See Notice on p. 194

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THE ANALYST

The Journal of The Society of Public Analysts and other Analytical Chemists

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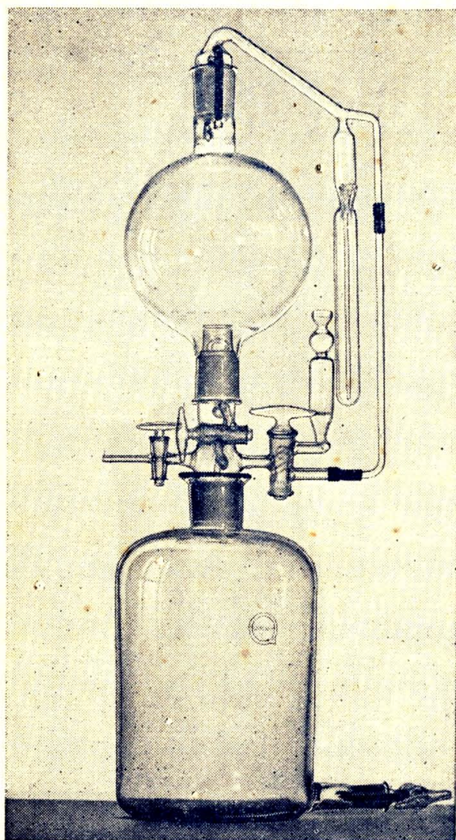

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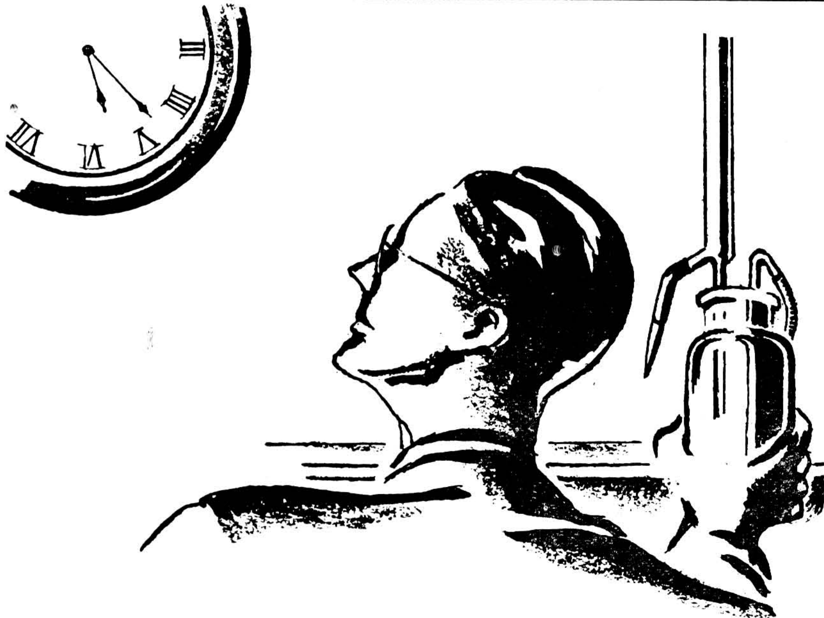

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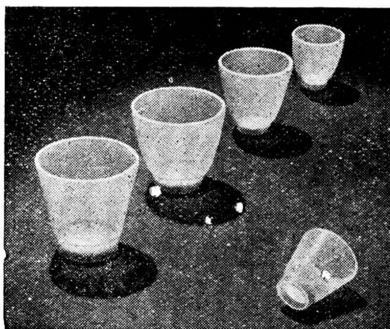

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 5 p.m. on Wednesday, April 4th, at The Chemical Society's Rooms, Burlington House, London, W.1, with the President, Dr. G. W. Monier-Williams, in the chair. The following papers were presented and discussed: "The Freezing Point of Sour Milk," by F. J. MacDonald; "The Electrometric Determination of Ascorbic Acid," by H. Liebmann, Ph.D., and A. D. Ayres, B.Sc.; "Magnetic Stirring in the Electro-Deposition of Metals," by H. W. Webb.

NEW MEMBERS

Arthur John Marson Bailey, B.Sc., A.R.I.C., M.P.S.; Francis Raymond Cropper, B.Sc., Ph.D.(Lond.), F.R.I.C.*; Edward Romer Dawson, M.Sc., Ph.D. (Leeds); Norman Entwistle, B.Sc. (Manc.)*; John Gandy, B.Sc. (Lond.)*; Milton Blackburn Ormerod, B.Sc. (Manc.), A.R.I.C.*; George Reginald Short, Ph.C.; John Valentine Smart, A.R.I.C.*; Donald Murgatroyd Smith, A.R.C.S., B.Sc. (Lond.), D.I.C., F.Inst.P.; Samuel Douthart Steele, B.Sc. (Glasgow), A.R.T.C., A.R.I.C.

DEATHS

WE regret to have to record the deaths of:

James Kear Colwell
Charles Edwin Corfield
Alan Percy Platt
Robert William Richardson

Address of the Retiring President, S. Ernest Melling

[Delivered at the Annual General Meeting, March 9, 1945]

MR. PRESIDENT, LADIES AND GENTLEMEN

Having introduced my successor and vacated this honoured Chair it is now my responsibility and privilege to address you.

During 40 years' membership of the Society, I have had the pleasure of attending many Annual Meetings and listening with profit to the Addresses of our successive Presidents, who have, quite appropriately, taken the opportunity to make some observations upon the progress and affairs of the Society, with special reference to important events during their respective periods of office. They have, for example, touched upon our relationship with outside bodies including, of course, various Government Departments, whose views and actions may very much concern the Public or other Official Analyst; they have focused attention upon legal enactments in being and the consequences of their implementation, or of impending legislation by which, on technical grounds, we may not have been too impressed; they have given some thought to ethical and professional considerations, based upon wide and mature experience; have proffered sound advice, in one way or another, to the younger members of the Society, and have had the melancholy task of making sympathetic reference to the loss sustained by the Society in the death of distinguished members during their tenures of office.

Hence, following these precedents, I should like to refer to one or two outstanding events of the past two years.

In the first place, the duty—inevitable and painful as it is—devolves upon me to join you in giving a passing salute to all those who have left vacant places in our midst, and, in silence, record our gratitude for their contributions not only to the furtherance, of our domestic interests, but also to the enrichment of analytical and technical knowledge, and, no less, to their support and comradeship.

* Through the North of England Section.

Whilst all those whom death has removed have their share in our regard, it is fitting that special reference should be made to our one lady Honorary Member, Miss M. B. Elliott, who, by her enthusiasm and exceptional abilities, strove, in health and sickness, to ensure that our business affairs should be efficiently conducted and the high reputation of our journal fully maintained. How well she succeeded we all gratefully recognise, and it is good to know that our Editor (Dr. Ainsworth Mitchell)—than whom no one is better qualified to speak—in a sympathetic tribute to her character and rich gifts, has placed on permanent record in THE ANALYST our immense indebtedness to her for loyal and devoted service throughout, at least, sixteen strenuous years.

I would, also, mention the further grievous, and, to most of us, quite unexpected loss we suffered only a few months ago. In the death of our distinguished member, Sir John Fox, a wide circle of chemists, inside and outside the Society, and officials representing many branches of the Public Services, deplore the passing of a man endowed with quite remarkable gifts. This is neither the time nor the place to attempt an account of his versatile achievements, nor a recital of his eminent services to analytical chemistry, in its widest sense, nor yet, again, of the trust reposed in him by the State. Such a tribute must come from an abler and a far more authoritative source, but, as one of many, I cannot forbear saying how deeply we, as Public Analysts and Official Agricultural Analysts, regret the loss of a Government Chemist in whom we all had supreme confidence, whose decisions we accepted without questioning, and whose wide vision, gift of friendliness and charm of manner have left an abiding impression of wholesome admiration and profound respect.

Only 8 weeks ago another prominent figure passed from sight by the painfully sudden death of Dr. G. D. Elsdon, whose contributions to our journal throughout many years reveal a rare catholicity of investigation in the domain of bromatology, and it is indeed deplorable that his later career as an administrator in the responsible public service of River Pollution prevention in Lancashire has so prematurely been brought to a close. As one, moreover, who played a prominent part in the inauguration of the North of England Section of the Society in 1924, we shall gratefully remember his friendly spirit and unselfish services and recall his enthusiasm in all matters pertaining to the enlargement and welfare of our corporate life.

The Council's Report for the current year embodies the Obituary List and gives all the relevant statistics as to the number and character of our meetings, membership and so forth, so that I need not repeat such information here, but one must certainly mention the extension of the scope of our activities by the adoption of the Group system—clearly within the framework of our constitution—which, in its fullest sense, has already given momentum to the Society and will enhance, still further, its high reputation. It is, indeed, difficult to conceive opposition to the creation of Groups, with their individual and special interests, provided that they are fundamentally allied to analytical chemistry. There is no justification in taking up your time now in recalling the negotiations, whereby the Microchemical Club—whose membership included several enthusiastic pioneer workers in this branch of chemical analysis—was encouraged to join us and so provide a powerful nucleus of the Microchemistry Group. This came into being last October, when Dr. Janet Matthews gave us a most interesting historical survey of the inception and rapid development of this elegant and precise technique. Already under the guidance of its Chairman (Professor Briscoe) the calibre of its work has been seen in valuable contributions towards our knowledge in several directions, and we have confidence that it will gather an ever-increasing impetus and success in the boundless field of analysis and research.

Then, only a month ago, the Physical Methods Group had its inaugural meeting, at which Mr. Chirnside was elected Chairman. His stimulating address, showing how new approaches to old problems are being made by refined methods based upon the use of instruments of far greater precision, stressed the necessity of keeping abreast of this work and, to the industrial chemist and Public Analyst alike, indicated the need of a common basis for papers and discussions of their problems.

The immediate future, indeed, is full of promise, for these and, doubtless, other Groups, the formation of which may be requested by various sectional interests.

