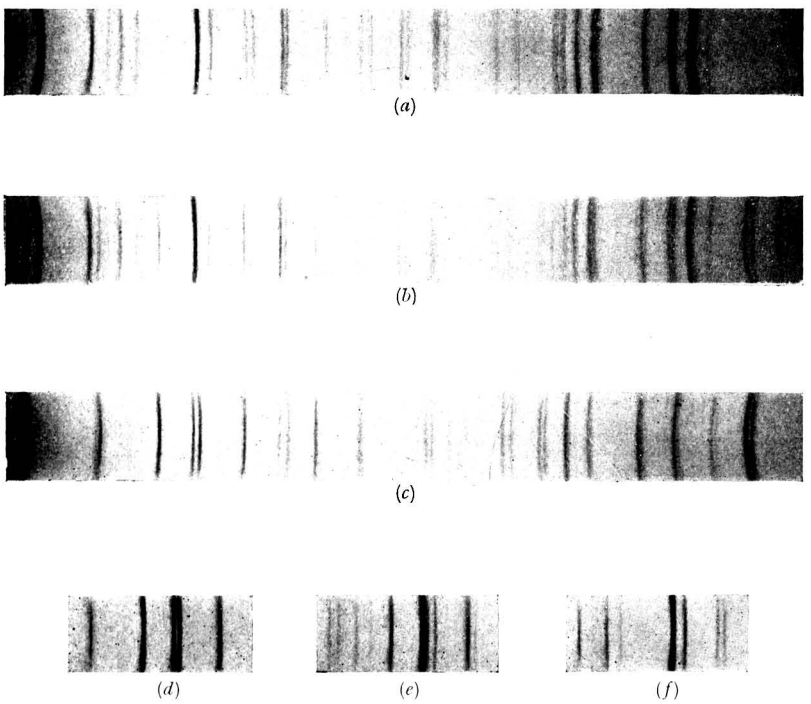

Fig. 2. X-ray powder photographs: (*a*) and (*f*), rutile; (*b*) and (*e*), 50 per cent. of rutile + 50 per cent. of anatase; (*c*) and (*d*), anatase

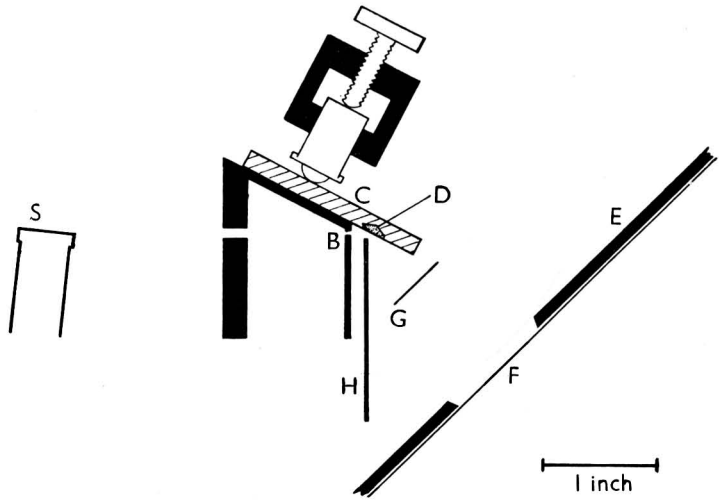

Fig. 3. Diagram of the flat powder layer camera.
B, single slit; C, removable Perspex specimen holder; D, depression; E, film-holder; F, film; G, β-filter; H, shield; S, source of X-rays

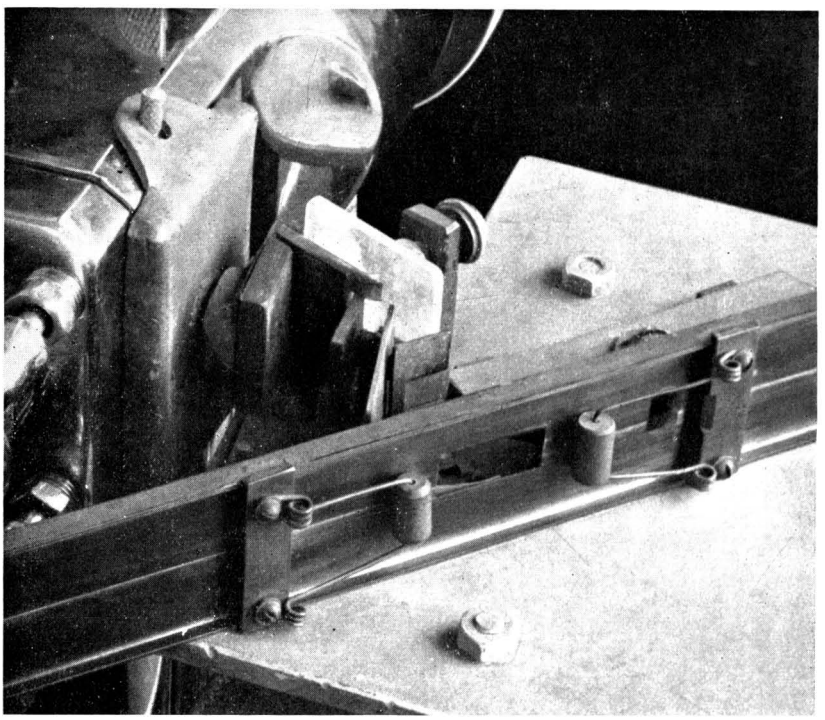

Fig. 4. The flat powder layer camera

a countersunk depression, D, to hold the powder centred on the area of incidence of the primary beam; distance BD is 0·5 cm. The film holder, E, is 24 inches long and is constructed from brass curtain rail to carry a strip of film 30 cm × 3 cm (the conventional strip-size in 9-cm Debye-Scherrer cameras) in a vertical plane inclined at an angle of 45° to the incident beam. The plane of the film is then perpendicular to a reflected beam, whose Bragg angle is 22·5°. A window 1 cm high and 2·5 cm long is cut in the film holder centred about this angular position. The distance, DF, of the window from the specimen is 4·0 cm. All reflected rays passing through the window will be focused to some extent as $\sin 28°/\sin 17° \simeq SD/DF$, *i.e.*, the focusing condition is satisfied for a beam passing through the centre of the window. The film strip is wrapped in black paper and pressed against the window by two spring-loaded rollers. Ten exposures can be made on one 30-cm strip of film by moving the film laterally after each exposure. A β-filter, G, is placed at this point so that it can also function as an attenuator of fluorescent radiation from the specimen. A shield, H, prevents diffracted rays from the edge of the slit, from falling on the film. The specimen holder is held against the face of the support by a ball and spring (a conventional door fitting) and is fixed in position by vertical and horizontal stops.

Specimen preparation is carried out simply by pressing the powder into the hollow drilled in the holder and by smoothing the powder surface flush with the face of the holder. With titanium dioxide pigments the powders are packed dry without diluent or binder, but when pastes or non-cohering powders are used, a strip of cellophane adhesive tape conveniently keeps the specimen in the holder. If the specimen cannot be sufficiently finely ground to give uniform diffraction lines in the stationary state, the specimen holder would have to be constructed so that it could rotate about a horizontal axis, perpendicular to the specimen face and passing through the centre of the powder.

Fig. 2, *d*, *e* and *f* show that the photographs taken with this camera are as satisfactory for visual comparison or photometric measurement as full Debye-Scherrer patterns. The great reduction in exposure times is illustrated by those for the photographs in Fig. 2. The full Debye-Scherrer patterns were obtained with a Unicam 9-cm camera after exposure for 30 minutes. The small photographs from the powder layer camera were obtained after exposure for 3 minutes. In practice there is a further considerable economy in time by virtue of the multiple exposures carried by the same strip of film. Under certain circumstances the time spent in processing film can be reduced tenfold.

The performance of the instrument can be assessed from the fact that from photographs of synthetic mixtures of known composition (in steps of 5 per cent.) as standards, four independent observers each estimated unknown mixtures to within 2 per cent. of the actual composition, and their results were within 2 per cent. of each other.

Apart from the analysis of rutile - anatase pigments for which it was specifically designed, the camera has been found very useful for rapid qualitative examinations, particularly of nearly pure minerals. In such circumstances a rapid preliminary examination can be a valuable guide to a subsequent full chemical analysis. In addition, it has been possible to examine pigmented vitreous enamel panels and applied paint films.

This type of camera could probably be applied with equal success to the analysis of any binary crystalline mixture of uniform quality, the necessary modification being made to give the preferred angular range.

THE GEIGER-COUNTER X-RAY SPECTROMETER METHOD—

In recent years the Geiger-tube with associated scaling circuits has been increasingly used in X-ray work for the direct measurement of intensity; the advantageous elimination of photographic processing, coupled with an increased range of linearity is likely to lead to a more widespread use of instruments that rely on this method of measurement. The instrument used for the work described here was the Norelco Geiger-counter X-ray spectrometer manufactured by North American Philips Co., and the method developed proved so simple and reliable that it has now become incorporated in factory process control.

The instrument is described by Bleeksma[3] and a critical examination of its performance has been carried out by Lonsdale.[4] The flat powder layer method is again utilised and consequently the camera has similar advantages to those of the camera described above. It consists essentially of a source of X-rays, a slit system defining a divergent beam, a specimen holder, a second slit system mounted in front of an end-window Geiger-tube which is carried on a graduated arc centred on the specimen. The specimen rotates at half of the angular

velocity of the Geiger-tube so that the para-focusing condition is satisfied for all settings round the arc. The output pulses from the Geiger-tube are passed through scaling circuits and are made to actuate an electro-mechanical counter from which the intensities can be read directly.

A strictly conventional application of the instrument would necessitate the scanning of two X-ray diffraction lines, one from the anatase pattern and one from the rutile pattern, and a subsequent comparison of the ratio of the integrated intensities or the peak heights of the two lines. In practice, however, the regulation of the instrument even with a voltage stabiliser in the mains supply, was not sufficient to give the desired reproducibility; also such a procedure would be too lengthy. It was found advantageous to open the receiving slit so that it subtended an angle of 2° at the centre of rotation of the specimen, an angle wide enough to take the whole of the diffracted beam simultaneously. This had the effect of increasing the accuracy of count of the weaker intensities and also allowed an alternate count to be made on either line in reasonably short periods. Each of the rutile 110 and anatase 101 lines was counted for 1 minute alternatively, three times over, and the total time of estimation was about 7 minutes. The alternation helped to eliminate longer period variations in the intensity of the primary X-ray beam. It would be more desirable to use two Geiger-tubes and to count the two lines simultaneously as was done by Richmond and

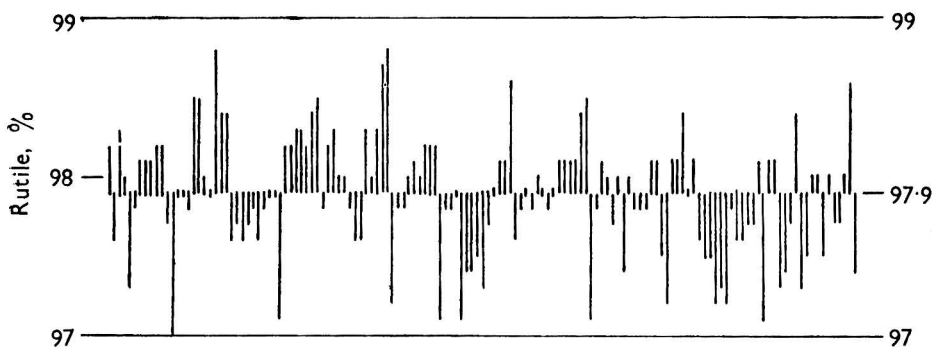

Fig. 5. Reproducibility of Geiger-counter X-ray spectrometer method

Watson.[5] They, however, used narrow slits and effectively measured peak heights; the present arrangement with wide slits effectively measures integrated intensities. The advantage of the former arrangement is in the tolerance of impurities allowed, provided the foreign material does not diffract at a Bragg angle identical with that of the chosen rutile or anatase line. The latter arrangement allows for variations in diffraction line contours brought about by lattice strain or small crystallite size, and bearing in mind pigmentary titanium dioxide which has an average crystallite size of about 0·2 μ, it can be seen that wide slits have their own advantageous field of application.

As in the previous method, standard rutile - anatase mixtures were prepared and used to provide a calibration curve. The calibration was checked daily at two points, and Fig. 5 illustrates the reproducibility of the method as the daily estimate of a 98·0 per cent. rutile standard over 139 consecutive days. The mean value of the 139 estimates was 97·9 per cent. of rutile with a standard deviation of 0·4 per cent.; this deviation arose from all irregularities inherent in the method, such as sampling, operational faults and fluctuations of count. It was found to be essential to make the daily check on two standards, not only to verify that the instrument had developed no fault but also because of long period variations which necessitated recalibration. In general, the instrument was recalibrated when the average of the previous six daily estimates differed from the standard value by more than $\pm$0·5 per cent. Errors arising from non-uniformity in specimen preparation were found to be quite small, as the figures in Table I show. The volume of the specimen holder was 0·34 ml, and by deliberately packing this volume under different pressures, specimen densities of between 1·03 g per ml and 1·50 g per ml were obtained.

It is instructive to consider these results in relation to the fluctuations inherent in a relatively short period measurement with a Geiger-counter tube. When a beam of X-rays

of constant intensity is being measured by a Geiger-counter, the X-ray quanta arrive in a random fashion, and repetitive counts made for a given period of time will in general, be different. It can be shown[6] that if $\bar{n}$ is the true mean value of the count in the given time,

TABLE I

EFFECT OF SPECIMEN DENSITY

Standard value 97·8 per cent. rutile		3 minute count			
Weight of TiO_2, g	Specimen density, g per ml	Rutile 110, R	Anatase 101, A	R/A	Rutile, %
0·349	1·03	1885 × 16	563 × 16	3·35	97·5
0·401	1·18	1992 × 16	595 × 16	3·35	97·5
0·422	1·24	2024 × 16	599 × 16	3·37	97·6
0·510	1·50	1852 × 16	569 × 16	3·26	96·8

as determined by a very long period measurement, and if a large number of counts over the given time be made, values of n will be obtained distributed about the mean value $\bar{n}$ according to Poisson's law,

$$P_n = \frac{(\bar{n})^n e^{-\bar{n}}}{n!}$$

where P_n is the probability or relative frequency of a count of n. The standard deviation of this distribution is $\sigma = (\bar{n})^{\frac{1}{2}}$ and, expressed as a percentage of the true mean, is given by

$$\frac{100(\bar{n})^{\frac{1}{2}}}{\bar{n}} = \frac{100}{(\bar{n})^{\frac{1}{2}}}.$$

If n is not too small this becomes approximately $100/(n)^{\frac{1}{2}}$ and the accuracy increases with the time of the count. With a pigment that is substantially anatase the count in 3 minutes on the rutile 110 line is approximately 9000 and on the anatase 101 line approximately 55,000. The standard deviations in repeated 3-minute counts are therefore 1·06 per cent. and 0·43 per cent. of the respective means. This accuracy was considered adequate for our purposes and, as shown below, leads to a standard deviation of less than 1 per cent. in the estimations of the percentage of rutile in a pigment.

The form of the calibration curve for the percentage of rutile plotted against the ratio N_R/N_A, where N_R is the rutile 110 count and N_A is the anatase 101 count, fits the theoretical curve given by

$$r = \frac{a + x\mathrm{R}}{b + (1 - x)\mathrm{A}}$$

sufficiently well for an estimate of the accuracy of the method to be attempted. In this relationship r is the ratio of the rutile 110 count to the anatase 101 count, a and b are the background contributions to the rutile 110 and anatase 101 intensities (independent of x), R and A are the counts from pure rutile and anatase, respectively, less the background, and x is the fraction of rutile. The relative values of R, A, a and b as estimated from many measurements are—

$$\mathrm{R} \simeq 3{\cdot}0, \quad \mathrm{A} \simeq 4{\cdot}0, \quad a \simeq b \simeq 1{\cdot}0.$$

By differentiation,

$$\frac{\partial x}{\partial r} = \frac{\mathrm{R}b + \mathrm{RA} + a\mathrm{A}}{(\mathrm{R} + \mathrm{A}r)^2}$$

and when the above values are substituted these become

$$r = \frac{(1 + 3x)}{(5 - 4x)}$$

and

$$\frac{\partial x}{\partial r} = \frac{2{\cdot}11}{(1 + 1{\cdot}33r)^2}$$

Now $r = N_R/N_A$ where N_R and N_A are the respective counts on the rutile 110 and anatase 101 lines,

so
$$\frac{dr}{r} = \frac{dN_R}{N_R} - \frac{dN_A}{N_A}$$

and
$$\partial x = \frac{2{\cdot}11r}{(1 + 1{\cdot}33r)}\left(\frac{dN_R}{N_R} - \frac{dN_A}{N_A}\right)$$

where ∂x is the error in x due to the errors dN_R and dN_A.

The standard deviations of N_R and N_A are however $(N_R)^{\frac{1}{2}}$ and $(N_A)^{\frac{1}{2}}$ respectively, therefore the standard deviation of x is given by

$$\Delta x = \frac{2{\cdot}11r}{(1 + 1{\cdot}33r)^2}\left(\frac{1}{N_R} + \frac{1}{N_A}\right)^{\frac{1}{2}}$$

Δx has been computed for values of x from 0 to 1·0 and plotted in Fig. 6. With a pigment containing 98 per cent. of rutile the theoretical standard deviation in a large number of

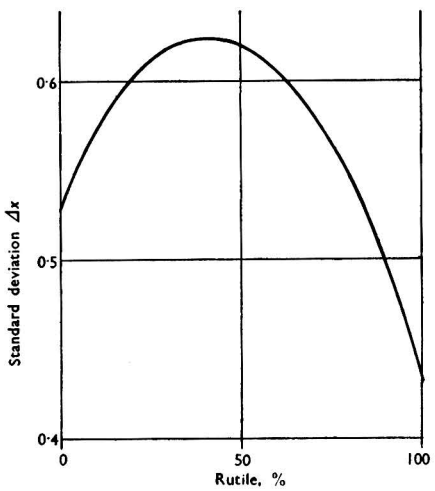

Fig. 6. Standard deviation of determination of 0 to 100 per cent. of rutile

counts is 0·44 per cent., which compares well with the value of 0·4 per cent. found in practice (Fig. 5), and it can be assumed that the errors of estimation are almost entirely due to the random nature of the diffracted X-ray beam, and that errors due to experimental variations in this method are of a different order. By taking longer counts the accuracy can be increased until the experimental variations become significant. The accuracy is dependent on the sensitivity of the Geiger-counter tube and if the latter is moved in its seating or replaced, a new calibration is necessary.

It should be pointed out that the high standard of accuracy attainable by this method is partly the result of the uniform and ideal nature of the titanium dioxide powders used. The pigments consist of crystallites which are remarkably uniform in size; their mean size is about 0·2 μ and standard deviation about 0·05 μ, so no complications owing to secondary extinction arise. When materials are used that contain coarser crystallites $> 1\ \mu$, the estimations may be out by several per cent., and it cannot be over-emphasised that high accuracy is only attainable when dealing with powders of uniform quality.