If one were to take war-time difficulties and problems as the text of an address, the field is so wide that an hour's consideration of them would be a modest estimate, but, in any

event, one should strive on such an occasion as this to reduce a recital of our difficulties to a minimum. I make passing reference to only one phase, affecting the Public Analyst and Official Agricultural Analyst in particular, and not altogether in a spirit of light relief, *i.e.*, the ever-increasing spate of Statutory Rules & Orders (mainly issued by the Ministry of Food) and other emergency measures, to keep pace with which is no light matter, and some of which present peculiar difficulty in their application. Many years have gone by since a colleague, discussing the value of the freezing-point test of milk, queried whether investigation of the test would have been so widely, vigorously and even enthusiastically pursued, in the absence of a certain type of adverse criticism—at times ventilated in Petty Sessional Courts which became, as it were, the cockpit of somewhat heated debate and dialectic discussion! Though the analogy may not be well chosen, since Necessity is the Mother of Invention, the call for investigation of certain problems in order to implement satisfactorily one or other of these Orders is clamant, and there is little doubt but that further demands will be made on the time and activities of the Analytical Methods Committee.

A more intimate note of our family life is struck by reminding you (if it be necessary) that we have this year achieved our seventieth birthday, coupled with the happy experience of celebrating the occasion by offering our congratulations and best wishes to our greatly-respected leader and head of the profession, Dr. Bernard Dyer, who was elected an Associate Member at the first General Meeting of the Society held on February 5th, 1875.

I suppose such a record is unique in the history of any scientific Society—certainly in this country.

Since the outbreak of war, Dr. Dyer, still young in spirit and with an ever-alert mind, has had to forego many of his activities (much against his personal wishes), and it may well be that some of our younger members who have joined us since 1939 know him only by name and reputation. Well, that is their loss, but I express the confident hope that—with an early termination of hostilities in Europe—they may have the opportunity of meeting him at one or other of our sessions and so realise something of our especial regard for one to whom the Society owes so much.

Before coming to the subject which forms the basis of my main observations, may I confess the difficulty one had when considering what form this address should take?

My first impulse was to trespass upon your patience by dilating on the subject of "Cheshire Cheese," possibly because one had (very many years ago it is true) to devote much attention to a consideration of its manufacture, composition and properties, with a view to fixing a fat-standard for the genuine product, which, in the absence of any statutory limit, would be accepted by a Bench of Justices. (This has no reference of course, to the Agricultural Produce—Grading and Marking—Act, 1928, under which aëgis a subsequent S.R. & O., issued in 1933, respecting this brand, defined a minimum fat-content, expressed on a dry basis, of 45%). Although I rejected it as too narrow and parochial, on such an occasion as this, one cannot resist the temptation to make passing reference to the staple product of a county which one has had the honour to serve for 40 years. There is almost as literary a flavour about this particular style of cheese as about wine, and it has been uncommonly interesting to dip into its lore while studying at close quarters the craft, the secrets of which have been handed down from mother to daughter for generations. Before it suffered a decline in social status—presumably owing to the fact that, by the eighteenth century, it had come to be regarded as food for the labouring classes—we find Thomas Cogan, English philosopher, physician and minister (who, by the way, had the gift of pithy expression*), interpreting an old Latin verse showing the qualities of a good cheese in this way:

> "Cheese should not be white as Snow is,
> Nor full of eyes as Argos was,
> Nor old as Methusalah was,
> Nor full of Whey or weeping as Mary Magdalene was,
> Nor rough as Esau was, nor full of spots as Lazarus!"

Even in those far-off days many people preferred the Cheshire brand made at Nantwich to the Banbury product, which was supposed to be the best.

* He was the author of the expression "But mum's the word; the least said, the soonest mended."

Coming to more modern times, you will recall that in *Cranford*, Mrs. Gaskell described how well-bred ladies regarded this article of food as plebeian, and were careful to refuse it—at least in public—and this prejudice seems to have spread abroad and lingered in unexpected places.

On the other hand, one must not forget the Cheshire merchant who visited Spain, and there met a native who extolled the rich fruits and spices of his country, claiming that "there's no such like in thine." Holding aloft one of his specimen cheeses, the Englishman riposted thus:

> "Your land produces twice a year
> Rich fruits and spice you say,
> But what I in my hands do hold,
> Our land gives twice a day . . ."

and so one might go on in this strain to describe how the introduction of the New Testament into Tranquebar (Hindustan) was the result of Cheshire cheese, or again, to extract from Thomas Fuller's *Book of Worthies*, an explanation why, despite the importation of kine and dairymaids from the north-west, farmers in the rich southern counties were disappointed in their attempts to produce the real article. They should, of course, have carted the land to give them a sporting chance!

WATER AND WATER SUPPLIES

I come now to the main theme in this address, which relates, from the hygienic standpoint, to "Water and Water Supplies"—and which, I venture to hope, may appeal to the Other Analytical Chemist as well as to the Public Analyst, since both are competent to be consulted in matters relating thereto. Moreover, my observations may be considered pertinent in view of the fact that the Water Bill had, only a few days ago, its second reading in the House. As a sequel to last year's White Paper, "A National Water Policy," the Bill is an earnest and voluminous effort on the part of the Government, and if it is not regarded with complete approval in certain quarters, it none the less makes a good start in a long-term programme and visualises an effective partnership between the State and the Authority. Moreover, as a most important phase of the social stability of the State, in post-war planning alone, it must naturally concern all of us, and it is upon its main aspects and implications that I wish to speak.

First, it should be briefly mentioned that, following quickly on the heels of the Government's policy, outlined in the White Paper, Parliament passed into law during last Session the Rural Water Supplies and Sewerage Act, 1944—a good example of preparatory legislation —which contains many of the essential ingredients of the Rural Water Supplies, Act, 1934, and follows the same general lines.

The inclusion of facilities for sewage disposal, however, is an altogether new departure, and makes good the main inadequacy of the previous Act, for without this the extension of piped water supplies to country districts may make an unsatisfactory position still worse. The combined cost, after the Government has contributed its quota, will be more equitably distributed, since it will be charged to the general rate spread over the whole rural district. Thus the estimated cost of one penny per day to consumers in small houses may be quite feasible, but whether the Treasury Grant of £15,000,000 will be sufficiently substantial is another matter. One has only to visualise the long stretches of mains to remote and scattered houses, the additional outlay on sewage disposal (however simple the method) and so on, with the concomitant low rateable values and the need to consider how we are to guard against a repetition of what happened subsequently to the 1934 Act, to realise the complexities as a whole.

In the formulation of these prior schemes it soon became evident that the *pro rata* financial responsibility borne by the Exchequer could not keep pace with their estimated cost. For example, one Authority propounded a scheme involving somewhere about £125,000. The grant-in-aid in this instance was only of the order of £3,000, and, since the County Council was only prepared to contribute a like amount, the scheme fell through.

Here, with no intention to question the broad principle, may there not be disappointments in store if the proposals for distribution are really intended as a vital factor in a sociological programme which should apply equally throughout? After all, water supply should be the major consideration in country planning, which is very much to the fore. You must have been impressed, when touring more-or-less remote rural areas in pre-war days, by the moribund

state and general appearance of decay of many of the smaller farms and outbuildings brought ibout, maybe, by uneconomic returns from agriculture, mortgage burden and so forth. Obviously, the small farmer's resources have been insufficient to keep the property in repair, :o say nothing of modern plant and machinery equipment. May there not be a chance, :herefore, of the disappearance of these farms, unless they undergo a drastic restoration to keep them abreast of the times and worthy of housing agricultural workers of the type competent to cope with the conditions of modern farming? Many of the farms can show ι long family history, having been occupied by successive generations, which throughout ılternating periods of prosperity and hardship have become rooted in the soil from which :hey have drawn their sustenance. To such as these the farm is not merely a means of ivelihood; it is home. If the State is prepared to play its part in a sound scheme of rehabilita-:ion of such premises, well and good, but until the Minister of Town and Country Planning reveals his plans it seems to me that, as matters stand, there is a potential weakness in that 'undamental unity which demands a comprehensive vision in dealing with the varying aspects of agricultural life.

Now as to the more recent Bill, the time at my disposal demands that I should limit ny remarks to only a few of its provisions which may be of direct or indirect interest to :he analyst.

Included in the Central and Local Planning proposals is the very important matter of ınderground supplies, whose potentialities of boundless yield, given favourable geological conditions, will relieve water undertakers, as a whole, of their anxieties where reservoir capacity is less than, say, 150 days' supply. Avoiding reference to the legal aspect—upon which, indeed, I am not competent to speak—one might reasonably suggest that, by tapping ıidden resources, the immobilisation, by water undertakers, of catchment areas might argely disappear. It is common knowledge that one Authority closed down about 35 farms on its gathering-ground in a matter of seven or eight years, thus making a considerable stride :owards the desolation of a countryside. Although our knowledge of these sources is, on the whole, somewhat vague, we do know that drought conditions do not affect the vast majority of deep-seated boreholes for a long time, if, indeed, at all. Some waters, no doubt, owing to ıardness, the presence of iron and manganese compounds or other mineral salts, may require pre-treatment, but invariably (or almost invariably) one finds a bacteriologically-sound condition accompanying a state of complete biochemical stability and high organic purity. [n the abstraction and development of future supplies, the pre-requisite is that, in every nstance where it is proposed to obtain water by well or borehole to a depth beyond 50 feet, :he person doing so must supply the Committee of the Privy Council for Scientific and Indus-:rial Research with all relevant information. This overcomes the objection, frequently raised in the past, concerning the strange state of affairs which allows anybody to sink a well on his property, without anyone's permission, withdraw as much water as he pleases, and without keeping any records, provided that he does not sell it to the public but uses it himself. Hence breweries, dairies and other large-scale users have, quite naturally, availed themselves of these facilities, and, should for any reason their industry close down, they are equally entitled to leave the bore-hole derelict. You will perhaps recall some Member giving the House a most amusing account of Lloyds Bank and the Bank of England entering into competition in this relation, but the ultimate reason for such extraordinary rivalry never :ranspired. Reverse such a procedure and one can well imagine *Punch's* reaction to the situation.

That the legislation now proposed is fully justified there can be little doubt. Up to the present, and not confining the issue to underground supplies, the recommendations of Regional Advisory Water Committees, set up by the Ministry of Health, to survey the resources of a listrict, have been rendered abortive, simply because they had no legal power to enforce :hem. Moreover, the collection of information has been slow and cumbersome, especially so in respect of the smaller Authorities, and the survey has been further complicated by re-arrangement of Local Government districts. Now, the Minister of Health's powers can nsure a drastic re-casting of the data already obtained regarding available resources and possible future development, and his decision will be absolute and binding.

As regards Local Organisation of Water Supplies (Part II of the Bill), my observations nay appropriately deal with Joint Water Boards, with their involved combination of water ındertakers and their supply to premises outside their boundary.

I have mentioned the foregoing in order to provide a simple illustration of the principle, with which some members of the Society may be confronted in the near future, by referring to the possibilities of the water-bearing strata of the triassic formations permeating certain Midland Counties, capable, when developed, of yielding unlimited volumes of pure and wholesome water. One would select, as a background, an area which is quite typical, the shire involved being essentially agricultural. In periods of drought it is one of severe contrasts. Inhabitants of one village, dependent on shallow wells, have to make long and tedious journeys to satisfy their meagre potable wants, or, alternatively, water is delivered by carts. These unfortunate people need little reminding of Benjamin Franklin's proverb "When the well is dry, we know the worth of water."*

A similar neighbouring community has water laid on from a source of upland supply—in one district 50 or 60 miles distant and in another 120 miles away. Such disparity has its inevitable repercussions and, with an awakened social conscience, little wonder, since we have in this country abundant water (actual and potential) for all our domestic needs and more than sufficient to meet the ever-increasing industrial and agricultural demands.

In furtherance of a project relating to this state of affairs, one can think of no more commendable example of a close co-operation of the practical geologist, the engineer, the medical officer and the analyst, resulting in the ideal team-work capable of affording a satisfactory answer to a many-sided problem.

In an opening sentence I made reference to Presidents having offered advice to younger members as the result of their personal experience, and if I venture to do so here it is because the truth of the saying of Marcus Aurelius: "We are born for co-operation as are the feet, the hands, the eyelids and the upper and lower jaws," has been driven home to me on many occasions. That spirit of *esprit de corps* which characterises our members, fostered as it has been by informal talks and discussions in a sociable atmosphere—coupled with a keen desire for effective co-ordination—is equally attainable in a much wider field of professional relationship.†

What I hope mainly to convey in this summary is that the natural survey of the country's water resources and subsequent control of water supplies in general necessitates: (1) consideration of the history of the supply, its source and the topography of the catchment area in question; (2) determination of its analytical and physical characters; (3) determination of its bacteriological condition, in order to arrive at a reliable and just opinion regarding wholesomeness or otherwise. As to the comparative value of these methods of appraisal one must not be too dogmatic, but I would like to say a word or two about the importance of chemical analysis and physical observations.

You may recall discussions in this room in by-gone years on the use of the term "pure and wholesome." That couplet has now largely disappeared. The exclusive use of the word "wholesome" (or wholesomeness) in Part IV of the Bill is very noticeable; indeed, reference to "purity" apparently is confined to one Clause only relating to "Agreements as to Drainage, etc. of Land." It must therefore be comprehensively interpreted in the light of a modern dictionary definition: "tending to promote health." If water is not looked upon favourably by the consumer—it may be owing to high colour, marked opalescence or turbidity, the presence of macroscopic débris, abnormal odour or taste, to all of which an aesthetic objection may be taken—presumably it is unwholesome! Add to this list, labile fermentable components (whose presence is revealed and break-down products measured by arbitrary methods), to say nothing of a remote history, as indicated by nitrification with or without denitrification, and it is manifest that the non-bacteriological aspect cannot be regarded as of secondary importance in the final adjudication, despite the last line of defence, *viz.*, efficient sterilisation.

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* This American philosopher and statesman interested himself in the physical properties of water. Having demonstrated the quiescent effect of a few ml of olive oil upon an extensive area of turbulent water, he put his knowledge to neat political use. Accredited to France as diplomatic representative of America, then struggling for independence, it is said that he was wont to illustrate his arguments with members of the French Court by shaking his stick, in which a little oil was concealed, over the rippled surface of the lakes at Versailles, so producing an apparently magical result and thus increasing his prestige.

† At this stage of the Address a rough plan was shown of the hydro-geological lay-out of a district under review for the establishment of a Water Board. This illustrated a commentary on the disposition of the Keuper marls and glacial drift deposits in relation to the surrounding outcrop area represented by the Keuper and Bunter sandstones; also on the respective positions of small towns in the district, with the outlying bore-holes supplying them.

The fact that plumbo-solvency determination falls within this category perhaps justifies me in mentioning the responsibility which a decision of the Court of Appeal about seven years ago puts upon a water undertaking, since even yet it does not appear to be universally recognised. The Irwell Valley Water Board (Lancs.) unsuccessfully appealed from an award to a husband and wife as compensation for having suffered from lead poisoning caused by the quality of the soft peaty water furnished by the Board, who undoubtedly knew that their supply was plumbo-solvent and was liable to erode lead. The question was this: had the Water Board a further duty in the light of this knowledge, to warn consumers to take due precautions? Now, although there was no breach of statutory duty to pass pure water through their mains, there were two practicable and reasonable means available whereby these specific complaints could have been met; neither of these had been taken and therefore the appeal was dismissed.

Just one more observation or query (and by no means a cynical one) about *wholesome* quality *vis-à-vis* chemical analysis. Without invoking analytical values, how could one possibly check, one way or another, the remarkable statement, ascribed to an eminent authority when speaking on water at a recent meeting of scientific people, that "We are even in some of our great cities eating soap by the ton and drinking other people's bath water"?

During the course of a Conference which representatives of a Joint Committee of the Royal Institute of Chemistry and ourselves had in May last at the Ministry of Health, concerning certain differences arising from the activities of Emergency Public Health Laboratories, the Minister (Mr. Willink) gave a very broad hint that the analyst's services would be greatly welcomed in a co-ordinated effort to implement the policy now disclosed, since he was persuaded that, by training and experience, the analyst or water examiner was well qualified to contribute materially to this common cause.

Nevertheless we must not minimise the difficulty frequently experienced in making a strict comparison of the nature and character of waters reported upon in different parts of the country, and therefore it would seem an opportune moment to suggest that a series of standard methods of chemical analysis should be formulated—particularly so for obtaining certain data such as, *e.g.*, total and temporary hardness, soda alkalinity, dissolved oxygen and free carbonic acid and an officially-recognised technique for the determination of plumbo-solvency. This would not, of course, prevent analysts from supplementing such standard methods by any other tests they might think fit to employ. Already we have in this country an analogous publication dealing with sewage and sewage effluents (*Methods of Chemical Analysis applied to Sewage and Sewage Effluents*).

The Analytical Methods Committee of the Society, through a special Sub-Committee, might usefully apply themselves to such a task along the lines of Standard Methods for the Examination of Water, approved and adopted by the American Public Health Association and the American Waterworks Association.

Agreed standard methods of chemical analysis, in conjunction with the Ministry of Health Bulletin No. 71 regarding bacteriological examination, would allow water examiners throughout the country to submit reports which would be strictly comparable one with the other, and thus permit a proper evaluation of the respective merits and demerits of waters where a choice of supply might be in question. There remains the ultimate responsibility of the correct interpretation of the analytical values. In this connection it is manifest that any limit of the organic components present in the water, as indicated by the yields of ammoniacal and albuminoid nitrogen and the amount of oxygen absorbed, cannot be generally applied to all supplies, whatever their source. Thus, whilst it may be desirable to have in mind some standard of purity or limits of impurity as measured by such empirical tests, judgment of supplies derived from deep-seated sources must be on a radically different basis from that employed when dealing with shallow supplies, lake water, etc., especially so if the gathering ground of the latter is of a peaty nature.

What is finally wanted, and with equal urgency, is a concise addendum to any such official methods setting out, after the most searching enquiries, through other and more competent channels, the maximum permissible amount of metallic contamination (notably lead, zinc and copper), together with fluorides or other potentially harmful salts in supplies of drinking water.

The Cobalticyanide Ion as a Precipitant for Metal Ions

Determination of: (a) Cadmium in Pb-Sb-Cd and Pb-Sn-Cd Alloys; (b) Silver in Lead; Manganese in Citrate Solutions

BY B. S. EVANS AND D. G. HIGGS*

POTASSIUM cobalticyanide has been recommended as a precipitant for nickel and cobalt[1] and for separating copper from selenium and tellurium,[2] and it has also been used by us as a spot test reagent for certain metals[3]; beyond this its use as an analytical reagent appears to have been completely overlooked. It seemed desirable therefore that an extended survey of its analytical possibilities should be undertaken.