Finally, some mention should be made of the standard mixtures of rutile and anatase pigments on which the absolute accuracy of the estimations depends. In general, all the titanium dioxide produced in the pigment industry has undergone relatively severe thermal treatment in the form of high temperature calcination at 800° to 1000° C, and consequently

anatase pigments, which are metastable with respect to rutile, usually contain a small percentage of rutile. Likewise, rutile pigments, which are usually manufactured by the high temperature transformation of anatase titanium dioxide, generally contain a residual amount of anatase. Under these circumstances it is necessary to use a method of successive approximations, to make an estimate of the absolute rutile content of the two pigments used to prepare the standards. The two pigments, which are substantially rutile and anatase, are first assumed to be 100 per cent. in their respective crystalline forms. Standard mixtures are made up and accurate counts taken on the rutile 110 and anatase 101 lines. These counts are plotted against the apparent percentage of rutile in the standards. The background under the respective lines is found by interpolation of background counts outside and between the lines. All intensities are expressed as fractions of the rutile 110 background which is given the arbitrary value 1. The two primary pigments both show counts in the position of the minor constituent line greater than the interpolated background count. It is possible to deduce the residual percentages in the primary pigments from the difference between the count on the minor constituent line and the interpolated background count taken in conjunction with the slope of counts against apparent percentage of rutile curves. These curves are linear over the ranges 0 to 10 per cent. of rutile and 90 to 100 per cent. of rutile, and the corrections are applied to make the linear extrapolation of the new curves to 100 per cent. of rutile and 100 per cent. of anatase coincide with the respective background values.

References

1. Rooksby, H. P., *Analyst*, 1945, **70**, 166.
2. Brentano, J. C. M., *Z. Phys.*, 1931, **70**, 74; *Proc. Phys. Soc., Lond.*, 1935, **47**, 932.
3. Bleeksma, J., Kloos, G., and di Giovanni, H. J., *Philips Tech. Rev.*, 1948, **10**, 1.
4. Lonsdale, K., *Acta Crystall.*, 1948, **1**, 12.
5. Richmond, J. T., and Watson, P. E., *J. Oil Col. Chem. Ass.*, 1952, **35**, 162.
6. Bateman, H., *Phil. Mag.*, 1910, **20**, 704.

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A "Difference" Formula for the Morton-Stubbs Correction of Vitamin-A Absorption Curves

By S. J. PROKHOVNIK

The application of the Morton - Stubbs correction geometrically shows that the precision of corrected values depends on the precision of differences between the readings at 328 mμ and the two fixation points. The usual Morton - Stubbs formula can be written in terms of these differences; the use of this modified formula has the advantages that the arithmetic is simpler and quicker and may be carried out with an ordinary slide rule and that the cause of poor duplication is more easily detected and possible experimental errors indicated in this way can be checked.

The Morton - Stubbs correction is usually applied in the form—

$$E \text{ (corrected)} = \frac{K}{K-1}\left(E_1 - \frac{\lambda_3-\lambda_1}{\lambda_3-\lambda_2}E_2 - \frac{\lambda_1-\lambda_2}{\lambda_3-\lambda_2}E_3\right)$$

where E_1 is the measured absorption at λ_1, a wavelength where the vitamin A absorption is maximum, or nearly so; E_2 and E_3 are the measured absorptions at λ_2 and λ_3, on either side of λ_1, and usually taken such that the vitamin A absorption at these points is equal, in which event $K = E_1/E_2 = E_1/E_3$ for pure vitamin A.

Using vitamin A acetate dissolved in *iso*propyl alcohol, we[1] obtained—

$$E \text{ at } 312\ m\mu = E \text{ at } 337\ m\mu, \text{ and } \frac{E \text{ at } 328\ m\mu}{E \text{ at } 312\ m\mu} = \frac{E \text{ at } 328\ m\mu}{E \text{ at } 337\ m\mu} = 1{\cdot}160$$

This reduces the formula to—

E at 328 mμ (corrected) = 7·25 E at 328 mμ − 2·61 E at 312 mμ − 4·64 E at 337 mμ,

which is valid for vitamin A assays with *iso*propyl alcohol as solvent and assumes that the extraneous absorptions at the three points considered lie on a straight line.

THE APPLICATION OF THE CORRECTION GEOMETRICALLY—

Like most workers in this field, we have found that the precision (that is, reproducibility of results) of vitamin A determination suffers considerably when the correction is applied.

In order to determine this loss of precision quantitatively, and the reason for it, the correction was applied geometrically, following the example of Morton and Stubbs[2] in their 1948 paper. The correction can be made rapidly as follows. Consider the three readings—

E at 312 mμ = 0·535
E at 328 mμ = 0·601
E at 337 mμ = 0·550

(*a*) As E at 337 mμ − E at 312 mμ = 0·015, the correction for the slope is obtained by subtracting 0·015 from E at 337 mμ and 16/25 of 0·015 from E at 328 mμ. After making this correction—

$E_{(a)}$ at 312 mμ = 0·535 = 0·535
$E_{(a)}$ at 328 mμ = 0·601 − 0·0096 = 0·5914
$E_{(a)}$ at 337 mμ = 0·550 − 0·015 = 0·535

(*b*) Now as E at 328 mμ/E at 312 mμ = 1·16 for vitamin A in *iso*propyl alcohol, $E_{(a)}$ at 328 mμ − $E_{(a)}$ at 312 mμ = 0·0564 and represents 0·16/1·16 of the vitamin A absorption, and therefore—

$$\text{E at 328 m}\mu\text{ (corrected)} = \frac{1{\cdot}16}{0{\cdot}16} \times 0{\cdot}0564 = 0{\cdot}409,$$

i.e., E at 328 mμ (corrected) = 7·25 ($E_{(a)}$ at 328 mμ − $E_{(a)}$ at 312 mμ).

This shows that the precision of the corrected value depends directly on that of the difference $E_{(a)}$ at 328 mμ − $E_{(a)}$ at 312 mμ. This difference is much smaller than the uncorrected reading. When K = $1\frac{1}{6}$ to $1\frac{1}{7}$, the difference can never be greater than $\frac{1}{7}$ to $\frac{1}{8}$ of the maximum absorption even for pure vitamin A, and for oils having considerable extraneous absorption it will be $\frac{1}{10}$ or even less of the reading for E at 328 mμ. Hence an error in the uncorrected determination becomes at least 7 or 8 times greater in relation to the above difference and it follows that the precision of determinations will suffer by a similar factor when the correction is applied. This conclusion agrees with Gridgeman's more exact analysis[3] by the theory of errors and with the experimental work of Adamson, Elvidge, Gridgeman, Hopkins, Stuckey and Taylor.[4]

It should be noted further that the difference $E_{(a)}$ at 328 mμ − $E_{(a)}$ at 312 mμ is in turn a function of two other differences, *viz.*, E at 328 mμ − E at 312 mμ and the slope difference E at 337 mμ − E at 312 mμ. Hence the geometrical procedure indicates that the corrected value is a function of the differences between the readings rather than of their absolute values. The ultimate form of this relationship is most easily obtained algebraically.

A MODIFIED ARITHMETIC PROCEDURE—

The usual form of the Morton - Stubbs correction—

$$\text{E (corrected)} = \frac{K}{K-1}\left(E_1 - \frac{\lambda_3-\lambda_1}{\lambda_3-\lambda_2}E_2 - \frac{\lambda_1-\lambda_2}{\lambda_3-\lambda_2}E_3\right)$$

can be written in the form—

$$\text{E (corrected)} = \frac{K}{K-1}\left(\frac{\lambda_3-\lambda_1}{\lambda_3-\lambda_2}(E_1-E_2) + \frac{\lambda_1-\lambda_2}{\lambda_3-\lambda_2}(E_1-E_3)\right).$$

When, as above, K = 1·16, λ_1 = 328, λ_2 = 312, λ_3 = 337,

$$\begin{aligned}\text{E (corrected)} &= 7{\cdot}25\,E_1 - 2{\cdot}61\,E_2 - 4{\cdot}64\,E_3\\ &= 2{\cdot}61\,(E_1-E_2) + 4{\cdot}64\,(E_1-E_3),\end{aligned}$$

i.e., E (corrected) = 2·61 ΔE_{12} + 4·64 ΔE_{13}.

Thus E (corrected) is here a simple function of the differences E at 328 mμ — E at 312 mμ and E at 328 mμ — E at 337 mμ, and its precision depends on the reproducibility of these differences. Poor duplication will occur when the respective differences are out of proportion to the weights taken, even though the respective readings may appear to be proportionately correct.

By focusing attention directly on the differences, the modified formula enables such discrepancies to be detected by inspection. Table I illustrates this. It is clear from the values of $|\Delta E|$ that either the readings are in error (and this can be checked) or the samples are intrinsically different in some respect. This type of inspection quickly indicates the cause of poor duplication and can sometimes lead to the elimination of errors.

TABLE I

DETECTION OF DISCREPANCIES BY INSPECTION OF DIFFERENCES

	Sample 1		Sample 2	
Weight of oil (diluted to 100 ml) ..	0·1358 g		0·1320 g	
λ, mμ	E	$\lvert\Delta E\rvert$	E	$\lvert\Delta E\rvert$
312	0·334		0·322	
		0·048		0·050
328	0·382		0·372	
		0·035		0·042
337	0·347		0·330	
E at 328 mμ (corrected)	0·292		0·325	

A further advantage of the modified formula is that the arithmetic involved is simpler and quicker than with the original form of the correction. The original correction requires three products correct to four significant figures. With the "difference" formula, only two products correct to three significant figures are required, and hence an ordinary slide rule can be used.

For instance, with the same three readings as before, *viz.*—

λ, mμ	E	$\lvert\Delta E\rvert$
312	0·535	
		0·066
328	0·601	
		0·051
337	0·550	

E at 328 mμ (corrected) = 2·61 × 0·066 + 4·64 × 0·051
= 0·172 + 0·237
= 0·409

It must be emphasised that the modified formula does not, of course, improve the precision of corrected determinations, although it may improve duplication by enabling experimental errors to be quickly detected and possibly eradicated. The precision suffers by a factor depending on the ratio between the uncorrected reading and a weighted average of the two differences. This leads to similar conclusions to those obtained by the geometric procedure.

Grateful acknowledgment is made to the directors of Nicholas Proprietary Limited for permission to publish this work.

REFERENCES

1. Turnbull, N. H., and Prokhovnik, S. J., *Roy. Aust. Chem. Inst. J. & Proc.*, 1949, **16**, 217.
2. Morton, R. A., and Stubbs, A. L., *Biochem. J.*, 1948, **42**, 195.
3. Gridgeman, N. T., *Analyst*, 1951, **76**, 449.
4. Adamson, D. C. M., Elvidge, W. F., Gridgeman, N. T., Hopkins, E. H., Stuckey, R. E., and Taylor, R. J., *Ibid.*, 1951, **76**, 445.

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The Determination of Sub-Microgram Quantities of Arsenic by Radioactivation

Part II*

The Determination of Arsenic in Sea-Water

BY A. A. SMALES AND B. D. PATE

The application of radioactivation to the determination of arsenic in small samples of sea-water, taken from points off the south-west coast of Cornwall, is described. An average value of 2·6 μg of arsenic per litre, with a range of 1·6 to 5·0 μg per litre, was found.

INTEREST in the estimation of the arsenic content of sea-water has not in the past been very widespread. When its determination was undertaken, it was usually supplementary to the determination of arsenic in fish and other edible marine organisms, on which interest was focused following an outbreak of arsenical poisoning at the beginning of this century. The arsenic content of these edible materials was relatively high, being of the order of tens of parts per million, and so comparatively crude techniques could be used for its determination. When applied to sea-water these often gave results of questionable accuracy, and successive reports were often contradictory.

The earliest determinations of the arsenic content of sea-water were made by Gautier[1,2] in 1903, as part of a programme of arsenic determinations in biological material. Water from the coast of Brittany was found to contain 10 micrograms per litre, while water from a deep-sea station near the Azores showed some variation with depth. At a depth of 10 metres, 25 μg per litre was found, at 1335 metres 10 μg per litre, and at 5943 metres 80 μg per litre. Gautier attributed the high figure at depth to volcanic influences and that at the surface to disturbances caused by marine organisms and evaporation; many investigators who subsequently worked on this problem assumed that this increase of arsenic content with depth was real.

The method of determination used in Gautier's investigation was the Marsh - Berzelius, as this was the most sensitive procedure for determining arsenic available at the time. It was also used by later workers.

Orton,[3] investigating the high mortality of oysters in the Thames estuary in 1921 and 1922, examined sea-water from the south shore, West Mersea, and from the Ham Grounds off Whitstable, and in both localities found up to 30 μg of As_2O_3 per litre. Water from the English Channel (near the Wolf light off the Lizard) was found to contain 25 μg of As_2O_3 per litre. An investigation by Chapman,[4] however, gave higher results; in samples of sea-water taken near the Nore lightship, he found arsenic contents (expressed as As_2O_3) ranging from 140 to 1000 μg per litre, with a mean for sixteen determinations of 330 μg per litre. These results formed a contrast to figures quoted by Chapman from earlier work by Frankland (1870). Samples of river water have been found to contain only 40 micrograms per litre.

The application of improved methods of determination to sea-water analysis was attempted by several workers in order to get better results and to remove the laborious, extended evaporations necessary in some older procedures. Atkins and Wilson[5,6] noted that arsenate present in sea-water would give a molybdenum-blue reaction and so interfere with phosphate determinations by this method. They did not, however, attempt the determinations of arsenic by such a procedure, and this was left to later workers.

Application of the Gutzeit technique was first successfully reported by Rakestraw and Lutz[7] in 1933. These workers concentrated 100 ml of sea-water to a small volume before the determination, evolved the arsenic as arsine in the Association of Official Agricultural Chemists' Gutzeit apparatus[8] and determined the quantity of arsenic present from the length of stain produced on a strip of mercuric bromide paper. This procedure they applied to

* For particulars of Part I of this series (not in *The Analyst*), see reference list, p. 195.

samples of sea-water drawn from several points off the eastern seaboard of America and from two deep-sea stations in the North Atlantic.

The samples from shallow stations varied in a random manner between extreme values of 6 and 28 μg per litre, with a mean of 15 μg per litre, whilst the samples from the deep-sea stations drawn from depths of up to 4000 metres showed similar values, and failed to corroborate Gautier's findings. They form an interesting contrast with the phosphate content, which increases ten-fold for a similar increase in depth.

A further investigation was conducted in 1940 by Fedossov,[9] who also used the Gutzeit method and confined his attentions to the Black and Caspian Seas and to the Sea of Azov. Water from the last he found to contain 1 to 15 μg per litre (average 5·7 μg per litre), whilst the Black Sea and Kerch Straits gave values of 5 to 15 μg per litre (averages 10 and 6 μg per litre) and the Caspian Sea water contained 3 to 12 μg per litre (average 6 μg per litre). As to variation with depth, Fedossov found that in the Caspian Sea, which allowed samples to be taken at depths of up to 875 metres, any slight change in arsenic content resulted in a smaller concentration at depth than at the surface, contrary to Gautier's findings. Insufficient evidence was forthcoming, however, on which to base definite conclusions.

The most recent determination, apart from that described in this paper, was by Gorgy, Rakestraw and Fox[10] in 1948. They used the Gutzeit procedure as modified by Jacobs and Nagler,[11] in which arsine, evolved in the normal manner, is absorbed in sodium hypobromite solution and subsequently estimated photometrically by the molybdenum-blue procedure. To ensure that all the arsenic present in the sample was converted to a form detectable by this procedure, an oxidation with sulphuric and nitric acids and hydrogen peroxide was used; comparison of results obtained with and without this pre-treatment confirmed earlier reports that about 60 per cent. of the arsenic was present in the sea-water as inorganic arsenite.

This procedure was applied to samples drawn from the Pacific Ocean and the figures for total arsenic contents ranged between 15 and 50 micrograms per litre, results considerably higher than those reported in the two previous publications. Examination of samples drawn from increasing depths showed no significant increases in arsenic content, which confirmed earlier observations.

Principles of the Determination of Arsenic in Sea-water by Activation Analysis

The method and procedure as outlined previously[12] were applied in this determination, with a few modifications as described below. The sea-water samples were irradiated, in the liquid state, in a specially-developed apparatus that has been described in detail elsewhere.[13]

The irradiation of sea-water—

Sea-water as a medium for the application of activation analysis is exceptional in two ways. First, the presence of a wide variety of elements in the material—over 40 are listed by Sverdrup[14]—provides a very stringent test of the separation methods used for extracting the element under examination from the mixture, in which it is only a minor constituent. The separation method outlined below proved entirely adequate, as no example of contamination of the ^{76}As activity with other activity was observed in any of the samples of arsenic separated.

Secondly, the presence of quantities of elements such as sodium and chlorine in sea-water leads to a considerable amount of activity being generated in these samples. This factor influenced the decay times and details of separation procedure finally selected, as it is of considerable importance to reduce the radiation hazard associated with all operations to as small a value as practicable.

The activity developed from four of the major constituents in 10 ml of sea-water after irradiation at a flux of 10^{12} neutrons per sq. cm per second for the normal period of 70 hours can be calculated, *e.g.*, as shown in Part I of this series,[12] and this has been done in Table I. Here the abundances given by Sverdrup[14] have been used; other elements present in sea-water but not listed in Table I produce lesser activities.

Hence it can be seen that from a 10-ml sample a total of approximately 2×10^9 disintegrations per second, *i.e.*, 60 millicuries, of activity is to be expected. As this is mainly due to ^{24}Na, which emits a powerful 2·76 MeV γ-ray, the radiation hazard associated with handling a sample immediately after unloading is impracticably large, even apart from the

doses incurred from the aluminium and silica containers during the unloading operations. A delay before handling the samples is therefore essential, and for sea-water irradiations 24 hours is suitable.

At the end of this time, the initial ^{24}Na activity, $1{\cdot}65 \times 10^9$ disintegrations per second, will have been reduced by decay through approximately two half-lives to about 4×10^8 disintegrations per second, *i.e.*, about 11 millicuries, which may be handled more safely with normal shielding.

TABLE I

ACTIVATION OF SEA-WATER WITH 70-HOUR IRRADIATION

Element	Abundance in sea-water, mg per litre	Weight present in 10 ml, g	Target isotope considered	Isotope produced	Cross-section for reaction $\times 10^{-24}$ sq. cm	Half-life of product isotope	Activity developed, disintegrations per second
Chlorine	18,980	0·19	^{37}Cl	^{38}Cl	0·56	37 minutes	$4{\cdot}2 \times 10^8$
Chlorine	18,980	0·19	^{35}Cl	^{35}S	0·34	87·1 days	$1{\cdot}63 \times 10^7$
Sodium	10,561	0·10	^{23}Na	^{24}Na	0·63	14·8 hours	$1{\cdot}65 \times 10^9$
Magnesium	1272	0·013	^{26}Mg	^{27}Mg	0·048	10·2 minutes	$2{\cdot}7 \times 10^6$
Potassium	380	0·004	^{41}K	^{42}K	1·0	12·44 hours	$3{\cdot}7 \times 10^6$

Self shielding, *i.e.*, decrease in neutron flux within the sample owing to absorption of neutrons passing through, can be calculated to be less than 2 per cent. for sea-water, and has therefore been neglected.

SENSITIVITY FOR ARSENIC—

An irradiation of a sample of arsenic for 70 hours will produce about 84 per cent. of the saturation activity, that is, $1{\cdot}68 \times 10^{12}$ disintegrations per minute per gram.

The delay period of 24 hours, combined with the time required for separating and measuring the activity—say a total of about 27 hours—will reduce this by decay through one half-life to $8{\cdot}4 \times 10^{11}$ disintegrations per minute per gram.