TABLE I

Metal ion	Acid: Hydrochloric	Acid: Nitric	Acid: Sulphuric	Colour of ppt.	Remarks
$Zn^{\cdot\cdot}$	N	C 15–20	C 15–20	White	
$Cd^{\cdot\cdot}$	N	C 15–20	C 15–20	,,	
$Cu^{\cdot\cdot}$	C 10	P 15	P 10	Blue	The HCl strength is rather critical for small amounts.
$Cu^{\cdot}$	C 1–20	—	—	White	
$Ni^{\cdot\cdot}$	C 5	C 5	C 5	Pale green	Acid strength not investigated
$Co^{\cdot\cdot}$	C 5	C 5	C 5	Pink	Acid strength not investigated
$Pb^{\cdot\cdot}$	N	N	—		
$Sn^{\cdot\cdot\cdot\cdot}$	N	—	—		
$Sn^{\cdot\cdot}$	N	—	N		
$Sb^{\cdot\cdot\cdot}$	N	N	N		
$Mg^{\cdot\cdot}$	N	N	N		
$Al^{\cdot\cdot\cdot}$	N	N	N		
$Zr^{\cdot\cdot\cdot\cdot}$	N	N	N		
Ba	N	N	—		
$Ca^{\cdot\cdot}$	N	N	—		
$Fe^{\cdot\cdot\cdot}$	N	N	N		
$Fe^{\cdot\cdot}$	C 30–50	—	C 5–20	H_2SO_4: Lemon HCl: White to pale lemon	
$V^{\cdot\cdot\cdot\cdot\cdot}$	N	N	N		
$V^{\cdot\cdot\cdot\cdot}$	N	—	C 20–25	Pale blue	
$Be^{\cdot\cdot}$	N	N	N		
$Ce^{\cdot\cdot\cdot}$	N	N	N		
$Ce^{\cdot\cdot\cdot\cdot}$	N	N	N		
$Ti^{\cdot\cdot\cdot\cdot}$	N	N	N		
$Ti^{\cdot\cdot\cdot}$	P	—	P	Pale buff	
$Ag^{\cdot}$	—	C 1–15	—	White	
$Bi^{\cdot\cdot\cdot}$	N	P	C 25+1% Na_2SO_4	,,	The H_2SO_4 conditions are critical.
$Cr^{\cdot\cdot\cdot\cdot\cdot\cdot}$	N	N	N		
$Cr^{\cdot\cdot\cdot}$	N	N	N		
$Cr^{\cdot\cdot}$	P 50	P 40	P 25	HNO_3: Yellow HCl } H_2SO_4 }	"$Cr^{\cdot\cdot}$" here merely means a stage of reduction below $Cr^{\cdot\cdot\cdot}$.
$Mn^{\cdot\cdot}$	P 20	P 15	C 25+5% $C_2H_4O_2$		Mn. The acid conditions are critical.
$Hg^{\cdot\cdot}$	N	P1–40	P 1–25	Yellow	
$Mo^{\cdot\cdot\cdot\cdot\cdot\cdot}$	N	N	N		Colour impossible to observe consistently owing to formation of molybdenum blue and cobalt cobalticyanide.
Mo (reduced)	P	P	P	Yellow	
$U^{\cdot\cdot\cdot\cdot\cdot\cdot}$	N	N	N		

The cobalticyanide ion closely resembles the ferricyanide ion in most of its reactions. It has, however, important advantages as compared with the latter, chiefly connected with its marked stability; above all it is not at all easy to reduce, and hence involves no difficulties akin to those associated with the production of ferrocyanides. The results of a qualitative survey of the behaviour of the cobalticyanide ion with a number of metal ions under varying conditions of acidity are given in Table I. It was early found that the influence of the acid

* Communication from Armaments Research Department, formerly the Research Department, Woolwich.

was not always merely a question of hydrogen ion content but that the anion seemed sometimes also to play a part; each ion therefore (where feasible) was tried in each of the three common acids—nitric, hydrochloric and sulphuric. In the table, C means apparently complete precipitation, P—partial precipitation and N—no precipitation; a stroke indicates that for some reason the trial could not be made. The numbers accompanying the above letters refer to acid concentration and represent the number of ml of the strong acid present in 100 ml of solution.

Quantitative investigation was, of course, concentrated solely upon those elements with which complete pptn. had been obtained; nickel and cobalt, having been already dealt with,[1] were not tried. The determination of chromium in the lower stages of oxidation seemed to have little practical interest and was not pursued further.

Processes for the quantitative determination of the following elements, with prior separation as cobalticyanides, were worked out: cadmium, zinc, copper, silver, bismuth, manganese, iron and vanadium. In certain instances these processes seemed to have direct practical applications, with considerable simplification of existing methods; for such separations directions are given at the end of the appropriate sections.

Quantitative Investigations

(1) Cadmium—Quantitative pptn. is obtained in an acid concn. of 15 to 20% of conc. nitric or sulphuric acid; hydrochloric acid dissolves the ppt.; hence only a partial, if any, precipitate is obtained in presence of chlorides. Large amounts of alkali or ammonium salts appear to cause partial pptn. As with most cobalticyanide ppts., it is necessary to wash with an acid soln. containing cobalticyanide; omission of the latter ingredient causes some of the ppt. to redissolve or to pass through the filter.

Varying amounts of cadmium as sulphate were each dissolved in 20 ml of strong sulphuric acid diluted to 100 ml with water; 20 ml of a 10% soln. of potassium cobalticyanide were added to each, and the resultant liquids were vigorously stirred and allowed to settle for ½ hour. The cadmium cobalticyanide ppts., which were white and flocculent, were filtered off on well pressed-down pulp filters and washed several times with 20% sulphuric acid containing 1% of potassium cobalticyanide. The washing was continued with 20 to 30 ml of 10% sodium hydroxide soln., part of which was used to rinse-in the precipitation beaker; this treatment converted the cadmium cobalticyanide into hydroxide, which was finally washed several times with water. The cadmium hydroxide was dissolved through into a beaker with 50 ml of water containing 7 ml of diluted sulphuric acid (1+3) followed by washing with 50 ml of water in successive small portions. The cadmium was pptd. as sulphide and determined by weighing as $CdSO_4$ in the usual manner. The weight so obtained was corrected for traces of silica and of residual acidity (H_2SO_4).

Cd taken	$CdSO_4$ (impure) found	SiO_2	H_2SO	Cd found
g	g	g	g	g
0·0050	0·0104	0·0006	0·0002	0·0052
0·0100	0·0212	0·0015	—	0·0106
0·0200	0·0317	0·0007	—	0·0196
0·0300	0·0584	0·0014	—	0·0307
0·0400	0·0772	0·0013	—	0·0409
0·0500	0·0944	0·0010	—	0·0503

Determination of Cadmium in Cable-making Alloys—As lead, tin and antimony are not pptd. by cobalticyanide, while cadmium can be pptd. completely in nitric acid soln., it seemed likely that a simple method for determination of cadmium in these alloys could be devised. The specifications of two of these alloys containing cadmium are as follows.

"C"—cadmium 0·15%; tin 0·40%; lead remainder
"D"— ,, 0·25%; antimony 0·50%; ,,

Methods were worked out for these two alloys. In making the trials quantities of lead and tin (or antimony) representing a 5·0-g sample of the alloy in question were weighed out and known amounts of cadmium, representing a range of 0·20 to 0·30%, were added. Details of the methods are as follows.

Alloy "C"—Dissolve a 5·0-g sample in 30 ml of diluted nitric acid (sp.gr. 1·2) and evaporate to dryness. Take up the residue with 100 ml of a solution containing 5% of potassium nitrate and 5% of strong nitric acid; boil the liquid for a minute or two and filter through a

tight pulp filter, washing the "metastannic acid" residue with a solution of the same composition as that used for taking up the first residue. Adjust the nitric acid concn. of the filtrate to 15 to 20 v/v, cool and add 20 ml of a 10% soln. of potassium cobalticyanide. From here on follow the method described above for the determination of cadmium.

Alloy "D"—Dissolve a 5·0-g sample in a mixture of 20 ml of citric acid soln. (50%) and 30 ml of diluted nitric acid (sp.g. 1·2) and evaporate until the salts have crystallised out almost completely. Dissolve the residue in 150 ml of diluted nitric acid (1 in 5) and make up to a total vol. of 200 ml. Cool, add 20 ml of potassium cobalticyanide soln. and finish as before.

Lead taken	Tin taken	Antimony taken	Cadmium added	Cadmium found	Cadmium added	Cadmium found
g	g	g	g	g	%	%
5·0	0·020	—	0·0100	0·0106	0·20	0·212
5·0	0·020	—	0·0125	0·0127	0·25	0·254
5·0	0·020	—	0·0150	0·0157	0·30	0·314
5·0	—	0·025	0·0100	0·0103	0·20	0·207
5·0	—	0·025	0·0125	0·0127	0·25	0·254
5·0	—	0·025	0·0150	0·0151	0·30	0·302

(2) ZINC—The conditions for complete pptn. of zinc are very much the same as for cadmium. Zinc is completely pptd. in either 20% nitric or 15% sulphuric acid; below this acid strength pptn. is not complete. Hydrochloric acid apparently dissolves the ppt., so that in its presence pptn. is either nil or very partial. The following acids either do not allow of complete pptn. or their salts tend to redissolve the ppt.; citric, formic, acetic, phosphoric and sulphurous. Of the two acids giving complete pptn., sulphuric is by far preferable, as 20% nitric acid tends to cause slight decomposition of the reagent and hence a brown discoloration of the zinc sulphide finally obtained.

Trials of the process were made on solns. of varying amounts of zinc sulphate in 100-ml quantities of diluted sulphuric acid of concentration between 15 and 20% v/v. Each test soln. was treated with 10 ml of 10% potassium cobalticyanide soln. The white powdery ppt. was allowed to settle for ½ hour and then filtered off on a tight pulp filter and washed several times with 15 to 20% sulphuric acid containing 1% of potassium cobalticyanide. The ppt. was dissolved through into a flask by treatment on the pulp with 50 ml of 10% sodium hydroxide soln. in repeated small quantities, and the pulp was washed with water up to a total bulk of 150 to 200 ml. The filtrate was neutralised to methyl red with diluted sulphuric acid (1+3), 25 ml of 20% ammonium sulphate solution and 20 ml of 50% citric acid soln. were added and followed, after neutralisation with ammonia, by 5 ml of formic acid. In this liquid the zinc was thrown down as sulphide by passing hydrogen sulphide at boiling temperature and allowing to cool under pressure of the Kipp's apparatus. The zinc sulphide was finally filtered off, washed, ignited, converted into zinc sulphate and weighed as such according to directions given in a former paper.[5] Results obtained were as follows.

Zinc taken, g	Zinc found, g
0·0050	0·0042
0·0100	0·0096
0·0150	0·0145

The reaction should be available for the direct separation of zinc from lead (in nitric acid solution) tin, antimony and aluminium, but in default of any immediate practical application it was not further investigated.