The sensitivity with which arsenic can be determined can be calculated as follows—

With the Geiger-counter normally used in this work, an efficiency of 6 per cent. is readily available, *i.e.*—

$$1 \text{ g of arsenic} \equiv 5 \times 10^{10} \text{ counts per minute, } or$$

$$1 \text{ count per minute} \equiv 2 \times 10^{-11} \text{ g of arsenic.}$$

If the working limit of determination is taken as about 10 counts per minute from the arsenic above a counter background of 10 counts per minute, then this represents 2×10^{-10} g of arsenic.

The smallest quantity of arsenic determined in a 10-ml sample of liquid was $1{\cdot}7 \times 10^{-9}$ g in conductivity water (see below). The value of this result as an indication of the normal arsenic content of conductivity water is doubtful, but it serves to illustrate that the theoretically predicted sensitivities can be approached in practice, as well as giving a maximum value for any contamination. The amount of ^{76}As activity found in this experiment permitted absorption and decay curves to be plotted and an estimate of the half-life and maximum β-energy of the emission to be made. The accuracy was adequate to confirm the identity of the ^{76}As isotope.

EXPERIMENTAL PROCEDURE

Determination of the arsenic in sea-water by the activation method involved (*i*) activation of the material in the liquid form by slow neutron irradiation, (*ii*) chemical separation of the arsenic activity with a carrier and (*iii*) estimation and characterisation of the activity radiometrically.

IRRADIATION—

The sea-water samples were irradiated in the liquid form. Previous workers had been obliged, owing to the relative insensitivity of their determination methods, to evaporate a large bulk of sea-water to get a determinable quantity of arsenic. The sensitivity of the

activation method, however, is such that an analysis of as little as 10 ml of sea-water directly, *i.e.*, without any pre-treatment, is readily possible. Hence the possible contamination of the samples with arsenic during preliminary treatment is avoided.

The only time when contamination of the sample must be avoided with this procedure is before or during the loading of the irradiation tubes, as once the samples have been irradiated, any further small contamination will not affect the analytical results. Extensive precautions were therefore adopted when dealing with the sea-water samples before irradiation. The sea-water was transported and stored in clean polythene bottles until required for analysis, and the loading procedures were carried out in a separate laboratory from that in which the loading of the standard solution and the subsequent chemical separation were performed. Further precautions taken during the loading of samples included the use of clean protective clothing, surgical gloves and so on, and the success of these techniques may be judged from the reproducibility of the results for the many sea-water samples examined and the results of the conductivity water analyses, given in detail later.

The apparatus and techniques used in the irradiation of liquid samples have been adequately described elsewhere,[13] but the precise technique used for loading the irradiation tubes was as follows.

Loading the irradiation tubes—Clean the silica irradiation tubes thoroughly, finally boiling them three times with distilled or conductivity water. Then heat them to redness to remove the purple tint produced in silica by previous neutron irradiation and to remove the last traces of moisture. Allow them to cool, mark each with grease-pencil for subsequent identification and weigh them.

Thoroughly clean and dry an adequate number of beakers or similar vessels. To load an individual sample, transfer 15 to 20 ml of sea-water to a beaker and place an inverted silica irradiation tube in the beaker with its neck resting on the bottom under the surface of the liquid. Carefully heat the bulb of the tube with a Bunsen burner until no further air bubbles are expelled. Allow to cool, when the sample is drawn in and fills the tube to about nine-tenths of its total volume.

Determine the quantity of sample taken by weighing. About 10 ml of sample are taken in each tube, and the irradiation apparatus accommodates eight tubes. This allows the simultaneous irradiation of three duplicate samples of sea-water and two samples of the standard arsenic comparison solution, prepared as described below.

Load the irradiation apparatus in the pile and subject the samples to a week-end (70-hour) irradiation. Set aside for 6 hours after withdrawal from the pile, then unload the apparatus and set aside the silica tubes and contents for a further 18 hours. Then apply the separation technique described below.

The standard arsenic solution, used for comparison throughout the work involving liquid irradiation, contained 1 microgram of arsenic per millilitre of solution and was prepared by dissolving A.R. quality arsenious oxide in a small quantity of sodium hydroxide solution and then diluting the resulting solution appropriately.

CHEMICAL SEPARATION—

The separation procedure used was essentially the same as that outlined previously[12] and applied to the separation of arsenic from germanium, the distillation procedure being modified so that there was only one distillation under oxidising conditions. This was used as a preliminary oxidation to destroy any organic arsenic compounds present in the sea-water. The details of the separation procedure are as follows.

Separation procedure—Assemble the distillation apparatus, which is a round-bottomed glass flask fitted with splash head and connected by a swan-neck joint to a condenser. Then transfer the activated sea-water sample to the distillation flask through a dropping funnel by warming the irradiation tube in an inverted position, when the contents are rapidly expelled. Then add in the order given 1 ml of 10 per cent. sodium hydroxide solution, 5·0 ml of a carrier solution of sodium arsenite containing 10 mg of arsenic per millilitre and a few drops of 100-volume hydrogen peroxide; wash the dropping funnel with a few millilitres of water. Reduce the volume of the resulting mixture in the distillation flask by boiling until about 2 ml remain. Allow this solution to cool. Mix 10 ml of concentrated hydrochloric acid and 5 ml of 100-volume hydrogen peroxide in the dropping funnel and set it aside until it is evolving chlorine freely. Then add it to the distillation mixture and wash the funnel with a few millilitres of water. Distil the resulting solution until 2 ml remain and then allow it to cool.

Remove the receiver, replace it by another containing 10 ml of cold water and arrange the delivery tube of the condenser so that it dips just below the surface of the water. Add 10 ml of 40 per cent. hydrobromic acid to the flask through the dropping funnel, wash through with distilled water and continue the distillation. Distil to a small volume and repeat the addition of hydrobromic acid and distillation twice more. Add to the combined distillates from this step 1 to 2 g of solid ammonium hypophosphite and heat to 90° to 95° C for 30 minutes, during which time the arsenic precipitate coagulates. Collect the precipitate by centrifugation, wash it thoroughly with water and finally transfer it to a tared aluminium counting tray of 2 to 3 ml capacity; dry the precipitate under an infra-red lamp, allow it to cool and weigh it to establish the chemical yield. Measure the radioactivity of the samples and standard under identical conditions and correct each count for coincidence losses, for any decay of significance between the times of counting samples and standard, for chemical yield and, if necessary, for self absorption. By keeping the weight of standard and sample as nearly as possible the same, the last correction can be made negligible. Then—

$$\text{Mass of arsenic in sample} = \text{Mass of arsenic in standard} \times \frac{\text{Corrected count of sample}}{\text{Corrected count of standard}}$$

Finally check the decay and β-energy of samples and standard to ensure that ^{76}As is being counted, *i.e.*, that the half-life is 26·8 hours and the maximum β-energy is 3·1 MeV.

Experimental results

Examination of procedure—

The examination of the chemical separation procedure by means of tracer techniques with ^{76}As has already been described.[12] The over-all yield of about 90 per cent. found there was confirmed by the chemical yields determined by means of the carrier recoveries in the sea-water separation.

When this procedure was applied to sea-water, certain further aspects required examination.

Organically combined arsenic—Previous work[10] indicated that a proportion of the arsenic in sea-water was present in organic combination. This might disturb the analysis by the present method in two ways.

(*a*) If extremely resistant to oxidation, organic arsenic compounds might suffer only partial mineralisation by chlorine attack, and so exchange only incompletely with the inorganic carrier, which would lead to low results. This possibility was examined by comparison of the analytical results from four samples from the same specimen of sea-water. Two were analysed by means of the procedure outlined above, making use of the normal chlorine ($HCl + H_2O_2$) oxidation, and two by a similar procedure that embodied a preliminary oxidation with a nitric - sulphuric acid mixture and were then finished as usual. The results are shown in Table II. The increases observed with mixed-acid mineralisation are comparable with the experimental deviation observed between identical duplicates and are probably without significance.

Table II

Analysis of sea-water by different oxidation techniques

Oxidation agent	Sample	Sample weight, g	Arsenic found, g	Arsenic content, μg per litre
Chlorine	1	4·3200	$1·5 \times 10^{-8}$	3·5
Chlorine	2	8·0545	$3·2 \times 10^{-8}$	4·0
Nitric - sulphuric acid ..	1	9·5434	$4·1 \times 10^{-8}$	4·3
Nitric - sulphuric acid ..	2	12·3227	$6·1 \times 10^{-8}$	4·9

(*b*) If the organic arsenic compounds in sea-water were sufficiently volatile, some proportion might escape oxidation by volatilisation from the oxidation mixture and so would appear in the distillate obtained under oxidising conditions, which is rejected; this again would lead to low results. To test this possibility, an activated sample was distilled in the normal way, except that the distillate under oxidising conditions and the flask residue were separately reserved in addition to the distillate under reducing conditions. After the addition of 50 mg of arsenic carrier to each of the former two fractions, all three were adjusted to contain about 50 per cent. of hydrochloric acid and a hypophosphite precipitation was performed on them in the usual way. The arsenic precipitates were separated, mounted and

examined radiometrically. That from the oxidative distillate showed no β-activity, whilst that from the distillate under reducing conditions showed only the normal ^{76}As activity. The precipitate from the distillation residue showed no activity from ^{76}As, but did show a little 1·4 MeV β-radiation and γ-radiation, presumably from ^{24}Na, present in activated sea-water, carried down on the precipitate.

These results serve to confirm that no significant quantity of arsenic escapes separation in the manner suggested earlier.

Recovery of arsenic—The recovery of arsenic added to sea-water in known amounts was next examined. A sea-water specimen, the arsenic content of which was already known, was treated with various amounts of arsenic, added as known volumes of standard arsenic solutions. The volumes used were such that the composition of the sea-water was not disturbed (by dilution) by more than 5 per cent. in any one solution, and the results of analysing the mixtures are shown in Table III. Evidently the presence of the other constituents of sea-water does not affect the recovery of arsenic to any apprèciable extent.

THE ANALYSIS OF SEA-WATER SAMPLES—

The sea-water specimens examined by the method described were all drawn from an area south-west of Land's End in Cornwall. This location was not so far west as to be off

TABLE III

RECOVERY OF ADDED ARSENIC FROM A SEA-WATER MEDIUM

Arsenic added, μg per litre	Sample weight, g	Arsenic found, g	Arsenic content, μg per litre	Added arsenic recovered, μg per litre
Nil	8·0545	$3·2 \times 10^{-8}$	4·0 (normal value)	—
5	11·9973	$9·2 \times 10^{-8}$	7·7	3·7
10	11·1014	$1·7 \times 10^{-7}$	15·3	11·3
20	10·4234	$2·75 \times 10^{-7}$	26·3	22·3
50	11·5685	$6·8 \times 10^{-7}$	59	55
100	6·3437	$6·8 \times 10^{-7}$	107	103
500	9·9151	$5·15 \times 10^{-6}$	520	516

the "continental shelf," but was sufficiently far removed from the English Channel proper to ensure that the specimens were representative of Atlantic Ocean water. They were all collected between April and July, 1950, mostly in July, and great care was exercised during collection (*a*) to avoid areas and depths where the water was known to be unduly contaminated with detritus and (*b*) to avoid contamination of the specimens with extraneous matter prior to their sealing in the polythene storage containers used.

For the analysis, duplicate 10-ml samples from each specimen were used. The results shown in Table IV are arranged in order of depth at which the specimens were collected and are discussed in detail later.

In an attempt to detect any contamination that might occur, *e.g.*, during loading, samples approximating to conductivity water, prepared by means of an ion exchange column, were analysed by the same procedure as that described earlier for the sea-water analysis. Two typical results of the activation analysis of this material are shown in Table V, and it can be seen that the arsenic content was approximately one-tenth of that of the sea-water samples. There is no reason to believe that this amount of arsenic was not in fact present in the conductivity water, but at least the results show that contamination was not serious.

COMPARISON WITH THE EXAMINATION OF SEA-WATER BY THE METHOD OF GORGY *et al.*—

In view of the deviation of the above results from those recorded by Gorgy, Rakestraw and Fox[10] in 1948, it was decided to apply their procedure to one of the sea-water samples examined by the activation method. For the comparison, 500 ml of sea-water were evaporated to a small bulk in carefully cleaned "Vitreosil" silica beakers under an infra-red heater and the residue was examined by the Jacobs and Nagler modification of the Gutzeit test, as described by Gorgy *et al.* The results are shown in the first part of Table VI.

It was decided further to check these results by using a different method of concentrating the arsenic, namely co-precipitation with magnesium ammonium phosphate, and by starting with a larger quantity of sea-water.

Comparison procedure—Accurately measure 1 litre of sea-water, add to it sufficient aqueous bromine to impart an orange tint to the solution, and set it aside. Add 25 ml of a combined magnesium and phosphate solution (containing 10 per cent. of magnesium chloride, 5 per cent. of sodium dihydrogen phosphate and 10 per cent. of ammonium chloride) and add diluted ammonium hydroxide until the first permanent precipitate is formed. Set aside

TABLE IV

THE ANALYSIS OF SEA-WATER BY ACTIVATION ANALYSIS

Sample	Location		Depth, metres	Sample weight, g	Arsenic found, g	Arsenic content, μg per litre
	N	W				
44	50° 02′	4° 22′	Surface	11·12	$3·9 \times 10^{-8}$	3·5
44	50° 02′	4° 22′	Surface	10·58	$3·0 \times 10^{-8}$	2·8
319	50° 02′	4° 22′	10	7·83	$1·7 \times 10^{-8}$	2·2
319	50° 02′	4° 22′	10	7·90	$1·6 \times 10^{-8}$	2·0
325	48° 31′	5° 56′	10	8·33	$1·8 \times 10^{-8}$	2·2
17	50° 33′	8° 51′	20	10·18	$2·9 \times 10^{-8}$	2·9
17	50° 33′	8° 51′	20	10·38	$3·6 \times 10^{-8}$	3·4
24	48° 38′	6° 20′	40	9·90	$2·2 \times 10^{-8}$	2·2
24	48° 38′	6° 20′	40	8·74	$2·2 \times 10^{-8}$	2·5
18	48° 19′	7° 26′	40	4·32	$1·5 \times 10^{-8}$	3·5
18	48° 19′	7° 26′	40	8·05	$3·2 \times 10^{-8}$	4·0
317	50° 02′	4° 22′	50	8·12	$2·4 \times 10^{-8}$	3·0
317	50° 02′	4° 22′	50	9·75	$1·8 \times 10^{-8}$	1·8
352	50° 41′	7° 00′	75	10·21	$5·1 \times 10^{-8}$	5·0
352	50° 41′	7° 00′	75	8·69	$2·0 \times 10^{-8}$	2·3
323	48° 31′	5° 56′	90	12·47	$2·0 \times 10^{-8}$	1·6
323	48° 31′	5° 56′	90	8·59	$1·5 \times 10^{-8}$	1·8
346	49° 49′	7° 43′	100	6·11	$1·7 \times 10^{-8}$	2·8
346	49° 49′	7° 43′	100	8·19	$1·8 \times 10^{-8}$	2·2

for a short time and then add an excess of ammonium hydroxide, sp.gr. 0·880. Filter and wash the precipitate, and then dissolve it in 10 per cent. sulphuric acid and digest it with sodium bisulphite for 60 minutes, as described by Gorgy *et al.* Then boil to remove sulphur dioxide, adjust the acidity to that required for the evolution, and finish the determination by the Jacobs and Nagler method.

TABLE V

ANALYSIS OF CONDUCTIVITY WATER

Sample	Sample weight, g	Arsenic found, g	Arsenic content, μg per litre
1	11·3846	$1·7 \times 10^{-9}$	0·15
2	8·6033	$3·0 \times 10^{-9}$	0·35

Before being applied to sea-water, this procedure was applied to a litre of solution containing ^{76}As tracer and arsenic carrier in concentration equivalent to that of sea-water. Results indicated that the co-precipitation gave 96 per cent. recovery and the evolution 95 per cent., with a recovery of 91 per cent. for the whole procedure, which was considered adequate for the present purposes.

The results of applying the procedure to sea-water and to sea-water with a known arsenic addition are shown in the second part of Table VI.

CONCLUSIONS

The results shown in Tables II and III confirm the validity of results obtained by the method of activation analysis as applied to sea-water. The examination of a number of samples of this material (Table IV) gave results ranging from 1·6 to 5·0 micrograms per litre. The results of the examination of conductivity water (Table V) permitted an estimate of the maximum value of the "blank" of the determination, due to contamination of sample before irradiation, and so on, to be made.

The mean of the results shown in Table IV is 2·6 μg per litre; although the more recent of previous workers (except Gorgy *et al.*) found slightly higher mean results than this, the variation in their results covered a range including the present values, and so it may be said that there is some agreement between them.

The results of Gorgy, Rakestraw and Fox in 1948, however, require some comment. Their lowest arsenic content, 15 μg per litre, is six times as high as those obtained by the present method, and their highest, 50 μg per litre, is higher by a factor of about twenty. The reason for these higher figures is not immediately evident. The application of the procedure used by these workers (as far as it can be reproduced from the few details they give), when applied to the sea-water specimens used for the present work, gave the results shown earlier in Table VI and these, together with those obtained from the co-precipitation procedure, confirm the general order of the activation analysis results. Because of the size

TABLE VI

ANALYSIS OF SEA-WATER BY THE PROCEDURE OF GORGY *et al.*

Procedure	Sample	Arsenic found, μg	Arsenic content
Evaporation and Jacobs and Nagler	500 ml of sea-water	2·5	5 μg per litre
	500 ml of sea-water	1·5	3 μg per litre
$MgNH_4PO_4$ co-precipitation + Jacobs and Nagler ..	1 litre of distilled water	5	5 μg per litre (blank)
	1 litre of sea-water	7·5	2·5 μg per litre
	1 litre of sea-water	9	4 μg per litre
	1 litre of sea-water + 20 μg of arsenic	23	18 μg recovered

of the blanks involved, little further significance can be attached to them, but the results of Gorgy *et al.*—ten times those of the activation method—were certainly not obtained.

However, it may well be that the differences of arsenic content reported by the more recent investigations are in fact real, *i.e.*, that the approximate constancy of the relative composition of sea-water, true for a number of elements, does not hold for arsenic.

We are grateful to Dr. L. H. N. Cooper, of the Marine Biological Laboratory, for providing the samples of sea-water and for helpful discussion, and to the Director, A.E.R.E., for permission to publish this work.

REFERENCES

1. Gautier, A., *Compt. Rend.*, 1903, **137**, 232.
2. ——, *Ibid.*, 1903, **137**, 374.
3. Orton, J. H., *Min. of Agric. and Fish., Fishery Invest. Series* 2, Vol. 6, Nos. 3 and 4.
4. Chapman, A. C., *Analyst*, 1926, **51**, 548.
5. Atkins, W. R. G., and Wilson, E. G., *Biochem. J.*, 1926, **20**, 1223.
6. ——, ——, *J. Mar. Biol. Ass.*, 1927, **14**, 609.
7. Rakestraw, N. W., and Lutz, F. B., *Biol. Bull.*, 1933, **65**, 397.
8. Association of Official Agricultural Chemists, "Methods of Analysis," Second Edition, 1925, p. 171.
9. Fedossov, M. V., *Compt. Rend. Acad. Sci., U.R.S.S.*, 1940, **27**, 236.
10. Gorgy, S., Rakestraw, N. W., and Fox, D. L., *J. Mar. Res.*, 1948, **7**, 22.
11. Jacobs, M., and Nagler, J., *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 442.
12. Smales, A. A., and Pate, B. D., *Anal. Chem.*, in the press.
13. Pate, B. D., "An Apparatus for the Pile Irradiation of Liquids," Atomic Energy Research Establishment Report C/R 643, H.M. Stationery Office, 1951.
14. Sverdrup, H. U., Johnson, M. W., and Fleming, R. H., "The Oceans," Prentice-Hall Inc., New York, 1946.

NOTE—Reference 12 is to part I of this series.

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The Determination of Sub-Microgram Quantities of Arsenic by Radioactivation

Part III

The Determination of Arsenic in Biological Material

BY A. A. SMALES AND B. D. PATE

The determination of minute amounts of arsenic in "normal" biological material, such as human hair, nails, urine and blood and the internal organs of a mouse, by the radioactivation method is described and results are tabulated. Amounts of arsenic as small as 10^{-10} g can be determined so that very small samples suffice. For example, the "normal" level of arsenic in human blood or urine can be readily determined in only one drop of sample.

The general advantages of the activation method are summarised.

THERE have been a number of attempts in the past few years to lower the limit of determination of traces of arsenic in biological material. Probably the most successful of the methods are those of Satterlee and Blodgett,[1] How[2] and Kingsley and Schaffert,[3] in each of which a sensitivity of 0·01 micrograms of arsenic has been reached. Although it is hard to prophesy what wonders future chemical manufacturers may achieve in the production of reagent chemicals, it seems safe to say that in the application of analytical methods of this type there is always a finite limit to sensitivity owing to the reagent "blank," *i.e.*, the presence, in the reagents used in the determination, of the actual nuclide being determined.

Whilst it is not suggested that the method described in this paper will ever displace, on a general routine basis, methods for arsenic such as those referred to above, it would undoubtedly be of value to have, even for occasional special use, a method that, theoretically at least, offers the possibility of avoiding a reagent blank as well as having a sensitivity greater by a factor of a hundred than that of the methods of Satterlee, How or Kingsley.

For this reason the radioactivation method previously applied to the determination of arsenic in germanium dioxide[4] has been extended to the analysis of some biological material.

PRINCIPLES OF THE ACTIVATION METHOD

The principles of the method have been outlined in Parts I and II of this series[4,5] and need not be repeated here. It will be sufficient to recall that only major amounts of germanium, selenium or bromine are likely to prevent the attainment of a sensitivity of 10^{-10} g of arsenic when a flux of 10^{12} neutrons per sq. cm per second is used, and it is unlikely that the amounts of these elements present in the samples described will exert any interference. In essence, the method consists of irradiation of the samples, together with suitable standards, in a nuclear reactor, after which the arsenic activity is separated chemically with a carrier and finally measured.

CHEMICAL SEPARATION—

The separation method used in the earlier parts of this work was preceded for the biological samples by a conventional oxidation step, followed by a preliminary precipitation of elementary arsenic. This precipitate was dissolved in hydrogen peroxide and acid and subsequently treated as described previously.[4,5]

SELF SHIELDING—

The neutron absorption cross-sections of the major elements present in biological material, *i.e.*, carbon, hydrogen and oxygen, are so small that self-shielding effects are negligible.

Procedure

Irradiation of samples—

(*a*) Relatively large volumes of liquid, such as the several-millilitre samples of urine, were irradiated in the silica containers described in connection with sea-water analysis.[5] Briefly these containers are tubes sealed at one end and joined at the other to a short length of capillary tubing, the total capacity being about 11 ml. These containers, which are irradiated unsealed, together with the graphite holders and aluminium cans used during their irradiation in the pile, are fully described by Pate.[6] The standard used for simultaneous irradiation was a similar tube containing a solution of arsenic at a concentration of 1 μg per ml.

With this method of irradiation, loading and unloading of samples can only be done when the pile is shut down, so that a convenient irradiation period is a week-end.

After irradiation, samples and standards were set aside for 24 hours to allow the radioactivity to decay and were then transferred to à beaker by heating the container in the inverted position.

(*b*) Small volumes of liquid, *i.e.*, up to 0·2 ml, were irradiated in small silica pipettes. Determinations have been carried out on urine by this technique (see Table III) as well as on human blood. The pipettes were drawn from silica tubing and, after thorough cleaning, were filled either by capillary attraction or, after sealing one end, by the normal method of warming, inserting the open end in the liquid and then cooling. After sealing, the pipettes were irradiated in small aluminium cans packed with cotton wool. Up to 12 pipettes can conveniently be packed into the normal 3-inch can.

Portions of a solution containing 1 μg per ml of arsenic, similarly treated, served as standards.

After irradiation for 24 hours, followed by a waiting period of 1 hour to allow short-lived radioactivity to decay, the small pipettes were thoroughly washed on the outside, transferred to a beaker and broken under water.

(*c*) Solid samples, such as hair, nails, mouse tissues and vegetable material, were irradiated either after sealing in short lengths of polythene tubing or, for the more bulky samples, in bags made from polythene sheet. The standard used was a mixture of alumina containing 100 μg of arsenic per g, prepared by grinding the two oxides together in a number of stages, a procedure commonly used in the preparation of standards in emission spectroscopy. A quantity of 0·2 to 0·3 g of such a standard gave a convenient arsenic activity.

After irradiation for 15 hours followed by a waiting period of 1 hour, the tubes or bags were cut open and the contents transferred to a beaker.

General chemical procedure—

Transfer the weighed sample after irradiation to a 250-ml beaker. Add 5·0 ml of a standard arsenic solution containing 10 mg per ml of arsenic, and then add 2 ml of 100-volume hydrogen peroxide solution, 5 ml of nitric acid, sp.gr. 1·42, 5 ml of sulphuric acid, sp.gr. 1·84, and 2 ml of 70 per cent. perchloric acid. Gently heat the solution until the initial reaction subsides and then heat more strongly, adding further nitric acid, if necessary, to prevent charring. Finally heat until fumes of sulphuric and perchloric acid appear and until all the organic matter is completely oxidised. Dilute the solution, after cooling, to a volume of 20 ml, add 20 ml of hydrochloric acid, sp.gr. 1·19, and precipitate the arsenic by adding 1 g of ammonium hypophosphite and heating to 90° to 95° C for sufficient time to allow the precipitate to coagulate. Collect the precipitate by centrifugation, wash it with water and dissolve it by adding 5 ml of water, 2 ml of hydrogen peroxide solution and 2 ml of hydrochloric acid. Transfer the solution to a double-necked round-bottomed distillation flask, fit a dropping funnel to one neck and, to the other, a splash head connected through a swan-neck joint to a water-cooled condenser (ground-glass joints are used throughout).

Mix 10 ml of hydrochloric acid, sp.gr. 1·19, and 5 ml of 100-volume hydrogen peroxide in the dropping funnel and allow the mixture to stand until it is evolving chlorine freely. Add this mixture dropwise to the contents of the flask and proceed with the distillation under oxidising and reducing conditions, followed by hypophosphite precipitation and radiometric assay as described in Part II. Treat weighed standards similarly. For the solid standard, dissolve the arsenious oxide by boiling it with a small quantity of sodium hydroxide solution before acidifying.

Experimental results and discussion

Tracer investigation of the effect of organic matter on the separation procedure—

The chemical separation procedure was examined by adding a solution containing active arsenic, ^{76}As, to about 2 g of human hair, which is probably the most difficult of the samples examined to wet-oxidise, and 5 ml of the 10 mg per ml carrier arsenic solution. The procedure outlined above was then carried out, the various fractions of distillate and the residue being examined for activity in a liquid-counting Geiger tube.

The results are shown in Table I, from which it is clear that adequate recovery of arsenic is attainable, bearing in mind that correction for chemical yield is always made in the analysis.

Table I

Tracer investigation of separation from organic material

Stage	Count-rate, counts per minute	Equivalent recovery, %
Tracer added initially	33,215 ± 182	
Distillate from oxidation stages (HNO_3 - H_2SO_4)	888 ± 30	2·7
Oxidative distillate (Cl_2 - HCl)	755 ± 27	2·3
First reductive distillate	27,481 ± 165	82·9
Second and third reductive distillates	2133 ± 46	6·4
Residue in flask	1944 ± 44	5·9

Recovery and reproducibility experiments—

A series of human urine samples containing additions of standard arsenic solution was prepared, and approximately 10-ml portions were examined by the procedure outlined earlier, with the results shown in Table II.

Table II

Recovery of different amounts of arsenic added to human urine

Arsenic added, p.p.m.	Sample weight, g	Arsenic found, g	Arsenic content, p.p.m.	Arsenic recovery, p.p.m.
Nil (normal urine)	8·8174	$4·4 \times 10^{-7}$	0·050	—
0·005	5·3627	$2·9 \times 10^{-7}$	0·054	0·004
0·01	7·6069	$4·5 \times 10^{-7}$	0·059	0·009
0·02	8·8598	$5·9 \times 10^{-7}$	0·067	0·017
0·05	9·4094	$9·9 \times 10^{-7}$	0·105	0·055
0·1	9·5926	$1·4 \times 10^{-6}$	0·145	0·095
0·5	5·9450	$3·4 \times 10^{-6}$	0·570	0·520

The reproducibility of the method was tested on a sample of human urine by the semi-micro technique, *i.e.*, with quantities of about 0·1 ml in small silica pipettes. The results are shown in Table III, together with those for a sample of glass-distilled water, which served to set a limit to any possible contamination before irradiation.

Table III

Reproducibility of arsenic determination on a single urine sample by the small silica pipette procedure

Material	Sample weight, g	Arsenic found, μg	Arsenic content, p.p.m.
Urine	0·0885	0·0020	0·023
"	0·1411	0·0030	0·021
"	0·1005	0·0022	0·022
"	0·0790	0·0016	0·020
"	0·3322	0·0083	0·025
Glass-distilled water	0·1258	0·0003(0)	0·002
"	0·1210	0·0001(3)	0·001

ANALYSIS OF HUMAN URINE—

Arsenic determinations on human urine from normal persons are quoted by Cox[7] as varying between nil and 0·7 mg of As_2O_3 per litre, the most frequently occurring figures being between nil and 0·2 mg per litre. Griffon, Buisson and Bardou[8] report figures from 0·008 to 0·15 mg of arsenic per litre with three samples showing nil, while more recently Sultzaberger[9] reports values of about 0·03 mg per litre and Kingsley and Schaffert[3] quote a range between 0·046 and 0·206 mg of arsenic per litre. Some results by the radioactivation method are shown in Table IV, and it can be seen that the range for samples from ten people is from 0·013 to 0·33 mg of arsenic per kg, which is within the range quoted by Cox a quarter

TABLE IV

ANALYSIS OF URINE FOR ARSENIC

Subject	Sample weight, g	Arsenic found, g	Arsenic content, μg per g
1	10·5793	$1·4 \times 10^{-7}$	0·013
2	8·3505	$2·2 \times 10^{-7}$	0·027
3	5·3542	$8·9 \times 10^{-8}$	0·017
4	11·2746	$5·0 \times 10^{-7}$	0·044
5	9·8883	$7·4 \times 10^{-7}$	0·075
6	7·8574	$4·3 \times 10^{-7}$	0·055
7	5·2238	$1·71 \times 10^{-6}$	0·33
8	9·4405	$1·34 \times 10^{-6}$	0·14
9	9·9284	$5·65 \times 10^{-7}$	0·057
10	7·7696	$1·63 \times 10^{-6}$	0·21

of a century ago. The reason for quoting these further results on urine in this paper, apart from illustrating the application of the new technique, is that samples of hair, blood and finger and toe nails were also examined from the same ten healthy males and comparison between the tissues from a given individual may be desired. All subjects were laboratory workers but only one subject (No. 6) had specifically handled small quantities of arsenic compounds.

ANALYSIS OF HUMAN BLOOD—

Fewer references have been made to the determination of the arsenic content of blood from normal humans. Guthman and Grass[10] quote an average content of 0·64 p.p.m. and later Guthman and Heinrich[11] give 0·59 p.p.m. More recently Satterlee and Blodgett[1] found 0·255 p.p.m., while Kingsley and Schaffert[3] quote results of 0·055, 0·098 and 0·10 p.p.m. Results by radioactivation are shown in Table V, and it will be seen that the values for samples from the same ten subjects lie between 0·09 and 0·50 p.p.m. Of particular interest is the small weight of sample used in this case.

TABLE V

ANALYSIS OF BLOOD FOR ARSENIC

Subject	Sample weight, g	Arsenic found, g	Arsenic content, p.p.m.
1	0·0533	$9·0 \times 10^{-9}$	0·17
2	0·0587	$1·3 \times 10^{-8}$	0·22
3	0·0624	$2·7 \times 10^{-8}$	0·44
4	0·0546	$1·1 \times 10^{-8}$	0·20
5	0·0538	$4·9 \times 10^{-9}$	0·09
6	0·0388	$5·6 \times 10^{-9}$	0·15
7	0·0281	$7·3 \times 10^{-9}$	0·26
8	0·0880	$2·0 \times 10^{-8}$	0·23
9	0·0496	$2·5 \times 10^{-8}$	0·50
10	0·0837	$2·2 \times 10^{-8}$	0·26

ANALYSIS OF HUMAN HAIR AND NAILS—

Reports on the arsenic content of hair have been quite frequent. Of these, Van Itallie[12] reported that the arsenic content of human hair was 0 to 0·9 p.p.m. and Wührer[13] gave figures ranging between 0·2 and 0·5 p.p.m. of As_2O_3 in men, observing that women's hair often contained 0·05 p.p.m. or less. Szep[14] in 1940 examined hair and nail clippings and

found that normal "healthy" levels were, for hair, 0·27 to 0·77 p.p.m., and for nails, 1·5 to 5·2 p.p.m. Kunkele[15] in the same year considered that indications of arsenic poisoning were given by contents of greater than 3 p.p.m. in head hair and 0·1 mg per litre in urine. This latter figure is perhaps a little too low. Results by the radioactivation procedure on samples from the same ten subjects as before are shown in Tables VI to VII, and it can be

TABLE VI

ANALYSIS OF HAIR FOR ARSENIC

Subject	Sample weight, g	Arsenic found, g	Arsenic content, p.p.m.
1	0·4237	$3·44 \times 10^{-7}$	0·81
2	0·1399	$8·5 \times 10^{-8}$	0·61
3	0·8835	$6·7 \times 10^{-7}$	0·76
4	0·8970	$8·6 \times 10^{-7}$	0·96
5	0·1099	$1·05 \times 10^{-7}$	0·96
6	2·1022	$4·4 \times 10^{-6}$	2·1
7	0·6400	$1·1 \times 10^{-6}$	1·7
8	0·5172	$2·66 \times 10^{-7}$	0·51
9	0·6585	$7·5 \times 10^{-7}$	1·1
10	1·5556	$1·84 \times 10^{-6}$	1·2

TABLE VII

ANALYSIS OF FINGER NAILS FOR ARSENIC

Subject	Sample weight, g	Arsenic found, g	Arsenic content, p.p.m.
1	0·0474	$7·7 \times 10^{-8}$	1·6
2	0·0525	$1·49 \times 10^{-7}$	2·8
3	0·0997	$9·7 \times 10^{-8}$	0·97
4	0·0808	$7·6 \times 10^{-8}$	0·94
5	0·0453	$4·3 \times 10^{-8}$	0·95
6	0·0355	$5·3 \times 10^{-7}$	15
7	0·0569	$1·45 \times 10^{-7}$	2·6
8	0·0625	$2·18 \times 10^{-7}$	3·5
9	0·0122	$1·4 \times 10^{-8}$	1·1
10	0·0765	$6·3 \times 10^{-8}$	0·82

TABLE VIII

ANALYSIS OF TOE NAILS FOR ARSENIC

Subject	Sample weight, g	Arsenic found, g	Arsenic content, p.p.m.
1	0·0082	$4·6 \times 10^{-8}$	5·6
2	0·1590	$4·63 \times 10^{-7}$	2·9
3	0·1684	$8·6 \times 10^{-8}$	0·51
4	0·0257	$1·18 \times 10^{-7}$	4·6
5	0·0442	$4·16 \times 10^{-8}$	0·96
6	0·0484	$1·25 \times 10^{-7}$	2·6
7	0·1022	$2·24 \times 10^{-7}$	2·2
8	0·1064	$7·5 \times 10^{-8}$	0·70
9	0·0127	$2·4 \times 10^{-8}$	1·9
10	0·1163	$6·0 \times 10^{-8}$	0·52

seen that, for hair, the results range from 0·51 to 2·1 p.p.m.; for finger nail, from 0·82 to 3·5, with one exceptionally high result of 15 p.p.m.; and for toe nail, from 0·52 to 5·6 p.p.m. It is of interest to note that a determination of the arsenic content of a single normal hair (of weight about 1 mg) should be possible by this method if necessary.

ANALYSIS OF TISSUES FROM A NORMAL MOUSE—

Although there are records in the literature of the determination of arsenic in individual organs of normal animals as small as a rabbit or a guinea-pig, no previous results for a single mouse have been noted, presumably because of lack of a sufficiently sensitive method of analysis. With the advent of the radioactivation method such a determination becomes readily possible and some results are shown in Table IX; significant figures only are quoted.

TABLE IX

ANALYSIS OF THE ORGANS OF A MOUSE

Organ	Sample weight, g	Arsenic found, g	Arsenic content, p.p.m.
Bone (femur)	0·016	3×10^{-9}	0·2
Liver	0·760	$1{\cdot}14 \times 10^{-7}$	0·15
Kidney	0·208	$3{\cdot}0 \times 10^{-8}$	0·14
Heart	0·134	$1{\cdot}6 \times 10^{-8}$	0·12
Small intestine	0·743	$7{\cdot}8 \times 10^{-8}$	0·10
Skin and hair	0·120	$1{\cdot}1 \times 10^{-8}$	0·09
Stomach	0·429	$3{\cdot}8 \times 10^{-8}$	0·09
Spleen	0·097	6×10^{-9}	0·06
Muscle	0·296	$1{\cdot}1 \times 10^{-8}$	0·04
Brain	0·398	7×10^{-9}	0·02
Blood	0·0420	$1{\cdot}9 \times 10^{-8}$	0·45

ANALYSIS OF BROAD BEANS—

As a final illustration of some advantages of the activation method, an example was chosen in which contamination of the sample before irradiation (the only contamination that is significant in this method) was impossible. A broad-bean pod containing five beans was irradiated intact and was not opened until after irradiation, when the individual beans were analysed by the wet oxidation and separation method outlined above. The results are shown in Table X, bean No. 1 being that nearest the stem of the plant.

TABLE X

ANALYSIS OF ARSENIC IN BEANS

Bean	Sample weight, g	Arsenic found, g	Arsenic content, p.p.m.
1 (nearest stem)	1·0015	$3{\cdot}5 \times 10^{-7}$	0·35
2	0·7824	$1{\cdot}3 \times 10^{-7}$	0·17
3	0·8688	$1{\cdot}7 \times 10^{-7}$	0·20
4	0·8348	$1{\cdot}6 \times 10^{-7}$	0·19
5	0·8887	$1{\cdot}5 \times 10^{-7}$	0·17

INTERFERING ELEMENTS

Elements that could interfere from the nuclear viewpoint, such as selenium, bromine and germanium, which if present in major quantities might give rise to some ^{76}As, have already been mentioned.