(3) COPPER—Cobalticyanide produces a ppt. in solutions of copper in all the three acids—nitric, hydrochloric and sulphuric; where, however, small amounts of copper are involved, complete pptn. is only obtained in hydrochloric acid soln. Experiments were tried only on amounts of copper sufficiently small to be susceptible to colorimetric determination by the ammonia process (*i.e.*, 0·1 to 0·005 g). Precipitation was made in solns. of 100 ml volume containing 10 ml of conc. hydrochloric acid, the precipitant being 10 ml of a 10% soln. of potassium cobalticyanide. After pptn. the liquid was allowed to settle for ½ hour, a little filter pulp was added and well stirred in, and the liquid was filtered through a tight thick pulp filter. After washing with 10% hydrochloric acid containing 1% of cobalticyanide the copper compound was dissolved through into a clean beaker with 15–20 ml of diluted ammonia (1+1), which was also used to rinse the pptn. beaker, and was added a little at a time.

The filter was washed two or three times with 5% ammonia and finally with cold distilled water; the copper soln. was then evaporated to about 20 ml and cooled, 5 ml of diluted ammonia (1+1) were added and, after transference to a Nessler tube, the liquid was made up to 100 ml. The copper was then determined colorimetrically by matching against standard copper solns. containing the same concn. of ammonia. The results, which were accurate within the limits of colorimetric measurement, were as follows.

Copper added, g	Titration, ml (1 ml=0·001 g Cu)	Copper found, g
0·0010	0·95	0·00095
0·0020	2·00	0·00200
0·0030	3·00	0·00300
0·0040	4·00	0·00400
0·0050	5·00	0·00500

Results obtained from precipitations made in 10% nitric acid and 10% sulphuric acid were uniformly slightly low; thus 0·004 g of Cu gave 0·0038 in both acids. The presence of 5 g of ammonium, potassium or sodium chloride in the solution before pptn. had no influence on the result of precipitations in hydrochloric acid. As there seemed no immediate application of this reaction no further work was done but two applications have been already published: (*a*) the determination of cobalt when present as cobalticyanide[1]; (*b*) the prevention of the deleterious effect of copper on titrations of selenium and tellurium.[2]

(4) SILVER—Silver forms one of the most satisfactory of the cobalticyanide ppts., not only is it quantitative over a wide range of acid concentration (1 to 15% of conc. HNO_3) but also the ppt. is flocculent and easily filtered off, thereby differing markedly from many of these ppts. which filter extremely slowly. For obvious reasons hydrochloric acid cannot be used as the acid medium; sulphuric acid was not tried because the most likely metal to be separated is lead. Pptns. were made of varying amounts of silver in 100-ml volumes of 5% nitric acid; 10 ml of 10% cobalticyanide soln. were added to each, and the ppts. were allowed to settle for ½ hour. Each ppt. was filtered off on tight pulp and washed with 5% nitric acid containing 1% of cobalticyanide; the filter and ppt. were returned to the pptn. beaker, treated with 100 ml of 5% sodium hydroxide soln., boiled for a minute or two and cooled. The silver hydroxide mixed with pulp was filtered off and washed with cold 5% sodium hydroxide soln. After solution of the silver hydroxide in nitric acid the silver was determined gravimetrically as chloride in the usual manner; results were as follows.

Silver taken g	Weight of silver chloride g	Silver found g
0·0184	0·0239	0·0180
0·0368	0·0487	0·0366
0·0552	0·0730	0·0549
0·0736	0·0980	0·0737
0·1472	0·1957	0·1472

The cobalticyanide precipitation should obviously effect a separation of silver from lead. The following process was worked out for the determination of small amounts of silver in lead.

Determination of Silver in Lead—Dissolve a 50-g sample in 200 ml of nitric acid (sp.gr. 1·2), boil out the nitrous fumes and dilute with 400 ml of hot water; cool the soln. (a slight turbidity is probably due to a trace of chloride in the nitric acid used and will not affect the final result). Add 10 ml of 10% potassium cobalticyanide soln. and allow to settle for 2 hr. Filter off the ppt. on a tight pulp filter and wash thoroughly with 5% nitric acid containing 1% of cobalticyanide. Place the funnel in a clean flask, and dissolve the ppt. through by treatment with

Lead taken g	Silver added g	Titration, ml	Silver found (corr. for blank) g	Silver, % corrected for blank: added	Silver, % corrected for blank: found
50·0	—	5·35–5·25 = 0·10	—	—	—
50·0	0·0018	5·35–4·75 = 0·60	0·0018	0·0036	0·0036
50·0	0·0037	5·65–4·55 = 1·10	0·0037	0·0074	0·0074
50·0	0·0054	5·25–3·65 = 1·60	0·0054	0·0108	0·0108
50·0	0·0074	5·45–3·40 = 2·05	0·0072	0·0148	0·0144
50·0	0·0148	8·60–4·45 = 4·15	0·0150	0·0296	0·0300
50·0	0·0222	9·75–3·65 = 6·10	0·0222	0·0444	0·0444
50·0	0·0296	13·70–5·60 = 8·10	0·0296	0·0592	0·0592
50·0	0·0368	13·95–3·85 = 10·10	0·0368	0·0736	0·0736

40 ml of diluted ammonia (1+3) added in small quantities at a time; wash the pulp several times with cold water. The filtrate should be bright; any haziness is due to a trace of lead not washed out, and must be removed by filtration after digestion for ½ hour on the steam-bath. Neutralise the soln. with nitric acid and then add 10 ml of diluted ammonia (1+1); determine the silver cyanometrically. The results on previous page were obtained from aseries of trials made on lead to which varying amounts of standard silver soln. had been added.

(5) BISMUTH—There appears to be no precipitation of bismuth in hydrochloric acid media and in nitric acid the pptn. is partial. From sulphuric acid solns. the pptn. of large amounts (down to, say, *ca.* 0·01 g) seems to be complete, but smaller amounts are not pptd. completely even on prolonged standing. It was found that the presence of an electrolyte (Na_2SO_4) ensures complete pptn. of even small quantities; the sulphuric acid strength required in approx. 25% v/v. The ppt., which is white and powdery, is best decomposed by treatment with ammonia; there seems to be some slight decomposition of the reagent, which causes discoloration of the ppt. if sodium hydroxide is used.

Trials of the method were carried out on varying amounts of bismuth each dissolved in 100 ml of diluted sulphuric acid (1+3) containing 1 g of sodium sulphate. The bismuth in each soln. was pptd. with 10 ml of 10% potassium cobalticyanide soln.; it was allowed to settle for 2 hr. and then filtered off on a close-packed pulp filter and washed with diluted sulphuric acid (1+3) containing 1% of cobalticyanide. The filter was transferred to the pptn. beaker, treated with 100 ml of diluted ammonia (1+3) and broken up by stirring, and the liquid was boiled for 2 or 3 min. After complete cooling the ppt. and pulp were filtered off and washed with diluted ammonia (5%); the filter was then re-transferred to the beaker and the bismuth hydroxide was dissolved by similar treatment with dilute nitric acid. After the pulp had been filtered and washed with 5% nitric acid the bismuth in the filtrate was determined by Schoeller and Lambie's method.[6] Results were as follows.

Bismuth taken	Weight of bismuth phosphate	Bismuth found
g	g	g
0·1000	0·1469	0·1009
0·0500	0·0729	0·0501
0·0200	0·0286	0·0197
0·0100	0·0162	0·0101
0·0050	0·0072	0·0049
0·0020*	0·0029	0·0020

* This sample had to be left for 24 hr. for the ppt. to form.

This reaction should prove a ready means of separating bismuth from tin and antimony and might have applications; separation from lead is precluded by the fact that sulphuric acid has to be used. The possible applications of the reaction were not investigated.

(6) MANGANESE—This metal in large amount (say, above 0·01 g) is completely pptd. by cobalticyanide in nitric, hydrochloric or sulphuric acid media, as a white powdery ppt.; of the three acids, sulphuric has proved most satisfactory. With smaller amounts of manganese, however, the ppt. appeared not to separate completely. It was found that the addition of a small amount of acetic acid before the cobalticyanide caused pptn. to be complete even with small amounts. For the recovery of the manganese from the ppt. it is best to use a mixture of sodium hydroxide soln. and hydrogen peroxide; the digestion should be done cold, as we have found that warming causes the pulp partially to reduce the manganese dioxide, with the effect that small amounts of the ppt. pass through the filter.

Trial of the method was made as follows. Varying amounts of manganese as sulphate were dissolved in 100-ml quantities of diluted sulphuric acid (1+3), and to each 5 ml of glacial acetic acid were added. The manganese in each soln. was pptd. by addition of 10 ml of 10% potassium cobalticyanide soln.; after standing for 2 hr. the manganese cobalticyanide was filtered off and washed with sulphuric acid (1+3), to which had been added 5% of glacial acetic acid and 1% of potassium cobalticyanide. These trials being made before we discovered the somewhat harmful effects of heating the sodium hydroxide and peroxide mixture during digestion of the pulp, the pulp and ppt. were transferred to the pptn. beaker and treated with 20 ml of 10% sodium hydroxide soln. and 10 ml of hydrogen peroxide, and the mixture was boiled for 5 min. After thorough cooling the pulp and manganese dioxide were filtered

off and washed with cold 5% sodium hydroxide soln. The pulp and ppt. having been again returned to the beaker, the manganese was dissolved by warming with 30 ml of nitric acid (sp.gr. 1·2), 30 ml of water and 5 ml of saturated sulphur dioxide soln. The pulp having been filtered off and the soln. boiled down to *ca.* 30 ml, the manganese was determined by the usual bismuthate method. Results were as follows.

Manganese taken g	Titration ml of $N/10$ $KMnO_4$	Theoretical titration ml of $N/10$ $KMnO_4$	Manganese found g
0·0490	50·00–5·70 = 44·30	44·50	0·0487
0·0392	40·00–4·70 = 35·30	35·60	0·0388
0·0294	30·00–3·40 = 26·60	26·70	0·0292
0·0196	20·00–2·10 = 17·90	17·80	0·0197
0·0098	10·00–1·40 = 8·60	8·90	0·0095
0·0049	10·00–5·70 = 4·30	4·45	0·0047

These results, while reasonably close, tend to be somewhat low; this is undoubtedly due to the heating of the hydroxide with the pulp, as mentioned above; time did not permit these results being repeated, but *vide infra.*

Applications—The removal of manganese from a strong citrate soln. has always been time consuming and cumbersome, requiring either the complete destruction of the citric acid or the pptn. of the manganese as sulphide, a somewhat tricky and uncertain operation.[7] It seemed worth while to find out if citric acid interfered with the pptn. of manganese by cobalticyanide.

Trials were made exactly as before up to the end of the filtration of the manganese cobalticyanide except for the fact that 20 ml of 50% citric acid soln. were added in each test. It was found necessary to wash the ppt. very thoroughly in order completely to eliminate all traces of citric acid. The improved method of dealing with the cobalticyanide ppt., referred to above, was adopted. The pulp and ppt., having been re-transferred to the original beaker, were treated with 90 ml of 10% sodium hydroxide soln. and 10 ml of hydrogen peroxide (20 vol.), thoroughly broken up by stirring and allowed to stand (cold) for 10 min. with frequent stirring. They were then filtered off on a filter made from pulp which had previously been boiled for ½ hour with dilute (10% of 20 vol. strength) hydrogen peroxide. The manganese dioxide was washed with cold water and then dissolved, and the manganese was determined exactly as in the first set of trials. Results were as follows.

Manganese added g	Citric acid added g	Titration ml of $N/10$ $KMnO_4$	Theoretical titration ml of $N/10$ $KMnO_4$	Manganese found g
0·0011	10·0	8·52–7·65 = 0·87	1·00	0·0010
0·0022	10·0	8·52–6·60 = 1·92	2·00	0·0021
0·0055	10·0	8·52–3·60 = 4·92	5·00	0·0054
0·0110	10·0	16·77–6·90 = 9·87	10·00	0·0109
0·0220	10·0	22·36–2·70 = 19·66	20·00	0·0216
0·0440	10·0	44·72–5·10 = 39·62	40·00	0·0436

(7) IRON—As with manganese, large amounts of ferrous iron are pptd. completely in 30 to 50% hydrochloric acid; with small amounts, however, even up to 0·10 g, a few mg are left in the filtrate. It was found that addition of a little sodium chloride caused complete pptn. of quite small amounts. To ensure the iron being in the reduced condition, potassium iodide was added; this had a doubly beneficial effect as the iodine liberated caused the finely divided ppt. to flocculate. The behaviour of ferric iron is somewhat doubtful; it is marked in the table at the beginning of this paper as not being pptd., but with high acidity and concentrated solns. a slow formation of a yellow ppt. is observed; this appears to be ferric cobalticyanide.

Quantitative trial—Varying amounts of iron as ferric chloride were dissolved in 50 ml of water; 2 g of sodium chloride were dissolved in each soln., and 50 ml of hydrochloric acid (sp.gr. 1·16) were added. Precipitation was made by addition of 10 ml of a 10% soln. of cobalticyanide followed by 0·5 to 1 g of solid potassium iodide, and the whole was well stirred. After the ppt. had formed, 10 ml of sulphur dioxide soln. were added, and the liquid was allowed to stand for 1 hr. The ppt. was filtered off on pulp and washed with 50% hydrochloric acid in which was dissolved 2% of sodium chloride and 1% of potassium cobalticyanide. The pulp was returned to the beaker and treated with 100 ml of 20% ammonium sulphate soln., 50 ml of diluted ammonia (1+4) and 10 ml of hydrogen peroxide (20 vol.); after boiling,

the pulp and pptd. ferric hydroxide were filtered off, washed with 5% ammonium sulphate soln. and ignited and the iron was determined volumetrically. Results were as follows.

Iron taken g	Titration ml of $N/10$ $KMnO_4$	Iron found g
0·1004	18·10	0·1011
0·0502	9·00	0·0502
0·0251	4·50	0·0251
0·0100	1·93	0·0108
0·0050	0·99	0·0055

A few trials made by this method, in presence of 5·0 g of aluminium, with subsequent gravimetric determination of the iron, gave (after deduction of a blank due to iron present in the aluminium) substantially correct results.

(8) VANADIUM—Vanadium in the V^{V} condition is not pptd. at all by cobalticyanide but V^{IV} is pptd. completely from a 25% sulphuric acid soln. Hydrochloric acid appears to prevent pptn. altogether. The ppt. is gelatinous and sky blue, and, after a few min. shaking, flocculates; it is best to stir in a little paper pulp and after 30 min. standing it is readily filterable.

Quantitative trial—Varying amounts of vanadium in the V^{IV} condition were treated as described above and the ppts. so obtained were washed with 25% sulphuric acid containing 1% of cobalticyanide. A mixture of 50 ml of diluted ammonia (1+1) and 10 ml of 10% potassium cyanide soln. was made in each of the precipitation beakers and poured through its appropriate filter into a 600-ml beaker, and the filter washed two or three times with cold water. A 30-ml quantity of diluted sulphuric acid (1+7) was heated to boiling in the beaker and poured a little at a time through the filter to remove traces of vanadyl hydroxide which might have been formed. The filter was finally washed with cold water and discarded. The filtrate was evaporated to about 250 ml and cooled; it was then made slightly acid with sulphuric acid, and sodium bicarbonate was added to slight alkalinity; 10 ml of 10% potassium cyanide were added, followed by 2 to 3 g of sodium hydrosulphite and 50 ml of saturated borax soln. The soln. was boiled for 5 min., 20 ml of 20% sodium hydroxide soln. were added, and boiling was continued for a further 5 min. The ppt. (presumably vanadyl hydroxide) was filtered off hot on a tightly pressed pulp filter and washed with 5% sodium sulphate soln.; the filter was then burnt off at not too high a temperature in a platinum basin. (This separation is a modification of one worked out by one of the authors.[8]) The residue in the dish, consisting of sodium vanadate or vanadium pentoxide and sodium sulphate, but free from cobalt and traces of iron, was fused with a little fusion mixture and taken up with water, and the vanadium was determined volumetrically by the method published earlier.[9] Results obtained were as follows.

Vanadium taken g	Titration, ml of $N/100$ $Na_2S_2O_3$: actual	Titration, ml of $N/100$ $Na_2S_2O_3$: theoretical	Vanadium found g
0·0090	17·70	17·66	0·00902
0·0070	13·65	13·74	0·00695
0·0050	9·95	9·82	0·00507
0·0030	5·69	5·89	0·00290

No trials were made of separation from other metals with a view to the determination of vanadium, though it should be available for separating vanadium from titanium which is not precipitated in the Ti^{IV} condition. A problem has arisen, however, connected with the determination of aluminium in alloys containing vanadium. Trials made of separating the vanadium first by the above method and subsequently pptng. the aluminium with 8-hydroxyquinoline gave the following results.

Vanadium taken, g	Aluminium taken, g	$Al(C_9H_6ON)_3$, g	Aluminium found, g
nil	0·0159	0·2768	0·0162
0·050	0·0053	0·0910	0·0053
0·020	0·0021	0·0363	0·0021
0·020	0·0055	0·0913	0·0054
0·020	0·0106	0·1798	0·0105
0·020	0·0159	0·2747	0·0161
0·020	0·0212	0·3481	0·0204

(9) OTHER METALS (PARTIAL PRECIPITATIONS)—*Titanium*—Ti^{IV} does not seem to give any precipitate at all with cobalticyanide. Ti^{III} gives a pale buff precipitate in both sulphuric

and hydrochloric acids. After filtration, however, titanium was invariably found in the filtrate; no quantitative work was, therefore, attempted.

Mercury—Hg^{II} salts give yellow ppts. in both sulphuric and nitric acid solns. The ppts. seem extremely colloidal in character and apparently never separate completely and no adequate means of filtering them was discovered. Hydrochloric acid appears to inhibit pptn. altogether.

Chromium—The behaviour of chromium with cobalticyanide is interesting. Neither Cr^{VI} nor Cr^{III} is pptd.; if, however, the chromium is reduced to a lower stage of oxidation (*e.g.*, by reduction with magnesium under CO_2 or by solution of chromium itself in absence of air) a pale blue ppt. is obtained on addition of cobalticyanide. On allowing the soln. containing this ppt. to stand in contact with the air it slowly changes colour, that obtained in sulphuric or hydrochloric acid becoming pink and that in nitric acid yellow. This explains the yellow ppt. obtained as a spot test by treating chromium metal with a drop of cobalticyanide soln. in dilute nitric acid.[3] This examination was not pursued further.

Molybdenum—Mo^{VI} gives no ppt. In lower stages of oxidation molybdenum gives ppts. but these are apparently always contaminated with co-precipitated molybdenum blue. These ppts. appear in a great variety of colours (there is reason to believe that yellow is the true colour) and, in view of their obvious contamination, were not investigated further.

Uranium—The higher states of oxidation give no precipitate and it seems probable that this metal is not precipitated in any stage.

METALS GIVING NO PRECIPITATE WITH COBALTICYANIDE IN ANY OF THE THREE ACIDS TRIED (HCl, HNO_3, H_2SO_4)—In addition to those metals already mentioned the following were found not to give any precipitate. Al, Sb, Ba, Be, Ca, Ce^{IV} and ^{III}, Pb, Mg, Sn^{IV} and ^{II}, Zr, Sr, As, Na, K, NH_4, Li, Se and Te.

SUMMARY—(i) The behaviour of the cobalticyanide ion as a reagent for a number of cations has been examined qualitatively and, where complete precipitation appears to occur, quantitatively. Trials were made, where possible, in each of the three common acids.

(ii) The following new separations, using cobalticyanide precipitation, were worked out: (*a*) Cadmium in lead-tin-cadmium and lead-antimony-cadmium ("cable-making") alloys. (*b*) Silver in lead. (*c*) Manganese in citrate solution. (*d*) Iron in aluminium. (*e*) Vanadium from solutions containing aluminium with a view to the subsequent determination of aluminium. Three separations have already been published:—(*f*) Separation of nickel and cobalt from iron solutions.[1] (*g*) Separation of cobalt from solutions in which it occurs as cobalticyanide.[1] (*h*) Separation of copper from selenium and tellurium (making possible direct titration of either metal in presence of copper[2]). (*j*) A spot test for detection of chromium, cobalt,[3] etc.