The other type of interference that might arise in this method is that the chemical separation of the arsenic may not always give radiochemical purity. For example, if the distillation were to be carried out carelessly, a proportion of the active potassium or sodium might be carried over into the distillate mechanically, and some might even reach the final elementary arsenic precipitate. If this were to happen to a significant degree, the fact would become obvious from the decay and absorption measurements, because the true half-life and β-energy of ^{76}As, 27 hours and 3·1 MeV, would not be found. In all the experimental work described in this paper no such interference was observed. If it were to occur, the arsenic precipitate could be redissolved and put through the distillation and precipitation steps again for further purification.

HEALTH ASPECTS

Certain samples, with their containers, are very active after irradiation; in particular, the urine samples in large silica containers. With such samples the unloading, and even the distillation step, may have to be carried out behind some lead shielding in order to avoid excessive radiation doses to the operator. This is not difficult in practice, and is a common feature of radiochemistry. Frequent monitoring is desirable, and the active residues from samples should be retained in shielded containers until the activity, normally relatively short-lived, decays to a sufficient extent for disposal of the material.

Conclusions

Comparison of this activation method with the chemical methods of analysis described by earlier workers is of interest. The advantages of the former can be summarised as follows—

Sensitivity—Amounts of arsenic as small as 10^{-10} g can be determined in favourable circumstances with present British facilities.

Specificity—The identity of the nuclide used for the determination can be confirmed by decay and energy measurements in addition to the chemical separation.

Blanks—The method is free from difficulties caused by reagent blanks or pick-up during chemical operations after irradiation, as only active arsenic, ^{76}As, is measured.

Separation—There is no need for quantitative separation steps, as a correction is applied for losses by measuring chemical yield.

Scale of operation—The avoidance, by the use of carriers, of the necessity for chemical operations with sub-microgram quantities.

It should be added that facilities for irradiation in the Harwell Pile are generally available.[16]

Our thanks are given to the ten people who provided the samples of blood, hair, etc.; to Messrs. K. C. Parry and P. C. Long of the Haematology Group of the Medical Division at A.E.R.E., who took the blood samples; to Dr. R. H. Mole of the M.R.C. Radiobiological Unit at Harwell, who dissected the mouse; to members of the Pile Operating Group of Engineering Division, A.E.R.E., for their unfailing help with irradiation facilities; and finally to the Director, A.E.R.E., for permission to publish this paper.

References

1. Satterlee, H., and Blodgett, C., *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 400.
2. How, A. E., *Ibid.*, 1938, **10**, 226.
3. Kingsley, G. R., and Schaffert, R. R., *Anal. Chem.*, 1951, **23**, 914.
4. Smales, A. A., and Pate, B. D., *Ibid.*, in the press.
5. ——, ——, *Analyst*, 1952, **77**, 188.
6. Pate, B. D., "An Apparatus for the Pile Irradiation of Liquids," Atomic Energy Research Establishment Report C/R 643, H.M. Stationery Office, 1951.
7. Cox, H. E., *Analyst*, 1925, **50**, 3.
8. Griffon, H., Buisson, M., and Bardou, P., *Compt. Rend. Soc. Biol.*, 1934, **116**, 478.
9. Sultzaberger, J. A., *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 408.
10. Guthmann, H., and Grass, H., *Arch. Gynaekol.*, 1932, **152**, 127.
11. Guthmann, H., and Heinrich, K., *Ibid.*, 1941, **172**, 380.
12. Van Itallie, L., *Pharm. Monatsh.*, 1932, **13**, 220.
13. Wührer, J., *Biochem. Z.*, 1937, **294**, 401.
14. Szep, O., *Z. Physiol. Chem.*, 1940, **267**, 29.
15. Künkele, F., *Chem. Z.*, 1940, **64**, 29.
16. "Radioactive Materials and Stable Isotopes," Catalogue No. 2, Isotope Division, A.E.R.E., Harwell, Berks.

NOTE—References 4 and 5 are to parts I and II of this series.

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A Polarographic Method for the Estimation of Tetrachloronitrobenzene Residues on Potatoes

By J. G. WEBSTER and J. A. DAWSON

A method is described for the polarographic estimation of small amounts of tetrachloronitrobenzene. Details are given of the application of this method to the determination of tetrachloronitrobenzene residue on potatoes. Recovery rates are shown to be of the order of 84 to 93 per cent. from tubers treated with 10 to 500 μg of tetrachloronitrobenzene.

The use of 2:3:5:6-tetrachloronitrobenzene (TCNB) as a fungicide and sprouting depressant on stored potatoes necessitated the development of a quantitative method of analysis for small residual amounts of TCNB on potatoes.

The colorimetric technique of Auerbach[1] was found to suffer from interference by water, and consequently was insufficiently sensitive for the determination of the very small amounts of TCNB remaining on tubers after prolonged storage.

The polarographic method devised in these laboratories has been used satisfactorily for the past two years.

Experimental

Preliminary work—

Polarographic determinations were attempted on standard solutions of TCNB in a number of solvents, including acetone, ethyl alcohol and *iso*propyl alcohol. Sodium sulphite, sodium pyroborate, hydrochloric acid, sodium hydroxide, sodium acetate and acetic acid were tried as supporting electrolytes.

The most satisfactory results were those with *iso*propyl alcohol as solvent for the TCNB and an equi-molar mixture of sodium acetate and acetic acid in water as the supporting electrolyte.

The method finally adopted consisted in dissolving the TCNB in a known volume of absolute *iso*propyl alcohol and making up to twice that volume with equal parts of 0·2 *M* sodium acetate solution and 0·2 *M* acetic acid. The solution was deoxygenated with nitrogen and the polarogram recorded over the range −0·3 to −1·2 volts, the temperature being maintained at 25° ± 0·5° C.

The half-wave potential of TCNB in a mixture of 0·05 *M* sodium acetate and 0·05 *M* acetic acid containing 50 per cent. of *iso*propyl alcohol, measured against the saturated calomel electrode, is −0·53 volt.

A photographic-recording Cambridge polarograph was used. The value of $m^{\frac{2}{3}}t^{\frac{1}{6}}$ for the dropping-mercury electrode was approximately 2·16 $mg^{\frac{2}{3}}$ $sec.^{-\frac{1}{2}}$.

Method

Reagents—

All reagents should be of recognised analytical quality.

Sodium acetate solution, 0·2 *M*—Dissolve 8·2 g of sodium acetate in 500 ml of water.

Acetic acid, 0·2 *M*—Dissolve 6 g of glacial acetic acid in 500 ml of water. Titrate 20 ml of this solution with standardised 0·5 *N* sodium hydroxide solution, phenolphthalein being used as indicator, and calculate the volume of water required to be added to the bulk of the acetic acid solution in order to make it exactly 0·2 *M*. After adding this water, mix thoroughly and check the molarity of the acetic acid by a further titration.

iso*Propyl alcohol*—Absolute (see Note 1).

Procedure—

Dissolve 0·522 g of purified TCNB in 100 ml of *iso*propyl alcohol. Dilute 10 ml of this solution to 100 ml with *iso*propyl alcohol to give a $2{\cdot}0 \times 10^{-3}$ *M* solution of TCNB.

To 5 ml of this dilute solution add 2·5 ml of sodium acetate solution and 2·5 ml of acetic acid. Place the resulting solution in a polarographic cell in a bath maintained at 25° ± 0·5° C.

Deoxygenate the solution with nitrogen that has previously been bubbled through a wash bottle containing *iso*propyl alcohol, 0·2 *M* sodium acetate solution and 0·2 *M* acetic acid in the ratio of 2:1:1. Bubbling for 15 minutes was sufficient to remove all the dissolved oxygen.

Record the polarogram over the range −0·3 to −1·2 volts. The wave-height is proportional to the concentration of TCNB in the solution under test.

CALIBRATION OF THE POLAROGRAPH—

Repeat the above procedure with standard solutions containing from 5 to 500 μg of TCNB per ml and record the waves for different sensitivity settings of the galvanometer.

Measure the wave-heights and on suitable graph paper plot them against concentration of TCNB for the various sensitivity settings. Draw straight lines through these sets of points to pass through the origin. Some typical results are recorded in Table I.

TABLE I

TYPICAL RESULTS

Concentration, *C*, of TCNB, μg per ml	Diffusion current, i_d, μ-amp.	$K = i_d/C$
300*	8·1	0·027
300*	7·9	0·026
300*	8·0	0·027
200	5·2	0·026
75	2·0	0·027
60	1·6	0·027
30	0·76	0·025
15	0·36	0·024
10	0·25	0·025
2·5	0·07	0·026

* The first three results were obtained at three different sensitivity settings of the galvanometer.

The mean value of K calculated from measurement of thirty curves was found to be 0·026.

APPLICATION OF THE METHOD TO THE ESTIMATION OF TCNB RESIDUE ON POTATOES—

Place the weighed tuber in a porcelain dish and pour over it 12 ml of light petroleum, boiling range 40° to 60° C (see Note 2). Scrub the surface of the tuber with a plug of cotton wool held between forceps. Filter the light petroleum extract into a 25-ml graduated flask and repeat the washing with two 5-ml portions of light petroleum. Make up the total volume to 25 ml with light petroleum.

Place a suitable aliquot of the light petroleum solution in a 50-ml beaker. Evaporate the solvent at room temperature by blowing a gentle stream of dry air over the surface. The light petroleum extract must *not* be warmed or loss of TCNB by evaporation may occur.

Dissolve the residue in 5 ml of *iso*propyl alcohol and add 2·5 ml of 0·2 *M* sodium acetate and 2·5 ml of 0·2 *M* acetic acid. Deoxygenate this solution with nitrogen as described above and record the polarogram at a suitable sensitivity setting of the galvanometer.

Measure the wave-height and from the calibration graphs, plotted as described above, determine the concentration of TCNB in the test solution.

RESULTS

Known amounts of TCNB in light petroleum, boiling range 40° to 60° C, were applied to the surfaces of potatoes, at the rate of 1 ml per tuber. After the solvent had evaporated, the TCNB residues were determined by the method described above. Results (see Table II) show that recoveries of the order of 84 to 93 per cent. were attained and that residues as low as 0·05 p.p.m. could be determined.

NOTE 1—The reagents must be tested for impurities every 3 or 4 days by taking a blank through the normal procedure. If any irregularities appear in the "blank polarograms," the *iso*propyl alcohol must be redistilled. The impurity remains in the distillation flask.

NOTE 2—The use of light petroleum of boiling range 40° to 60° C is recommended for the following reasons: (*a*) it does not extract as much material from the potatoes themselves as would a water-miscible solvent and, hence, the polarograms are easier to interpret, and

TABLE II

RECOVERY OF KNOWN AMOUNTS OF TCNB ADDED TO POTATOES

Weight of potato, g	TCNB added, μg	Recovery, %
211	500	86
84	500	93
206	50	85
62	50	92
182	10	84
76	10	93

(*b*) losses of TCNB during the evaporation of the light petroleum are much less than those occurring when a solution of TCNB in a higher-boiling solvent is evaporated.

The assistance and advice given by Mr. R. P. Tew and Miss J. Muir in the course of this work and the facilities provided by the Chief Chemical Inspector, Ministry of Supply, at Woolwich Arsenal are gratefully acknowledged.

REFERENCE

1. Auerbach, M. E., *Anal. Chem.*, 1950, **22**, 1287.

FUNGICIDE AND INSECTICIDE RESEARCH CO-ORDINATION SERVICE
c/o CHEMICAL INSPECTORATE
ROYAL ARSENAL
WOOLWICH, S.E.18

November, 1951

Quantitative Determination of Sodium Cetyl Sulphate in its Solution in Water

BY G. R. EDWARDS, W. E. EWERS AND W. W. MANSFIELD

The determination of sodium cetyl sulphate by its quantitative separation from dilute aqueous solutions of the detergent is described. The compound of the detergent with methylene blue is precipitated, filtered and washed on a column packed with ground quartz, dissolved in ethyl alcohol and determined colorimetrically. In the concentration range 10 to 40 mg per litre, the deviation of the mean of duplicate analyses from the straight line relationship between light absorption and amount of sodium cetyl sulphate was 0·17 ± 0·15 mg per litre.

MOST methods that have been proposed for the determination of long-chain anionic detergents, such as sodium cetyl sulphate, depend upon the ease with which these compounds combine with long-chain cationic reagents such as cetyl trimethylammonium bromide and cetyl pyridinium bromide.[1] The anionic and cationic compounds have been titrated one against the other in the presence of methylene blue or bromophenol blue (depending on the titrant) and a layer of chloroform or benzene. The end-point is indicated by the appearance of a blue colour in the non-aqueous phase, owing to the formation of an oil-soluble compound between the dye and the excess of reagent. Epton[2] has suggested an alternative procedure in which the partition of methylene blue between aqueous and chloroform phases is affected by the addition of sodium sulphate and sulphuric acid so that the end-point is reached by matching the colours of the two layers. A review of these methods has been given recently by Swanston and Palmer,[1] who have suggested a modification of the titration method in which no indicator is used.

These methods are effective for concentrations of the reagent higher than 0·001 *M* (340 mg of sodium cetyl sulphate per litre[1]). For lower concentrations a more direct method proposed by Jones[3] was used for some time in this laboratory. Methylene blue solution was

added to the acid solution of sodium cetyl sulphate and the methylene blue - cetyl sulphate complex formed was extracted by shaking with four successive quantities of chloroform and was estimated colorimetrically in the extract. This method is tedious; as some of the aqueous phase is emulsified in the chloroform it is necessary to wash the chloroform layer with water; a considerable personal factor is involved and the results suffer accordingly. This difficulty is obviated in our method, which consists essentially in precipitating the coloured complex, filtering through a column packed with ground quartz, washing, extruding the column and redissolving the complex in ethyl alcohol for colorimetric determination. The methylene blue solution used for the precipitation was similar to that described by Epton, the purpose of adding sulphuric acid and sodium sulphate being to decrease the solubility in water of the methylene blue - cetyl sulphate compound. A quartz column was chosen for the filtration because other filter media, such as cellulose and glass, adsorb methylene blue much more strongly than does quartz.

METHOD

APPARATUS—

The Pyrex glass apparatus in which eight separate determinations can be made simultaneously is shown in Fig. 1. The cups in which the reagents are mixed are each of 30 ml capacity. These are sealed to tubing of 5 mm bore in which the quartz columns are formed. Each is fitted by a ground joint to a tap attached to a manifold through which the columns can be drained by gentle suction from the short syphon leg. A Spekker H454 photo-electric absorptiometer was used for measuring the light absorption of the solutions; a combination of orange-yellow 2 and orange-red 2 filters gives the maximum sensitivity.

REAGENTS—

Sodium cetyl sulphate—Purify sodium cetyl sulphate by alternately recrystallising the sample from 90 per cent. alcohol and extracting with ether; this reduces contamination by inorganic salts and free cetyl alcohol to a minimum.

Quartz—Heat ground quartz for 4 hours at 80° to 90° C in constant-boiling hydrochloric acid and size the quartz by elutriation; take the fraction settling at between 25 and 380 cm per minute. After drying at 110° C, heat the material for 1 hour at 500° C.

Methylene blue hydrochloride solution A—Dissolve 0·2 g of methylene blue hydrochloride, 50 g of anhydrous sodium sulphate and 10 ml of concentrated sulphuric acid in distilled water and make up to 1 litre.

Methylene blue hydrochloride solution B—Dilute 100 ml of solution A to 1 litre.

PROCEDURE—

Fill the manifold with distilled water and, with all taps closed, place about 20 ml of water in each cup. Tamp a small pad of cotton wool down the tube on to the top of the ground joint and add a measured quantity of quartz to fill the tube to the level shown in Fig. 1. Drain the columns through the manifold until the water level in each tube is at the top of the quartz packing. Add to each cup 10 ml of methylene blue hydrochloride solution A with a pipette and then 10 ml of a solution of sodium cetyl sulphate of unknown strength. Mix the solutions thoroughly with the aid of a glass stirrer and after 20 minutes allow the mixture to percolate through the silica column. After draining to the top of the quartz, wash the column first with 5 ml of solution B and then with 3 ml of distilled water; rinse the stirrer and the walls of the cup each time. The appearance of the column at various stages in this procedure is shown in Fig. 1.

Remove the column unit from the manifold and, holding it horizontally, pull out and discard the cotton wool plug, which contains a large amount of adsorbed methylene blue. Place the column unit in the neck of a 100-ml standard flask and wash the contents into the flask with 70 per cent. ethyl alcohol. The dark precipitate in the upper portion of the column dissolves in a short time. Then make the solution in the standard flask up to volume. The volume of the quartz, about 0·4 ml, can be ignored, since it remains sensibly constant. The light absorption of the solution in a 1-cm cell is measured and compared with a calibration curve made with standard solutions of sodium cetyl sulphate.

RESULTS AND DISCUSSION

The relation between light absorption and the sodium cetyl sulphate content of standard solutions was investigated for a series of fifty-two solutions, covering the range 0 to 40 mg

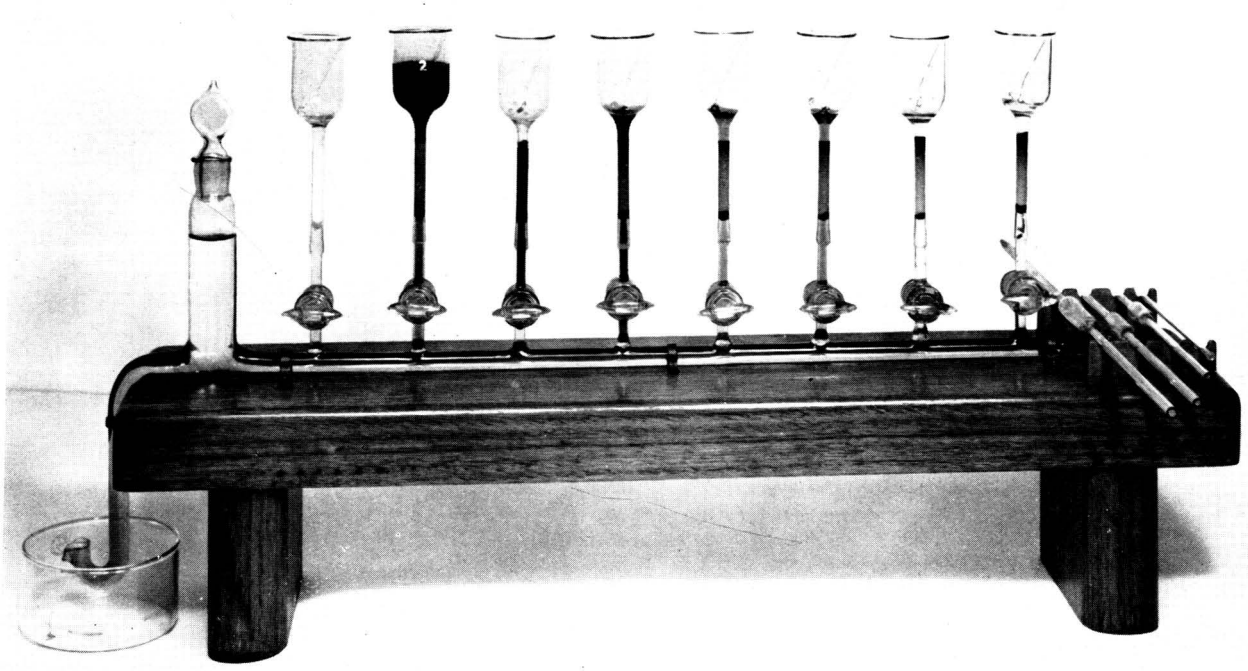

Fig. 1. Apparatus for precipitating, filtering and washing the methylene blue - cetyl sulphate compound

of sodium cetyl sulphate per litre. The data are plotted in Fig. 2. It will be noted that a reading of 0·012 was obtained with the absorptiometer for solutions containing no sodium cetyl sulphate.

For solutions containing from 10 to 40 mg per litre, the relationship between concentration and absorption was linear. The equation—

$$C = 79{\cdot}90\,S - 0{\cdot}50,$$

where C is the concentration of sodium cetyl sulphate in mg per litre and S is the instrument reading, fitted the data with a correlation error of about 0·003 per cent. With this equation, the deviation of the mean of duplicate analyses was found to be $0{\cdot}17 \pm 0{\cdot}15$ mg per litre.

At concentrations lower than 10 mg per litre the relationship is not linear owing to incomplete recovery of the methylene blue - cetyl sulphate complex. The preparation of a

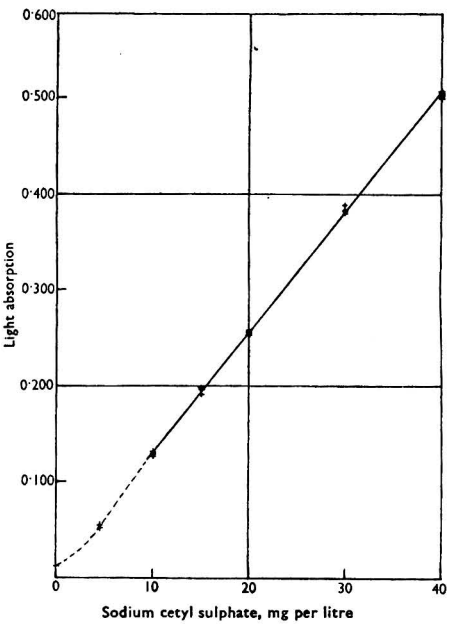

Fig. 2. Relation between light absorption and concentration of sodium cetyl sulphate in test portion

calibration curve allows determinations to be made at these lower concentrations with an error of about 0·3 mg per litre. The upper limit of concentration for which the method can be used in our apparatus is imposed merely by considerations of time. The filtration particularly and the redissolution of the complex become very much slower when the quantity of complex present is increased above that corresponding to 0·4 mg of sodium cetyl sulphate.

It is emphasised that the determined relation depends not only on the absorptiometer used, but also on the characteristics of the apparatus and the nature and area of the quartz surface.

This method was used successfully in measuring the adsorption of sodium cetyl sulphate on cassiterite.[4]

We wish to thank Mr. L. F. Evans for the sample of sodium cetyl sulphate used in developing this method.

References

1. Swanston, K., and Palmer, R. C., *J. Soc. Dyers & Col.*, 1950, **66**, 630.
2. Epton, S. R., *Trans. Faraday Soc.*, 1948, **44**, 226.
3. Jones, J. H., *J. Ass. Off. Agric. Chem.*, 1945, **28**, 398.
4. Edwards, G. R., and Ewers, W. E., *Austral. J. Sci. Res.* (*A*), 1951, **4**, 627.

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November, 1951

The Analysis of Zinc Residues

By E. F. PELLOWE and F. R. F. HARDY

A rapid chemical method is described for the analysis of galvaniser's ash or other zinc residues containing metallic zinc, zinc oxide and zinc chloride.

The zinc residue is shaken with ammonium acetate solution to dissolve zinc oxide and chloride. Zinc chloride is determined by titration with silver nitrate; zinc oxide plus chloride is determined by titration with potassium ferrocyanide in presence of sulphuric acid and the amount of zinc oxide is given by difference between the two determinations. Metallic zinc is determined on the residue from the dissolution stage.

In the hot-dip galvanising process one of the residual products is galvaniser's ash, a material that is essentially zinc oxide together with some metallic zinc, zinc chloride and heavy metal oxides as impurities. It is sometimes necessary to determine the amount of metallic zinc, zinc oxide and zinc chloride by a rapid and reasonably accurate method.

Many methods have been proposed for the determination of zinc oxide in zinc powder, but, except for some that require complicated apparatus and expert manipulation, they do not give accurate and reproducible results. Osborn,[1] however, has suggested a method based on the solubility of zinc oxide in ammonium acetate solution. It has been established that zinc oxide can be quantitatively dissolved in ammonium acetate solution, although metallic zinc remains unattacked. For instance, 2 g of pure metallic zinc powder were digested in 30 per cent. ammonium acetate solution for 8 hours; at the end of this the weight of the zinc had decreased by 0·0002 g, a negligible loss compared with other inherent errors. Zinc oxide, in contrast, has been found to dissolve completely provided it is given sufficient time to digest. This rate of digestion, which is quite rapid at first, but slowly diminishes as the concentration of dissolved zinc oxide increases, is dependent on several factors, such as the size of the zinc oxide particles, the presence of colloidal impurities and the temperature of the solution. As all these factors are variable, it would be difficult to apply a correction, but, with the knowledge that excessive digestion does not have any appreciable action on the metallic zinc, it is preferable to digest for longer than is necessary. It has been found that 2 hours is sufficient to dissolve all quantities of zinc oxide from nil to 100 per cent. if 2 g of sample are taken in 100 ml of 30 per cent. w/v ammonium acetate.

Method

Reagents—

All reagents must conform to recognised analytical standards of purity.

Potassium ferrocyanide solution—Prepare a solution containing 22 g of potassium ferrocyanide and 0·3 g of potassium ferricyanide per litre. Standardise the solution as described below.

Standard zinc solution—Dissolve exactly 4 g of pure zinc in the minimum of 20 per cent. sulphuric acid plus 4 drops of concentrated nitric acid. Boil, cool and make up to 1 litre in a volumetric flask.

25 ml of solution ≡ 0·10 g of zinc.

Silver nitrate—A 0·1 *N* standard solution.

Ammonium acetate—A 30 per cent. w/v solution.

Sulphuric acid—Diluted to 50 per cent. v/v.

Diphenylbenzidine indicator—Dissolve 0·5 g of diphenylbenzidine in 100 ml of concentrated sulphuric acid. Store in a well-stoppered dropping-bottle. This solution is only stable for about 6 weeks.

Diphenylamine blue indicator—Dissolve 0·1 g of diphenylamine in 10 ml of concentrated sulphuric acid and add 4·5 ml of this solution to 300 ml of 5 *N* sulphuric acid. Shake well and store in a stoppered bottle.

Standardise the ferrocyanide solution in the following manner. Transfer 25 ml of the standard zinc solution to a beaker, add 20 ml of 50 per cent. sulphuric acid and 50 ml of 30 per

cent. ammonium acetate, and make up to 100 ml with water. Add 6 drops of diphenylbenzidine solution and add about 8 ml of ferrocyanide from a burette. Shake well and leave until a deep purple colour is developed—this takes several minutes. Slowly add more ferrocyanide until the solution turns bluish-purple and again wait for the deep purple colour to return. When it does, add more ferrocyanide a drop at a time until the solution turns light green at the end-point of the titration. If the titre is *a* ml, then the factor of the ferrocyanide is $0{\cdot}10/a$ g of zinc per ml.

PROCEDURE—

Grind the ash to a fine powder and accurately weigh a 2-g sample. Transfer the sample to a 400-ml conical beaker and add 100 ml of 30 per cent. ammonium acetate solution. Shake at intervals of 15 minutes for 2 hours. (To obviate shaking by hand, the sample could be transferred to a stoppered bottle and shaken on a machine for 10 minutes.) After this time all the zinc oxide will have dissolved. Filter the solution through a Whatman No. 41 filter-paper and wash the residue thoroughly. Transfer the filtrate and washings to a 200-ml graduated flask and make up to volume. This is the stock solution.

Zinc chloride—Transfer a 50-ml aliquot of the stock solution to a 400-ml beaker and add 25 ml of 50 per cent. sulphuric acid. Add from a burette approximately 5 ml of standard 0·1 *N* silver nitrate solution, and then add 2·5 ml of diphenylamine blue indicator previously mixed with 0·5 ml of approximately 0·05 *N* potassium dichromate solution to give a deep blue colour. Continue titrating with the silver nitrate slowly and with constant shaking. In the early stages of the titration the precipitate is coloured blue; the end-point is indicated when the supernatant liquid turns purple. Calculate the zinc present as chloride.

Zinc oxide—Take a suitable aliquot of the stock solution containing about 0·1 g of zinc and add 50 per cent. sulphuric acid, 30 per cent. ammonium acetate and water so that the final bulk is 100 ml and the concentrations of sulphuric acid and ammonium acetate are 10 and 15 per cent. respectively. Titrate with standard potassium ferrocyanide as previously described. Calculate the amount of zinc present and subtract from this figure the previously calculated amount of zinc present as chloride. This gives the amount of zinc present as oxide.

Metallic zinc—Dissolve the residue on the filter-paper in 20 per cent. sulphuric acid, filter if necessary, and make up to 100 ml in a volumetric flask. Take a suitable aliquot, add the required amounts of ammonium acetate, sulphuric acid and water and titrate with standard potassium ferrocyanide. This gives zinc present as metallic zinc.

NOTES ON THE METHOD

Suitable concentrations of ammonium acetate and sulphuric acid as given in this method were found by titrating standard solutions of zinc dissolved in various concentrations of the two. Table I shows the results of these experiments.

TABLE I

EFFECT OF CONCENTRATION OF AMMONIUM ACETATE AND SULPHURIC ACID ON THE TITRATION

Concentration of sulphuric acid, % v/v	Concentration of ammonium acetate, % w/v	Titre, ml	End-point
5	15	26·80	Indecisive
8	15	26·60	Sharp; long waiting period
10	15	26·65	Sharp; in short time
12	15	26·65	Sharp; in short time
15	15	27·10	Masked and indecisive
10	10	26·65	Sharp
10	20	26·60	Sharp; long waiting period
5	5	26·70	Indecisive
20	20	—	No end-point

These results show that the best concentrations of ammonium acetate and sulphuric acid are about 15 and 10 per cent., respectively. On no account should the concentration of ammonium acetate and sulphuric acid be outside the ranges of 10 to 20 per cent. and 8 to 12 per cent., respectively.

Diphenylbenzidine is an oxidation - reduction indicator that is dark purple when oxidised and light green when reduced. When potassium ferrocyanide and ferricyanide are added to a zinc solution, the ferrocyanide reacts with the zinc to form zinc ferrocyanide, which is insoluble, and leaves the slightly oxidising ferricyanide in solution. When all the zinc has been precipitated, a very small excess of ferrocyanide will reduce the indicator. It is therefore essential that, if the indicator is to work properly, there must be no oxidising or reducing agent in the zinc solution to be titrated. As the analysis does not require the use of oxidising or reducing agents, interference could only come from the sample being analysed. The only compounds likely to interfere are soluble ferric salts, but the effect of these can be partly suppressed by addition of ammonium phosphate to form the relatively undissociated ferric phosphate.

Results

Table II shows the results of analysing synthetic mixtures of metallic zinc, zinc oxide and zinc chloride.

Table II

Analysis of synthetic mixtures

Synthetic mixture			Determined by analysis		
Zinc, %	Zinc oxide, %	Zinc chloride, %	Zinc, %	Zinc oxide, %	Zinc chloride, %
1	85	14	1·00	84·74	13·99
3	85	12	3·04	84·87	11·96
5	85	10	5·04	85·04	9·93
7	85	8	6·99	84·88	8·01
1	90	9	1·02	89·52	8·97
3	90	7	3·04	89·60	6·99
5	90	5	4·94	89·66	4·97
7	90	3	7·04	89·97	3·00

The zinc oxide, once it has been separated from the metallic zinc, could be estimated gravimetrically, electrolytically or polarographically. Gravimetric determination would, however, be somewhat longer than the volumetric determination described, and electrolytic or polarographic analysis requires special apparatus.

The method described could easily be adapted to the analysis of mixtures of metallic zinc and zinc oxide other than in galvaniser's ash. It has, in fact, been found that the accuracy of the method is maintained when small amounts of zinc oxide are determined, although it is then necessary to add standard zinc solution and to calculate the zinc oxide by difference.

Reference

1. Osborn, G. H., *Analyst*, 1951, **76**, 114.

Crittall Manufacturing Co. Ltd.
Manor Works
Braintree, Essex

December, 1951

Notes

QUALITATIVE DETECTION OF LONG-CHAIN ALKYL PYRIDINIUM HALIDES

IN a spot test for tertiary-ring bases detailed by Feigl,[1,2] the base is added to an alkyl halide (usually methyl iodide) or dimethyl sulphate, to form a quaternary compound that reacts with sodium 1:2-naphthaquinone-4-sulphonate, in the presence of sodium hydroxide solution, to give a red, violet or green coloured compound. The course of the reaction suggested by Feigl[1] depends on the work of Kröhnke.[3]

It has been found that the test can be applied to the detection of small amounts of cation-active compounds of the alkyl pyridinium halide type, such as lauryl pyridinium chloride, cetyl pyridinium chloride and Fixanol V.R.,* which is mainly cetyl pyridinium bromide.

PROCEDURE—

Place a drop of a saturated aqueous solution of sodium 1:2-naphthaquinone-4-sulphonate on a Whatman drop reaction paper (No. 120), add a drop (0·05 ml) of the test solution and make alkaline with a drop of 0·5 *N* sodium hydroxide solution. A red-violet coloration concentrated around the middle of the spot denotes the presence of an alkyl pyridinium halide in the test solution. With larger amounts of cation-active material the coloration diffuses outwards. Amounts of Fixanol V.R. as small as 25 μg, at concentrations as low as 1 in 2000, can be identified. The limits of identification were not investigated for lauryl pyridinium chloride and cetyl pyridinium chloride, but the reactions with these were similar to that with cetyl pyridinium bromide. The colour obtained on the reaction paper faded slowly. Similar results were obtained on a spot-plate, but the limit of identification was much less favourable and the colour obtained was not stable, especially at low concentrations, as it changed rapidly to reddish-brown and finally to brown. The addition of acetic acid to the alkaline solution, as recommended by Feigl,[1] resulted in an orange-yellow colour, as given by methyl pyridinium iodide, ethyl pyridinium bromide and so on; it was observed that a blank test without a pyridinium compound present gave exactly the same colour, so that the test was not conclusive. Cation-active materials of the quaternary ammonium class did not give the reaction and this is to be expected from the mechanism of the reaction as suggested. This test may be applied to the detection of alkyl pyridinium halides on textile materials.

Thanks are due to the Directors of British Enka Ltd. for permission to publish this note.

REFERENCES

1. Feigl, F., "Qualitative Analysis by Spot Tests," Third English Edition, Elsevier Publishing Company Inc., Amsterdam, 1947, pp. 392–394.
2. Feigl, F., and Frehden, O., *Mikrochemie*, 1943, **16**, 84.
3. Kröhnke, F., *Ber.*, 1933, **66**, 604, 1386.

RESEARCH AND DEVELOPMENT DEPARTMENT
BRITISH ENKA LIMITED
AINTREE, LIVERPOOL, 9

E. G. BROWN
November, 1951

DETERMINATION OF CARBON DISULPHIDE AS CUPRIC DIETHYLDITHIOCARBAMATE

DURING the determination of carbon disulphide by the D.S.I.R. Method,[1] AnalaR grade absolute ethyl alcohol was not originally available for dissolving the cupric acetate, and samples of commercial absolute alcohol were used instead. These caused the solutions to darken on warming, with subsequent precipitation of cupric oxide. AnalaR ethyl alcohol was found to be satisfactory, but *iso*butyl alcohol of the same grade also proved to be a cheap and efficient solvent of cupric acetate and can be used for routine work instead of ethyl alcohol. Another solvent that has been recommended for the method is 2-methoxyethanol (Methyl Cellosolve).[2]

Thanks are due to the Directors of British Enka Ltd. for permission to publish this note.

REFERENCES

1. Department of Scientific and Industrial Research, "Methods for the Detection of Toxic Gases in Industry," Leaflet No. 6, "Carbon Bisulphide Vapour," H.M.S.O., 1939.
2. Morehead, F. F., *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 373.

RESEARCH AND DEVELOPMENT DEPARTMENT
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November, 1951

* Fixanol V.R. is made by Imperial Chemical Industries Ltd.

British Standards Institution

NEW SPECIFICATION*

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DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to interested members of the Society, and can be obtained on application to the Secretary, Society of Public Analysts and Other Analytical Chemists, 7–8, Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee ISE/18—Sampling and Analysis of Iron and Steel.

CN(ISE)9196—Draft B.S. for the Determination of Vanadium in Carbon and Low Alloy Steels.

Draft Specification prepared by Technical Committee LBC/11—Microchemical Apparatus.

CN(LBC)9251—Draft B.S. for Capillary Pipettes (Revision of B.S. 797).

Chemicals in Food Products

AN interim report of the Select Committee of the United States House of Representatives, appointed under the Chairmanship of Mr. James J. Delaney, has recently been published under the title "Investigation of the Use of Chemicals in Food Products."† This Committee was appointed to conduct a full and complete investigation of—

"1. The nature, extent, and effect of the use of chemicals, compounds, and synthetics in the production, processing, preparation, and packaging of food products to determine the effect of the use of such chemicals, compounds, and synthetics (*A*) upon the health and welfare of the nation and (*B*) upon the stability and well-being of our agricultural economy;

2. The nature, extent, and effect of the use of pesticides and insecticides with respect to food and food products, particularly the effect of such use of pesticides and insecticides upon the health and welfare of the consumer by reason of toxic residues remaining on such food and food products as a result of such use; and

3. The nature, effect, and extent of the use of chemicals, compounds, and synthetics in the manufacture of fertiliser, particularly the effect of such use of chemicals, compounds, and synthetics upon (*A*) the condition of the soil as a result of the use of such fertiliser, (*B*) the quantity and quality of the vegetation growing from such soil, (*C*) the health of animals consuming such vegetation, (*D*) the quantity and quality of food produced from such soil, and (*E*) the public health and welfare generally."

Chemicals potentially hazardous to public health are classified under four headings—

1. Pesticides, including insecticides, fungicides, acaricides, herbicides, and plant-growth regulators.
2. Chemicals used as preservatives, anti-oxidants, mould inhibitors, emulsifying and other agents added to food during processing or storage.
3. Chemicals used to wash utensils in food production, processing, and wrapping.
4. Wax coatings, resins, plasticisers, and other ingredients of food-packaging materials.

The main points dealt with in this preliminary report and the conclusions arrived at by the Select Committee can be summarised as follows—

Many food crops cannot be produced without the help of toxic pesticides and precautions are necessary to prevent harmful residues in the edible crop. These pesticides cannot be safely used without fundamental knowledge of their chemical, pharmacological and therapeutic properties. Further, methods for the detection and determination of poisonous residues in the marketed crop must be available. For many of the agricultural poisons now in use these essential requirements are lacking.

* Obtainable from the British Standards Institution, Sales Department, 24, Victoria Street, London, S.W.1.

† Union Calendar No. 1139. 81st Congress, 2nd Session, Report No. 3254, published by the U.S. Government Printing Office, Washington.