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August, 1944

Some Examples of the Use of the X-ray Powder Diffraction Method in Quantitative Analysis; the Determination of Small Amounts of (a) Calcium Oxide in Magnesium Oxide; (b) Zinc Oxide in Zinc Sulphide

By H. P. ROOKSBY

(Communication from the Staff of the Research Laboratories of The General Electric Company Ltd., England)

(*Read at the Meeting, February* 7, 1945)

INTRODUCTION—The use of X-ray powder photographs for the quantitative determination of crystalline phases in a mixture is not new. It is fairly obvious that in an X-ray diffraction pattern of a specimen containing two (or more) constituents the densities of the lines characterising each constituent will be some function of the concentrations in the specimen. A phase present in high concentration will in general be expected to give lines of relatively high density, whilst one present in weak concentration may give lines of vanishing faintness, but it is hardly practicable to work out directly from the relative line densities quantitative figures for the amounts of the two phases. So many incalculable factors are involved that the usual methods of interpretation depend upon comparison with standards.

A usual technique, if it is required to determine the proportions of two phases in a mixture, is to make synthetic mixtures of the two constituents in various known proportions covering the likely range. One has to make sure, of course, that the materials employed for making up the mixtures are themselves above reproach in respect of purity. When the whole range from 0 to 100% has to be covered the synthetic mixtures can be varied by steps of, say, 10%. Standard X-ray powder photographs are then obtained of the series of synthetic mixtures. For quantitative evaluation of the proportions in an unknown mixture it is then necessary to take its X-ray pattern and compare this with the standard series. Such a comparison will generally enable the composition of the unknown to be found to the nearest 5%. This does not constitute such a high accuracy as is usually regarded as desirable in chemical circles, but it is sufficiently good to be quite useful in many applications. A particularly apposite example is the determination of the ratio of the two different crystal forms of titania, rutile and anatase, in titanium oxide pigments. Such an analysis could not, of course, in any event be made chemically, but the accuracy mentioned above, obtainable by X-ray methods, is extremely useful in this application.

What has been said so far refers to mixtures in which the constituents are present in relatively substantial proportions. Some improvement in accuracy is claimed if the densities of appropriate lines on the X-ray photograph are compared photometrically. By means of the photometer the variations in density along the X-ray negative are registered as a smooth curve. Peaks above the general level representing the background on the film correspond with the lines of the pattern, and the heights of the peaks are a measure of line densities. Such an instrumental comparison of line densities removes some of the uncertainties accompanying visual inspection. The eye, however, is a good photometer where simple comparisons of line densities are involved, and if nearby lines are chosen the sensitivity to small differences in relative density is quite high.

SENSITIVITY TO SMALL QUANTITIES—The sensitivity of the X-ray method to the detection of small proportions of one phase in another is not in general of a high order. The falling-away of intensity of reflections from a given substance is roughly proportional to the concentration in the mixture, neglecting differential absorption and scattering effects, and in X-ray analysis there can obviously be no technique analogous to the *raies ultimes* method in spectroscopic analysis. A figure of about 1% is regarded as being the average lower limit that can be expected to be detectable as a general rule, but in unfavourable circumstances 5 or even 10% may have to be set as a lower limit. There is, however, no rigid rule, and each problem has to be considered on its merits. It has been found that in certain instances the X-ray method is of very much higher sensitivity than this 1% figure, and in very favourable circumstances the limit is as low as 0·1%.

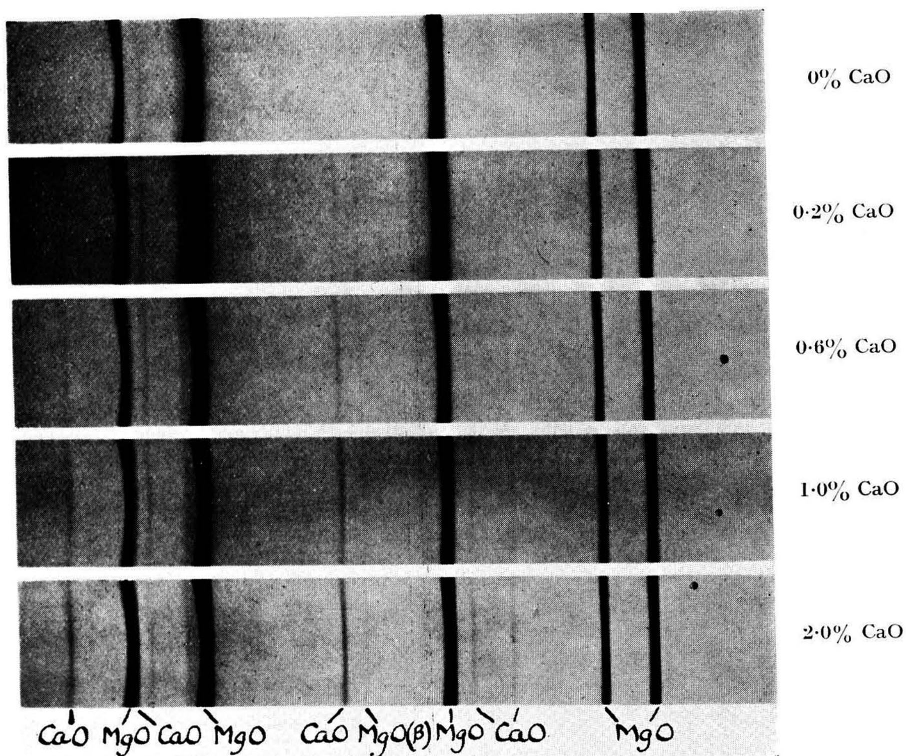

Plate I

Parts of X-ray powder photographs of magnesia containing various small amounts of calcium oxide impurity.

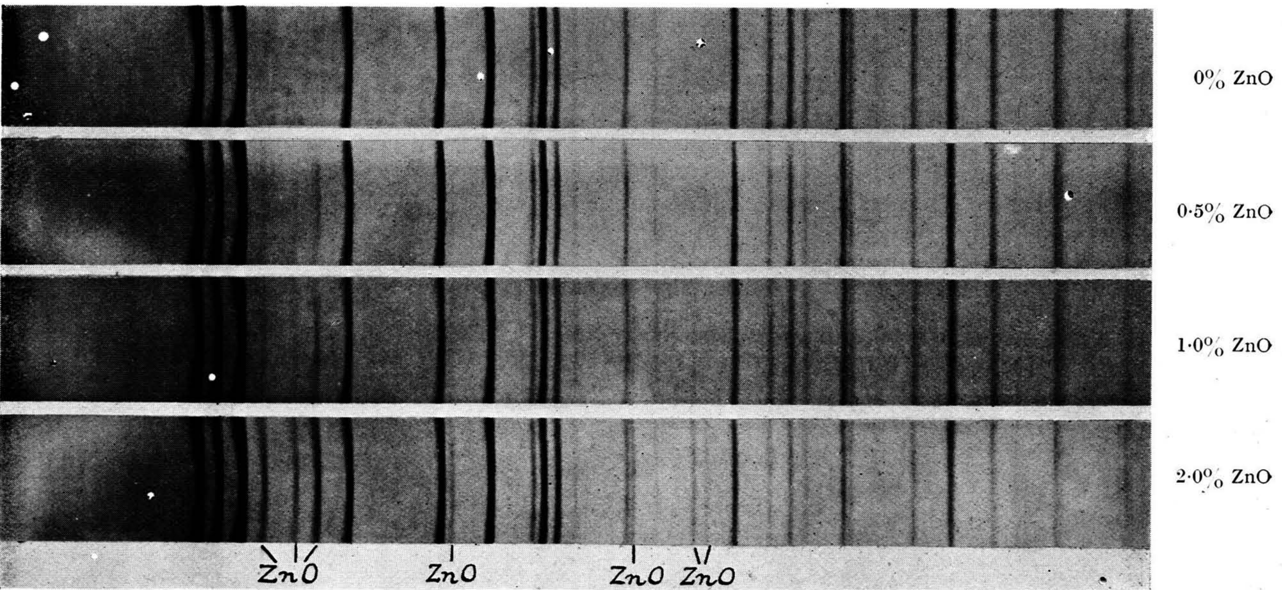

Plate II

Parts of X-ray powder photographs of luminescent zinc sulphide containing various small amounts of zinc oxide. The chief zinc oxide lines are indicated.

It must be explained that the examples to be described have not been worked out in any great detail. The possibilities of improving the accuracy by photometric means have not been explored, and the range of compositions over which it would be practicable to use X-ray technique has not been investigated with any completeness. The examples are given merely to indicate the potentialities of X-ray analysis in the field of small quantitative determinations, simple two-component systems being involved.

THE DETERMINATION OF CALCIUM OXIDE IN MAGNESIUM OXIDE—The first example concerns the detection of calcium oxide in magnesium oxide. For certain experimental purposes supplies of magnesia of very high purity were required; in particular the magnesia was only to contain calcium oxide in a proportion, say, not greater than 0·1%. It is, of course, possible to determine the lime by chemical methods, but these require considerable care and skill and are fairly lengthy and laborious. The X-ray powder technique, if it could be made sufficiently sensitive and reliable in the range of values to which interest attached, would effect a considerable saving in time and effort.

The X-ray powder photographic method adopted was conventional in character. The specimen in the form of a thin cylindrical rod, between 0·3 and 0·4 mm in diameter, was centred on the axis of a cylindrical camera of 19 cm diameter. The radiation from a copper target X-ray tube was filtered through Ni foil before entering the camera, so that the X-radiation giving rise to the diffraction pattern consisted mainly of the $K\alpha$ wavelength of copper with only a very weak $K\beta$ component. Although the aim of filtration through Ni is to reduce the $K\beta$ component to vanishing intensity, the fact that filtration was not 100% effective was of advantage in this instance, as will be indicated later.

The X-ray specimens were made by a standard procedure, by rolling a fibre of Lindemann type glass in the magnesia powder after "wetting" the fibre with a solution of Canada balsam in xylene. With the 19 cm diameter camera and the X-ray set operating under normal conditions an exposure of 2 hours was required to obtain a negative of adequate density. The specimen was continuously rotated during the exposure and the air in the camera was displaced with hydrogen to reduce background scatter.