The Food and Drug Administration have found that DDT is absorbed and stored in fatty animal tissue; when used in cow byres to kill flies, it was found in the milk in amounts up to 2 parts per million. Chlordane, an alternative to DDT with the same properties, is four to five times as poisonous.

Selenium compounds, used as insecticides, cause liver changes in experimental animals and have been found in apples in amounts up to 3 parts per million. Selenium accumulates in the soil, is absorbed by the plant, and appears in the edible crop.

Investigation of phenyl mercury compounds has shown that they are very poisonous and are stored in the kidney. The danger point in the human kidney is unknown.

There are other pesticides at present in use, or proposed, whose safety has not been established.

The authority to set tolerances under existing law cannot prevent the use of a chemical before a tolerance has been set and the safety of the chemical determined. Further testimony is required as to how far the existing law may be insufficient for the protection of public health.

In the processing, preservation and production of food there is no objection to the introduction of chemicals *per se*; they are often beneficial. But harmful pesticides, or pesticides that have not been proved to be harmless, have been used. Amongst these, nitrogen trichloride (Agene) was used for about 30 years in the milling industry before Mellanby, in 1946, showed that it caused canine hysteria; since when, although no definite injury to humans has been established, its use as a flour improver has been discontinued.

Thiourea, a poisonous fungicide, suggested for use on citrus fruits, was found by the Food and Drug Administration in the juice of the fruit.

A synthetic sweetening agent, *p*-phenetylurea (Dulcin), had been in use for more than 50 years before it was shown to be toxic when ingested in small amounts over a long period.

Lithium chloride, contained in a salt substitute for patients on a low-salt diet, has been shown to be poisonous when the sodium chloride of the body has been reduced. Its use was stopped, but not before several deaths had occurred.

Mineral oil, long regarded as harmless and used as a substitute for the usual food oils, has been shown to interfere with the absorption of various vitamins and to cause lipoid pneumonia in infants. It is no longer allowed to be used.

The evidence brought before the Select Committee did not permit a conclusion to be drawn on the debatable question of the long-range effect of synthetic emulsifiers or surface-active agents on human metabolism.

These substances, used largely in bread, cakes and ice-cream, are of four main types: (1) mono- and di-glycerides of fatty acids, (2) compounds formed from sorbitol and a fatty acid, (3) compounds produced by reacting the sorbitan ester of a fatty acid with polymerised ethylene oxide and (4) polyoxyethylene monostearate. By varying the fatty acid and the degree of polymerisation of the ethylene oxide many compounds are possible in each class.

Since 1937 mono- and di-glycerides have been used, mixed with shortening fat, to make bread, buns and cakes "more tender." In 1947 the polyoxyethylene type of softener became a competitive synthetic with super-glycerinated shortening in the bakery trade. Polyoxyethylene monostearate is also used to reduce by about half the quantity of shortening fats previously used. On the evidence, the Committee came to the conclusion that there was justification for the complaint that bread softeners were responsible for a reduction in the nutritive value of bakery goods and that further testimony was required to determine the extent of the loss.

Agricultural specialists testified that artificial fertilisers do not make crops more liable to attack by pests or less nutritious than those grown with natural organic manure. Moreover, the supply of organic fertilisers was inadequate for present-day needs.

With a few exceptions, the witnesses who gave evidence before the Select Committee were of opinion that the existing laws did not give sufficient protection to the public health from the risks incurred by chemicals in food products. The general consensus of opinion was that a section similar to the New Drug Section of the Federal Food, Drug and Cosmetic Act, which demands proof of safety before a new drug is permitted to be used, is required in the food chapter of the Federal Food, Drug and Cosmetic Act.

At present the Government bears the burden of proving that a chemical added to food is harmful. Under existing legislation this burden cannot always be met before injury occurs to consumers.

The Select Committee was of opinion that before specific recommendations were made to Congress, a further opportunity for presenting their views and comments should be given to those likely to be affected by a change in the law.

FURTHER HEARINGS

In accordance with the recommendations of the Select Committee in its preliminary report, further hearings of evidence took place during April, May and June, 1951.

The Select Committee was constituted as follows—

Representatives: Delaney of New York (*Chairman*); Abernethy of Mississippi; Hedrick of West Virginia; Jones of Missouri; Miller of Nebraska; McDonough of California; and Horan of Washington. Also present: Vincent A. Kleinfeld, chief counsel to the Committee, and Alvin L. Gottlieb, associate counsel.

In addition to taking oral evidence from 28 witnesses, sundry letters from interested bodies were admitted to the record.

The Association of State and Territorial Health Officers recommended that the existing Food, Drug and Cosmetic Act should be amended to prevent the incorporation into or on foods of chemical or other new ingredients before they have been viewed and approved by the Food and Drug Administration.

The National Farmers' Union expressed its concern that too little caution is exercised in the use of chemicals in food products, sprays and so forth.

In reply to a circular letter asking for their opinions on the investigation, and on whether further legislation is required, 43 chief health officers in States and territories recorded, for the most part, their desire for greater administrative control over the entry of chemicals of unknown potency into the food of the nation.

The views of the witnesses were strongly influenced by their professional or industrial outlook: entomologists, farmers, chemical manufacturers and public health officers were mostly of the opinion that there is already sufficient protection from hazard to health under existing legislation; whereas doctors, chemists, zoologists, physiologists, agriculturalists and nutritionists took the opposite view.

An important part of the record is a reprint from the *Bulletin of the Association of Food and Drug Officials* (Vol. 14, No. 3, July, 1950)* of a paper on "Some Toxicological Reasons Why Certain Chemicals May or May Not Be Permitted as Food Additives," by A. J. Lehman, Division of Pharmacology, Food and Drug Administration, Federal Security Agency.

The conclusions reached in this paper may be summarised as follows—

SWEETENING AGENTS—

p-Phenetylurea (Dulcin)—Harmful effects are produced on long-term low-level feeding.
1-*N-propoxy*-2-*amino*-4-*nitrobenzene* (P–4000)—Excluded on basis of low margin of safety.
Benzosulphimide (Saccharin)—Safe as a food additive.
Sodium cyclo*hexylsulphamate* (Sulphamate)—Considered safe for food and drug use.

ANTI-OXIDANTS—

n-*Propyl gallate, thiodipropionic acid* and its *dilauryl* and *distearyl esters, gum guaiac, butylated hydroxy anisole* (BHA) and Nor*dihydroguairetic acid* (NDGA)—Safe for use in edible fats and oils in the concentrations prescribed by the Bureau of Animal Industry.

Diphenyl-p-*phenylenediamine*—Very low order of toxicity, safe for use on alfalfa.

WAXING COMPOUNDS—

β-Hydroxybutyral aldehyde and *acetaldehyde*—Safe.

Trioxymethylene, borax and *boric acid*—Considered as poisons; unsafe in foods.

Sodium o-*phenylphenol, diphenyl, dehydroacetic acid* (DHA), p-*hydroxybenzoic esters*—Further study is necessary to determine their status.

Methyl ester of naphthalene acetic acid—Anti-sprouting agent. No objection to its use as an anti-sprouting agent.

Triethanolamine, Morpholine—Any toxicity would be due to alkalinity and not to the compounds.

* Reprints are obtainable from the Editorial Office of the Association, 2411, North Charles Street, Baltimore 18, Md., U.S.A. Price 30 cents.

WETTING AGENTS—

Quaternary ammonium, cationic—Considered as poisons. Their use in food cannot be justified.

Alkyl aryl sulphates and *sulphonates*, anionic—Considered safe for washing fruits and vegetables, etc., if followed by adequate rinsing.

Polyoxyethylene sorbitan fatty acid derivatives (Tweens); *sorbitan fatty acid derivatives* (Spans); *polyoxyethylene fatty acid derivatives* (Myris); *polyoxyethylene glycols* (Carbowaxes)—The status of some of these, as bread softeners, will be determined by a forthcoming order establishing standards for bread. Because of a number of important biochemical effects, which have not yet been solved, the addition of any of the other wetting agents is considered to be out of place at present.

SILICONES—

Methyl polysilicone (DC Antifoam A)—Safe for use as an anti-foaming agent if the concentrations do not exceed 10 p.p.m.

Methylphenyl polysiloxane (Pan glaze)—The insolubility of the resin precludes contamination of the bakery goods.

CELLULOSE DERIVATIVES—

Methyl cellulose and *carboxymethyl cellulose*—Unpublished data on these cellulose derivatives show that there is no intestinal absorption and that they are otherwise innocuous.

CHLORINATED INSECTICIDES—

DDT—The potential hazard of DDT may have been underestimated because (*a*) at very low levels of feeding there is storage and definite though minimal liver damage, (*b*) its elimination is slow, (*c*) milk can be contaminated as the result of spraying cow-sheds to kill flies, (*d*) it is deposited in human fat and has been found in human milk.

Chlordane—This is the most hazardous of the chlorinated insecticides.

Lindane—This is the least hazardous of its class.

Toxaphene—A method for the determination of this compound is still required.

Dieldrin and *Aldrin*—These compounds are about ten times more poisonous by the dermal than by the oral route; the chronic effects are unknown.

ORGANIC PHOSPHATES—

Parathion—Despite the deaths of a few operators, who were poisoned while applying parathion, the evidence goes to show that a food containing from 2 to 5 p.p.m. would not be a hazard to health.

Tetraethyldithionopyrophosphate and *ethyl*-p-*nitrophenylthionobenzene phosphonate*—The dermal and acute toxicity resemble those of parathion, but the chronic toxicity may be less.

Diethyl-p-*nitrophenyl phosphate*—Limited dermal toxicity data indicate a similarity to parathion.

Dimethyl parathion—The dermal toxicity is about one-tenth less than that of parathion and the oral toxicity similar to parathion.

NATURAL INSECTICIDES AND ACTIVATORS—

Rotenone—The chronic effects appear to be less than was at first thought; the residue hazard may also be less.

Pyrethrins—These are amongst the safest of the insecticides. The toxicological hazards under the conditions of use are remote.

Allethrin—The present evidence indicates a toxicity similar to that of the pyrethrins.

Pipironyl butoxide, *N-propyl isome* and *Octacide* 264—These activators are of too low an order of toxicity to be hazardous under ordinary conditions of use.

HERBICIDES—

2-4-*Dichlorophenoxyacetic acid*, *phenoxyacetic acid* and *naphthalene acetic acid*—The acute oral toxicity is of a low order. The chronic toxicity is unknown.

Methyl ester of naphthalene acetic acid—The acute toxicity is of a low order and the chronic effects appear to be slight.

FUNGICIDES—

Mercury compounds—The chronic toxicity is of a high order; any benefit to be derived from their use must be weighed carefully against the hazard.

Dithiocarbamates—No data are available for the appraisal of these compounds.

2-Heptadecylglyoxalidine—The acute and chronic toxicity is of a low order.

MITICIDES—

Dinitro derivatives (dinitro-o-cresol)—As a class these compounds are metabolic stimulants with a tendency to cause cataract. More study of these effects is required before an opinion as to their safety can be expressed.

Copper—There is little or no danger of chronic toxicity from copper salts. The danger from residues of copper lies in their irritant effect on the nerve endings of the stomach. In man, the amount required to produce this effect is about half a gram. Thirty parts per million in food is believed to be safe.

Whatever may be the outcome of this examination of a complex and contentious subject, the rest of the world will be indebted to the United States of America for the thorough and impartial way in which it has been carried out.

F. L. OKELL

International Union of Pure and Applied Chemistry

Analytical Section (Section V)

COMMISSION ON MICROTECHNIQUES

THE Committee held its first session in New York on Saturday, September 8th, 1951, with the President, Professor M. K. Zacherl (Vienna) in the Chair. Members present were: Dr. A. A. Benedetti-Pichler (New York), Professor H. Lieb (Graz), Dr. H. Malissa (Graz), Professor P. E. Wenger (Geneva), Dr. C. L. Wilson (Belfast). Apologies were received from Professor P. L. Kirk (Berkeley) and Dr. W. Zimmermann (Melbourne).

The President greeted the members at this, the first session, and explained briefly how the Committee had resulted from discussions on the occasion of the 1st International Microchemical Congress in Graz in July, 1950. The Committee was to consider as within its field all microchemical questions in so far as these are of international interest. This involves contact with the fields of interest of other Committees of the Analytical Section of the Union, and close co-operation must therefore be regarded as essential.

The Committee unanimously elected Professor Kirk as its Vice-President and Dr. Malissa as its Secretary; these members have agreed to act in these capacities. Professor Kirk will be primarily concerned with all matters directly affecting the United States of America; the President suggested that Dr. Malissa might consider the formation of an international card index that would embrace all chemists active in the microchemical field, and that would aim at making available reliable information on workers in the different branches of the subject.

The Section Committee had asked that the Committee should advise on an alternative to the title "Committee for Microchemistry" (which had up till then been used provisionally) having regard to the lack of precision of the term "microchemistry." The following titles were agreed—

Commission des Micro-méthodes.
Commission on Microtechniques.
Kommission für Mikromethoden.

Whilst the scope of the Committee's interest was considered, no definite allocation of duties to individual members was made other than those already mentioned above. However, the President was given authority to refer special problems to individual members where he thought this advisable, and, if need be, to form special sub-committees to study such problems.

The following four scientific journals were approved by the Section as the official publications of the Committee—

Mikrochemie vereinigt mit Mikrochimica Acta.
Analytical Chemistry.
The Analyst.
Analytica Chimica Acta.

In the interest of co-ordinated progress, a special Sub-Committee was constituted to consider the international standardisation of microchemical apparatus. It was explained that this Sub-Committee would have to work in close co-operation with the existing Committee for Standardisation of Laboratory Equipment (General Chemistry Section of the Union) through one of its members who would also sit on that Committee. Drs. Marteret and Gillis, representatives of that Committee, had discussed this and were in substantial agreement. The Sub-Committee is to consist of Professor M. K. Zacherl (President), Dr. H. K. Alber (Philadelphia), Mr. R. Belcher (Birmingham), Dr. G. Gorbach (Graz), Dr. D. Monnier (Geneva).

It was resolved to ask the Committee of the Analytical Section to use its influence to ensure (1) that all research papers on analytical chemistry should be published in journals devoted to analytical chemistry, and (2) that such papers should include summaries in French, English, German and, where possible, Russian.

In order to reach all chemists interested in microchemical methods, and thus to provide a sound foundation for international co-operation on microchemical questions, it was hoped that through the Secretariat contact might be made with various national microchemical organisations, colleges and universities, and large laboratories. It is hoped, in the near future, to issue a questionnaire distributed on this basis.

The Committee elected Dr. E. Abrahamczik (Ludwigshafen) as an additional member.

Finally, it was agreed that the next session of the Committee should be on the occasion of the International Congress on Analytical Chemistry to be held in Oxford in September, 1952, under the auspices of the Analytical Section of the International Union.

H. MALISSA, *Secretary.*

Book Reviews

TRACE ELEMENTS IN PLANT PHYSIOLOGY. Pp. xix + 144 + 10 plates. Waltham, Mass.: The Chronica Botanica Co. London: Wm. Dawson & Sons Ltd. 1950. Price $4.50; 36s.

This book is a record of the papers presented at a symposium organised by the International Union of Biological Sciences with the support of UNESCO at the Rothamsted Experimental Station, Harpenden, on November 5th and 6th, 1947.

The presentation of the papers is preceded by a preface by Professor T. Wallace and a short foreword by Dr. M. J. Sirks, of Groningen. Following the opening address by the late B. Nemec, of Prague, possibly particularly stimulating from certain human angles by reference to work in his laboratory on the power of some plants to absorb and accumulate gold and by the exhibition of gold obtained from the ash of a plant from volcanic soils in Bohemia, T. Wallace brings up to date his well-known work on visual diagnosis of trace element problems in plants; his colleague, E. J. Hewett, then deals with large-scale sand-culture methods for the study of their trace element nutrition. Leo Gisiger, of Berne, recounts the results of the work that arose from the necessity in Switzerland during the recent war to extend the area of its arable land, when it was first assumed that the crop failures that often occurred on newly-acquired arable land were caused by deficiencies of the trace elements boron and manganese, but "in the course of time it came to be of great practical interest to assume that the above-named difficulties were not primarily caused by a deficiency of minor elements, but by an excess of lime." His paper on "deficiencies of minor elements caused by excesses" is an attempt to show some of the relations existing between limed soils and the occurrence of the so-called "deficiency diseases," and in it he develops the theory that over-liming results in a concentration of hydroxyl ions that causes the roots to swell too much, the resulting reduction of their osmotic pressure preventing their penetration into the soil. Daniel I. Arnon, of California, pleads for the sentiment now strongly in evidence in America that elements required by plants in minute quantities should be termed "micronutrients" rather than "trace," "minor" or "rare" elements. He further deals with the criteria of their essentiality for plants, starting from the basis that, while soil experiments are often inconclusive, biochemical evidence on the specific function of a micronutrient element is decisive. To illustrate this point, he quotes the example of copper, which was found to be the prosthetic group of several oxidases such as polyphenol and ascorbic acid; the demonstration of the presence of those enzyme systems in plant metabolism constituted conclusive evidence of the indispensability of copper. In his view, it is therefore necessary that experiments intended to determine the essential status of a given element should be carried out in artificial culture media. The investigations in his laboratory, recounted in some detail, are of considerable interest, particularly as such elements as

chromium, arsenic, lead, aluminium and cadmium are included. One important underlying consideration was the possible essential effects of such minute traces that extraordinary precautions had to be taken to ensure the purity of chemicals and water. In attempting to determine the purity of water of the stock solutions, an adaptation of the diphenylthiocarbazone (dithizone) test was used because this permitted the detection of 0·001 mg of the *combined* content of a group of metals: zinc, copper, nickel, cobalt, lead, mercury, cadmium, thallium and bismuth. By this means the metal content of the water was reduced to less than 0·001 p.p.m., and it was possible to establish that 1 part of zinc in 200,000,000 parts of culture solution—amounting to 0·001 mg of zinc to a plant—produced consistent and reproducible responses in plants. Finally, it is suggested that, as the possibility arises that at some time elements now regarded as dispensable may be found to be essential to plant growth, but in such exceedingly small amounts as to be within the limits of contamination of the present reagents, one of the first necessities is *a search for more refined analytical methods.*

Incidentally, the above method of using dithizone adds emphasis to the contention, which I hold very firmly, that the present-day enthusiasm for dithizone as a quantitative analytical reagent is very seriously overdone. Here it is used as a collective reagent for some nine elements; in analysis it is used as a specific reagent for single elements by means of control through small pH ranges, and what is not always realised is that any error in controlling the pH may mean the inclusion of an element other than the one being determined; as the final assessment is usually that of the intensity of a colour, either visually or by means of an absorptiometer, no warning of any error due to foreign inclusions will be given.

The importance of copper and molybdenum in the nutrition of higher plants and micro-organisms is dealt with by E. G. Mulder, of Groningen. The microbiological assay method based on the accepted assumption that the fungus *Aspergillus niger* requires copper and molybdenum for normal functioning was used in this work, and here again the necessity for extreme purity of reagents and water arose. The intimate relation of copper and molybdenum, particularly the latter, with the nitrogen nutrition of the plant is demonstrated. On the thesis that copper, manganese and iron form a physiological unit, Jorma Erkama, of Helsinki, discusses the effect of the two former on the iron status of higher plants; most factors that have an effect on the uptake and availability of iron regulate its degree of oxidation, and views are advanced as to the oxidation - reduction conditions in plants. Two papers deal with manganese: one by M. P. Löhnes, of Wagenigen, narrates how the necessity for increased food production in the Netherlands during the German occupation led to the growing of beans where they had never been grown before, and how the cause of the continued partial failure of the crop was traced to an excess of manganese; the other, by H. Burström, of Sweden, is concerned with experimental work on roots, both attached and excised, to determine the part that manganese plays in nitrate assimilation. Of some interest to the organic school of thought is the statement by Steenbjerg, of Denmark, in his investigation of micro-elements (manganese and copper) from a practical point of view, that in some soils humus is capable of fixing copper so that it is unavailable to the plant, and therefore a practical measure in these parts would be to reduce the humus content of the soil substantially over some years. J. Lavollay, of Paris, describes the experimental work on the activity and interdependence of elements in the nutrition of *Aspergillus niger*, not, in this instance, with trace elements, but with those, such as magnesium, that are indispensable to the physiology of plants. He is able to demonstrate quantitative interaction of nutrients based on the results of this work. The last paper in this volume is by L. Seekles, of Utrecht, and is an account of the mode of action and occurrence of trace elements in pastures and in the blood and liver of farm animals; it deals with conditional copper deficiency in the north-east diluvial part of Holland, with manganese in relation to grass tetany and sterility in the Netherlands, and with a supplementary research on copper, cobalt, molybdenum and selenium. The author has worked particularly on the investigation of the action of ions of trace elements on metabolic processes in the animal body and postulates two lines of such action: one, as constituents of the molecules of enzymes, vitamins and hormones, the other, as activators of enzymes.

Brewer defines a symposium as, properly, a drinking together; hence, a convivial meeting for social and intellectual entertainment; hence, a discussion upon a subject, and the collected opinions of authorities printed and published in a review. We, of course, properly use the word to cover a meeting in the last sense, but nevertheless there must have been on this occasion a greater incentive than usual to accept the other parts of the definition as being also fitting to the event. When it is realised that, after years of war isolation, workers from the occupied countries of Europe were again able to foregather with their co-workers of America and the United Kingdom,

and when it is further realised that the sheer necessity for increased food production in Europe had compelled continuous and determined efforts in agricultural investigational work—often under the fear that soon the last dim shadow might fall—then it would be thought strange indeed if the social and intimate human approaches had not been greatly in evidence. There can be little doubt that this feeling marked the meeting to an outstanding extent. Yet there can be even less doubt that the new theories advanced, the records of experimental field work submitted for examination and the discussion arising thereon were of such great national importance as to constitute a notable occasion. Professor T. Wallace, in his preface, summarises the proceedings in considerable detail and draws attention to the various developments of thought and practice, particularly indicating that while investigations of trace element problems have progressed to some extent in respect of crop failures, the work undertaken to investigate the effects on plant growth has been slow because of the difficult nature of the problems involved. There is a great need for fundamental studies for which it is necessary to develop highly refined methods, but it may be expected that intensification of biochemical research on the rôle of trace elements in enzyme systems will yield a rich harvest of knowledge. The paper by L. Seekles on farm stock is quoted in support of the call for collaboration between plant and animal physiologists to co-ordinate their work and so ensure the progress needed "to increase the world supplies of food sufficiently fast to meet the needs of the rapidly expanding world population."

GEORGE TAYLOR

PHARMACOPOEA INTERNATIONALIS. Volume I. Pp. xviii + 406. Geneva: World Health Organisation. London: H.M. Stationery Office; The Pharmaceutical Press. New York: Columbia University Press. 1951. Price 35s.; $5.00; Sw. fr. 20.

The publication for the first time of an International Pharmacopoeia must indeed be regarded as a historic event and a triumph for the committee of international experts who have at last overcome the difficulties of its production. The British representative and Chairman, Dr. C. H. Hampshire, is especially to be congratulated on the culmination of his efforts extending over many years.

The need for an international pharmacopoeia was recognised as far back as 1874, when an International Pharmaceutical Congress was held in St. Petersburg. Nothing was done until 1902, when, as a result of a conference held in Brussels, the first International Agreement for the Unification of the Formulae of Potent Drugs was drawn up and subsequently ratified in 1906. A second agreement was drawn up in 1925 and ratified in 1929. In 1937 a serious attempt was made to produce an International Pharmacopoeia, a Technical Commission of Pharmacopoeial Experts was set up under the League of Nations, but progress was stopped by the second World War. In 1947 the World Health Organisation took over the work, and the present publication is the first result of their efforts. Volume I contains 199 monographs, which are almost entirely confined to crude drugs and pure chemical compounds, with a number of antitoxic sera, a few tinctures, injections and solutions. No antibiotics are included. These, with other newer drugs, injections, tinctures and tablets are reserved for Volume II. Some of the omissions are surprising, *e.g.*, insulin and thyroid, and it is sad to see that Epsom Salts are not regarded as worthy of international status. Aconite, after being dropped from the U.S.P. and scheduled for omission from the B.P., finds a place here, as does aconitine, which does not even appear in the B.P.C. This is evidently a concession to Continental opinion.

Some of the Latin names have a strange appearance. One had always thought that *Pharmacopoeia* was a perfectly good Latin word, and it is somewhat of a surprise to find the penultimate "i" dropped in the Latin title. *Coffeinum* for caffeine, *oxydum* for oxide, *natrium* for sodium, *oleum jecoris aselli* for cod-liver oil and *secale cornutum* for ergot seem unnecessarily archaic.

It is to be expected that a reviewer of an international work of this kind will avoid insularity, but one cannot suppress a feeling of pleasure that the British name "ergometrine" has been adopted as the principal name with "ergonovine" as a synonym. Is it really necessary, considering the existing confusion of drug nomenclature to invent new names in addition to those in existing pharmacopoeias? There may be good reasons why the compound known in the B.P. and French Codex as "chloramine" is called by the clumsy name "tosylchloramide sodium," but if so, it seems regrettable.

It seems a pity, too, that it should have been necessary to use true density (*i.e.*, density corrected for buoyancy) instead of uncorrected density at 20° C as in the B.P. Both the B.P. and B.P.C.

have recently changed from specific gravity to weight per millilitre at 20° C (uncorrected), which has required the alteration of a large number of standards.

Doses are not given in the monographs on the drugs, but in an appendix. In this form of presentation there seems to be some danger of a prescriber taking a figure from the wrong line, with possibly disastrous results.

The majority of the analytical methods are taken from the B.P. or U.S.P., but some of the standards have been altered; some are more severe, others less so.

For the assay of aconite the method published by the Analytical Methods Committee of the Society of Public Analysts, which was adopted by the B.P., has not been accepted. In its place is found a method for the estimation of aconitine by determining the amount of benzoic acid formed on hydrolysis, a method that on the face of it has several objectionable features, including the measurement of volatile solvents by weight instead of by volume, a practice that is not adopted in the assays of other crude drugs. It seems that in this, as in other examples, no attempt has been made to secure uniformity of analytical practice. The B.P. method for total and water-soluble alkaloids in ergot is given, though it has been shown that the method given in the U.S. National Formulary gives results agreeing better with the true ergometrine content. Sometimes alternative methods are given, *e.g.*, the B.P. test for arsenic is included along with the hypophosphorous acid method; the B.P. test for lead is accompanied by the U.S.P. test for heavy metals calculated as lead.

Although the cover is not very prepossessing, the printing and paper are remarkably good. It is evident that great care has been taken to avoid errors and misprints.

Regarded as a first instalment, the production of this book is a praiseworthy effort. It is something of an achievement to have started the machine at all, but there are still obvious adjustments to be made before smoothness of running is accomplished. N. EVERS

Publications Received

ANÁLISIS CUALITATIVO RÁPIDO DE LOS CATIONES. By G. CHARLOT, D. BÉZIER and R. GAUGUIN. Translated into Spanish and extended, with an appendix on the rapid qualitative analysis of anions, by FRANCISCO BERMEJO MARTÍNEZ. Pp. 135. Santiago de Compostela (Spain): Porto y Cia. Undated. Price 35.00 ptas.

STRUCTURAL CHEMISTRY OF INORGANIC COMPOUNDS. Volume II. By WALTER HÜCKEL, Dr.Phil. Translated by L. H. LONG, B.Sc., Ph.D., A.R.C.S., D.I.C. Pp. x + 441 to 1094. Amsterdam: Elsevier Publishing Co. Ltd. New York: Elsevier Press Inc. London: Cleaver-Hume Press Ltd. 1951. Price 90s.

TABLE OF DIELECTRIC CONSTANTS OF PURE LIQUIDS. By ARTHUR A. MARYOTT and EDGAR R. SMITH. National Bureau of Standards Circular 514. Pp. iv + 44. Washington: U.S. Government Printing Office, for United States Department of Commerce. 1951. Price 30 cents.

MEDICINAL CHEMISTRY. Volume II. By ALFRED BURGER. Pp. xv + 579 to 1084. New York and London: Interscience Publishers Inc. 1951. Price $10.00; 80s.

GERMAN BOOKS ON CHEMICAL AND COGNATE SUBJECTS. SUPPLEMENT 1951 TO SECOND EDITION, 1939–1950. By A. E. CUMMINS and S. VINCE. Pp. 32 + Addenda (1 p.). London: Lange, Maxwell & Springer Ltd. 1951. Free of charge on request.

MÉTHODES ET RÉACTIONS DE L'ANALYSE ORGANIQUE. Volume I. MÉTHODES DE L'ANALYSE GÉNÉRALE. By M. PESEZ and P. POIRIER. Pp. vi + 276. Paris: Masson et Cie. 1952. Price 1800 fr.

FUNDAMENTOS DE QUIMICA ANALITICA EN MICRO Y ULTRAMICRO ESCALAS. By M. C. ALVAREZ QUEROL, D.Sc. Pp. xvi + 218. Madrid: Aguilar, S.A. de Ediciones. 1950. Price 80 ptas. (paper); 95 ptas. (cloth boards).

A FULLY qualified Engineering Metallurgist, preferably University trained, is required to take complete charge of Metallurgical and Chemical Laboratory of large precision engineering works situated in West London area. Candidates must be between the ages of 30 and 38, and have first-class knowledge and experience of alloy steels, heat treatment, machineability and performance in highly stressed mechanisms. Also desired is some experience with light alloys, plastics and metal surface finishes. The appointment offers considerable scope for a man with initiative and a progressive outlook, both on production and research work. The successful candidate will be appointed to the senior staff at a salary in the region of £1,000 p.a., and will be eligible for the Company's pension scheme. Write Box No. 3810, THE ANALYST, 47, Gresham Street, London, E.C.2.

SOUTHERN RHODESIA

APPLICATIONS are invited for the post of Senior Chemist to new Electro-Chemical Works producing ferro-chromium alloys. Applicants, who should hold degree or equivalent qualification, should possess wide knowledge and practical experience of analytical chemistry of metals, ores and minerals. Successful applicant will be required to take responsibility for supervision and operational control of chemical laboratory, and be capable of applying himself to new or expanding developments involving wider scope and increased responsibility. Applicants should state age, full details of academic and works training, experience and present employment. Periods of service with respective employers should be given, and details of domestic responsibilities should be stated. The successful applicant will be required to take up his appointment in Rhodesia towards the end of this year. The position offered is permanent, and applications, which will be received in confidence, should be addressed to Box No. 3808, THE ANALYST, 47, Gresham Street, London, E.C.2.

NATIONAL COAL BOARD—NORTH-WESTERN DIVISION

APPLICATIONS are invited for the post of Scientist Grade II in the No. 4 (Burnley) Area Laboratory at Altham Coke Works, Altham, Accrington.

This vacancy was originally advertised in November and December, 1951, but it has been decided to re-advertise. Candidates who submitted applications in 1951 need not re-apply as they will be considered along with the new applications.

The appointment will be within the revised salary scale—£645 × £30–£1,095—and commencing salary will be according to qualifications and experience.

Candidates should possess an Honours Degree in Science or the equivalent, and have had several years industrial experience. Experience on Coke Oven analysis would be an advantage.

The successful candidate will be required to undergo a medical examination and membership of the Board's Superannuation Scheme is compulsory.

Applications, giving full particulars of age, education, qualifications and experience (with dates) should be sent to the Divisional Establishment Officer, National Coal Board, North-Western Division, 40, Portland Street, Manchester 1, within 14 days of the date of this advertisement.

GLAXO LABORATORIES LTD. required for their Fermentation Research Establishment at Stoke Poges, Bucks, a Graduate in Chemisty or Biochemistry who has had analytical experience in a biochemical or pharmaceutical laboratory. Some experience in the development of analytical methods would be an advantage. Staff superannuation and incentive schemes. Applications in writing to Senior Personnel Officer, Glaxo Laboratories Ltd., Greenford, Middlesex.

REQUIRED by Non-Ferrous Smelting Company in Yorkshire, Chemists (analytical and research), aged 28–35. Initial salary according to qualifications and experience. Good prospects of advancement. Apply with full particulars to the Secretary, Capper Pass & Son Limited, Bedminster, Bristol, 3.

METALLURGICAL CHEMIST required by Marconi's Wireless Telegraph Co. Ltd., Chelmsford. Experience in ferrous and non-ferrous analysis essential and applicants should preferably have some knowledge of oil and grease analysis. Five-day week. Pension scheme. Please apply, giving full details and quoting ref. "English Electric 1/814" to Westminster Employment Exchange, Chadwick Street, London, S.W.1.

CHEMIST, recently graduated, wanted for work in connection with the control and development of works' processes, by manufacturers of aluminium sheet, extrusions and paste (paint pigment). The Chemist's work would be largely practical, but would include laboratory work in both organic and inorganic chemistry. Box No. 3805, THE ANALYST, 47, Gresham Street, London, E.C.2.

MINISTRY OF AGRICULTURE AND FISHERIES, VETERINARY LABORATORY, WEYBRIDGE

THE Civil Service Commissioners invite applications for a permanent appointment as Principal Scientific Officer (or Senior Scientific Officer with prospects of promotion to Principal Scientific Officer). The successful candidate will be graded according to qualifications and experience. Applicants must have been born on or before December 31st, 1920. Candidates must have a first or second class honours degree in chemistry or physics or show evidence of exceptionally high professional attainment. Some biological knowledge and appropriate experience is necessary. Duties will include: (1) responsibility for the freeze-drying of *Br. abortus* vaccine, and (2) co-operation in studies on mechanisation of production flow, on problems of air-filtration and the practicability of deep culture of *Br. abortus* and *M. tuberculosis*.

Salary scales:—Principal Scientific Officer (men) £1033–£1377, (women) £907–£1218. Senior Scientific Officer (men) £781–£980, (women) £649–£875.

Exceptionally a starting salary above the minimum may be granted according to qualifications and experience.

Superannuation provision under the Federated Superannuation System for Universities.

Further particulars and application forms from Civil Service Commission, Scientific Branch, Trinidad House, Old Burlington Street, London, W.1, quoting No. S4133/52. Completed application forms must be returned by May 1st, 1952. Candidates born between January 1st, 1921, and December 31st, 1926 (inclusive), may be considered for appointment as Senior Scientific Officer, but must apply through the Open Competition already announced (No. S53/52).

ANALYST required for the Chemical Laboratory of a large light engineering works in the West London area. Duties will include work on electro-plating, enamelling and general process control. Degree standard required. Commencing salary £600 to £700, according to age, qualifications and experience. Write giving details to Box No. 3804, THE ANALYST, 47, Gresham Street, London, E.C.2.

ROCHE PRODUCTS LIMITED have an opening for a man of Inter. B.Sc. or B.Sc. standard with experience in analytical work. Letter stating qualifications, experience and salary required to the Secretary, Roche Products Ltd., Welwyn Garden City, Herts.

RESEARCH ASSOCIATION OF BRITISH RUBBER MANUFACTURERS invites applications for following post. ANALYST, must be graduate in chemistry or an Associate of the Royal Institute of Chemistry, with experience of analytical work, starting salary in range £525 to £600 p.a. Good prospects of advancement on scales similar to Scientific Civil Service. Previous knowledge of rubber not essential. Present location Croydon, moving to Shrewsbury early 1953. Applications with full written particulars to Secretary, 105, Lansdowne Road, Croydon, Surrey.

RESEARCH ASSISTANT

UNIVERSITY GRADUATE, female, with majors in chemistry and mineralogy/geology required for base metal research laboratory in Northern Rhodesia. Duties principally microscopic examination of minerals and analytical determinations. Salary according to qualifications and experience, minimum £700 per annum. Fluctuating cost of living allowance now about £8 per month. Life Assurance and Contributory Pension Scheme. Cash Bonus Scheme which at present rate equal to 60% of basic salary. Outward passage paid. Apply giving full details of age, qualifications and experience to Foster Turner & Everetts Limited, Box No. 3123/1, 11, Old Jewry, London, E.C.2.

RESEARCH ANALYST. Applications are invited for a senior appointment in the Research Department of a large modern industry. A varied experience in non-routine industrial analytical work with a bias towards organic chemicals are desirable qualifications. Only University graduates, or candidates with the A.R.I.C. by examination, can be considered, and the post offers an interesting and satisfying career to the right man. The starting salary will be up to £1,000 per annum, depending on qualifications. Write Box No. 3809, THE ANALYST, 47, Gresham Street, London, E.C.2, quoting Ref. 1524/1654.

THE Research and Development Dept. of Distillers Co. Ltd. at Epsom require a chemist for investigational work on chemical and biological-assay methods for antibiotics and other fermentation products. Applicants should be under 30 and possess an Honours Degree or equivalent in Chemistry, and preferably have some experience of biological assay. Salary will depend on qualifications and experience. Apply Staff Manager, Distillers Co. Ltd., 21, St. James' Square, London, S.W.1.

THE ELY BREWERY CO. LTD., of Cardiff, invite applications from young qualified chemists for the post of Brewing Chemist in their new laboratories. Applicants should preferably have experience in brewing or food chemistry, but this is not essential. Only applicants with experimental aptitude and initiative need apply. Attractive salary and prospects offered. Apply, giving full particulars to the above.

SENIOR ASSISTANT, with Branch E, required by a London public analyst. Commencing salary about £800, with good prospects, including early deputyship. Write 1, Southwark Street, London, S.E.1.

YOUNG LADY, aged approximately 25–35 years, required for experimental testing work on electrical domestic laundry equipment manufactured by a large engineering company with laboratories in the West London area. Applicants should have a good general education, previous laboratory experience, and be prepared to carry out tests which are of a practical nature. Interesting and varied work, good conditions and pay. Write giving details of age and previous experience to Box No. 3811, THE ANALYST, 47, Gresham Street, London, E.C.2.

YOUNG LADY, aged approximately 22–30 years, required for the Chemical Laboratory of a large electrical engineering company in the West London area. Applicants should have studied Chemistry up to H.S.C. or Inter. B.Sc. standard and should be experienced in chemical analysis. Five-day week, good conditions and pay. Write giving details of age and previous experience to Box No. 3812, THE ANALYST, 47, Gresham Street, London, E.C.2.

ANALYTICAL CHEMIST (28), B.Sc., A.R.I.C., 10 years' industrial and research experience—metallurgical, paper, food, organic, including modern instrumental methods of analysis—requires progressive position in London or Home Counties. Write Box No. 3806, THE ANALYST, 47, Gresham Street, London, E.C.2.

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