Samples of magnesia for X-ray examination were ignited at 1000° C. for a few minutes prior to taking the X-ray photograph. It has to be remembered that the X-ray analysis is specific to the calcium oxide content. If any calcium were present in the form of hydroxide or carbonate, there would be a corresponding decrease in the apparent lime content as determined from the X-ray pattern. Such an eventuality would only serve to confuse the analysis, even though the hydroxide or carbonate were present in sufficient quantity to be detected as separate phases. So, to avoid errors that could arise from partial (or even complete) weathering of the lime to hydroxide or carbonate, pre-ignition of the magnesia samples was invariably adopted.

Fig. 1 shows a set of X-ray patterns of magnesias containing various small amounts of added lime. In making comparisons one takes no notice of the strongest magnesia reflections. It so happens that, with the radiation employed and the photographic exposure adopted, faint β-reflections are obtained duplicating the strong α-reflections, and it is against these that the reflections characterising calcium oxide are compared. The pattern of a specimen containing 0·1% of lime was obtained but has not been included because, although the sensitive reflection for calcium oxide is just detectable on the original negative, it would not be visible on a reproduction. It will be seen, however, on the series shown, that at a concentration of 0·2% the most sensitive calcium oxide reflection is slightly weaker than the adjacent magnesia line, at 0·6% it is decidedly stronger, whilst at higher concentrations several more calcium oxide reflections can be distinguished. The range of concentrations between 0·1 and 1·0% can thus be covered in some detail.

It may be useful to give some comparison of the relative times required for an X-ray analysis and a chemical analysis of a sample of magnesia for calcium oxide. In the X-ray analysis we have to take account of the times taken for pre-ignition of the sample, for preparation of the specimen for the X-ray camera, for photographic exposure, for the processing of the film, and for inspection of the final negative and comparison with standards. At a conservative estimate this would involve a total time of, say, three hours, although during the photographic exposure the investigator would probably be occupied in other activities. Even if one took the precaution of doing duplicates, the time would not be greater than six hours. On the other hand, a careful chemical analysis will require a total time something of the order

of three days, and it is only fair to add that equally skilled attention to detail is required if a reliable result is to be achieved.

THE DETERMINATION OF ZINC OXIDE IN ZINC SULPHIDE—It is not proposed to describe the other example in detail. From time to time it has been required to examine fluorescent zinc sulphide powders for evidence of oxidation. The necessity for the examination arises because, in certain applications of the powders to glass and other surfaces, superficial oxidation of the particles can on occasion occur during baking processes. Such oxidation is usually accompanied by a deterioration in the luminescent efficiency of the powder.

The presence of small amounts of zinc oxide would be very difficult to detect with certainty by conventional chemical methods. Analysis for sulphur and zinc could perhaps be made, but the subsequent calculation for oxide would not be expected to give an irreproachable answer. The important point to be established was the approximate limit of sensitivity that could be reached by the X-ray method.

Synthetic mixtures of the untreated zinc sulphide powder and "Analar" zinc oxide were made up in various known proportions, and X-ray powder photographs obtained in the manner described above. A few of the series of standard X-ray reference patterns are shown in Fig. 2. It will be observed that the lines of zinc oxide are easily detectable for a concentration as low as 0·5%, and it was estimated from the series taken that the oxide could be revealed if present to the extent of 0·2%.

SUMMARY—Two examples of the use of X-ray powder diffraction methods in quantitative analysis are described. It is shown the calcium oxide can be detected in magnesia in as low a concentration as 0·1%. With zinc oxide in zinc sulphide the lower limit is approximately 0·2%.

DISCUSSION

Mr. F. L. OKELL asked if it would be possible to shorten the somewhat lengthy exposure by means of a fluorescent screen behind the photographic film.

Dr. R. R. GORDON said that if the X-ray method of quantitative analysis were to be made accurate some form of calibration relating to plate blackening with intensity was necessary. Could the author give any method by which this could be accomplished.

Mr. N. L. ALLPORT enquired if the X-ray method could be applied to the determination of the constituents of alloys. For instance, would the method be applicable to the determination of lead present in "tin" foil?

Mr. ROOKSBY, replying, said that fluorescent screens had not been found to be of much assistance for shortening exposures of powdered crystal photographs. They had been used in X-ray diffraction work involving Laue photographs, but, for these, shorter wave-length radiation was generally employed to which the screen made a better response. One had to remember also that the use of fluorescent screens would almost certainly impair the definition to some small extent and this might offset any gain in respect of the photographic exposure.

With regard to Dr. Gordon's question, the intensities corresponding to densities of different levels might be evaluated by preparing a calibrated photographic wedge for comparison with the X-ray diffraction pattern. Since the reciprocity relation between time and intensity held for X-rays, the wedge could be made by exposing a film to wide X-ray beam behind a specially shaped rotating sector, to give density steps corresponding with suitable increments of intensity. Quite good quantitative work, however, could be done without using a comparison wedge, the line densities being treated empirically in the manner described, so long as the photographic exposures, processing, etc., of the negatives were reasonably well-controlled.

In reply to Mr. Allport, the author said that the X-ray method could be used for quantitative measurements on alloys, where the question of a simple mixture of two phases was involved. Of course X-rays were employed extensively for studying alloys that were solid solutions, but the technique was quite different from that under discussion in the paper. He had no experience of the particular example mentioned, but thought that a very small percentage of free lead in presence of tin should be detectable by X-rays. On the other hand, chemical analysis or spectroscopic analysis might well be more suitable. X-rays would probably not be used where other methods were available.

The Measurement of Spoilage in Herring Stored under Moderately Low Temperature Conditions

BY F. CHARNLEY

As shown in previous papers,[1,2] the acid value of the oil of canned herring and the carbon dioxide value of the drained muscle tissue of canned salmon are closely related to examiners' ratings for freshness. Over the range from fresh to tainted samples both these characteristics afford relatively sensitive measures of the degree of post-mortem spoilage that may have taken place in the fish prior to canning. Furthermore, unlike the *p*H of the aqueous

liquid of canned salmon, these two characteristics are apparently unaffected by the seasonal condition of the fish. They thus fulfil the primary requisites of a valid test for spoilage, and it seems reasonable to conclude that under controlled conditions both characteristics will increase steadily as spoilage proceeds.

The object of the present investigation was twofold: first, to test this deduction and, secondly, to investigate the feasibility of measuring spoilage in uncooked herring tissue by means of the carbon dioxide value or, more correctly, the amount of volatile acids determined by the diffusion method.

METHODS—The experimental samples were obtained by storing freshly caught fish outdoors in a covered box on the north side of the cannery where the samples were packed. The tests on the samples were made at intervals of 24 hr. and were applied to both the minced, uncooked tissue and the canned samples packed at the same stage of spoilage. The samples for the analysis of the minced, uncooked tissue were prepared by mincing six fish in a meat grinder and thoroughly mixing the ground product, while the samples for the tests on the processed herring consisted of six 1-lb. tall tins of herring packed under standard cannery procedure at the cannery laboratory. The experiment was carried out during the eight days, December 4th to 11th, inclusive, 1943.

The progress of spoilage in the cooked samples was followed by simultaneous determination of the carbon dioxide value of the drained muscle tissue, the acid value of the oil, the *p*H of the aqueous liquid, and examiners' organoleptic ratings. The first of these tests was made as previously described,[2] and the second by the rapid procedure also mentioned previously.[1] The procedure for determining the acid value of the oil was modified by measuring out the sample volumetrically in a Mohr pipette calibrated to deliver, when rinsed with solvent, 1·2 ml of cleared oil at room temp., the solvent being a 50% mixture of benzene and alcohol. The third characteristic, *i.e.*, the *p*H of the aqueous liquid, was determined by means of a Beckman glass electrode instrument and was corrected to 18° C.

Only three of these tests, however, were applied to the uncooked samples, *viz.*, the organoleptic examination, the *p*H of the ground muscle tissue, and the carbon dioxide value or volatile acids of the uncooked tissue. The third test was carried out in a large diffusion apparatus, the outer chamber of which was a Dominion Seal pint jar. The inner receptacle was made by cutting down a 250-ml Pyrex beaker to a height of approx. 1½ in. and then grinding the edge flat with carborundum powder. To prevent bumping against the side of the outer chamber, a rubber band was stretched around the outside of the inner chamber about ⅜ in. from the top. Lastly, to allow free circulation of the gases above the sample, the inner chamber was set on a stand made by folding a strip of thin sheet metal, ⅜ to ½ in. wide by 5½ in. long, and bending the free ends from the middle of the folded strip at an angle of approx. 120°. When pressed firmly into the layer of tissue on the bottom of the Dominion Seal jar this stand raised the bottom of the absorption chamber ⅜ in. above the bottom of the jar.

To carry out an analysis, the rubber layer on the cover was lubricated with a mixture of 25% of hard paraffin and 75% of petrolatum. About 40 g of the ground tissue were introduced into the outer chamber (weighed with cover) and spread evenly over the bottom, and the whole was weighed. The metal stand was then pressed firmly into the layer of tissue, and the inner chamber, containing 5 ml of $N/10$ baryta, was inserted. The cover was tightly screwed down and the apparatus was set aside for 30 min. in an incubator maintained at 50° C. It was then removed from the incubator and allowed to cool for 10 min., after which the absorption chamber was removed and the baryta soln. titrated with $N/10$ hydrochloric acid. The difference between the result and that of a blank titration, divided by the weight of the sample, gave the volatile acids expressed as ml of $N/10$ hydrochloric acid per g.

In the present investigation duplicate determinations of the volatile acids in the samples of uncooked herring tissue were made.

RESULTS—The results of the expt. are shown in Table I. As will be observed, the examiners' ratings and the *p*H of the uncooked tissue have not been listed. These were omitted because the examiners were aware of the order in which the samples were drawn, and also because the *p*H values of the uncooked tissue, although increasing from 6·57 in the first sample to 7·05 at the end of the expt., represented single readings only, in contrast with the data of column 3, which represent averages of two analyses, and the data of columns 4, 5 and 6, which show averages of 6. Also, it should be mentioned that the temperatures listed

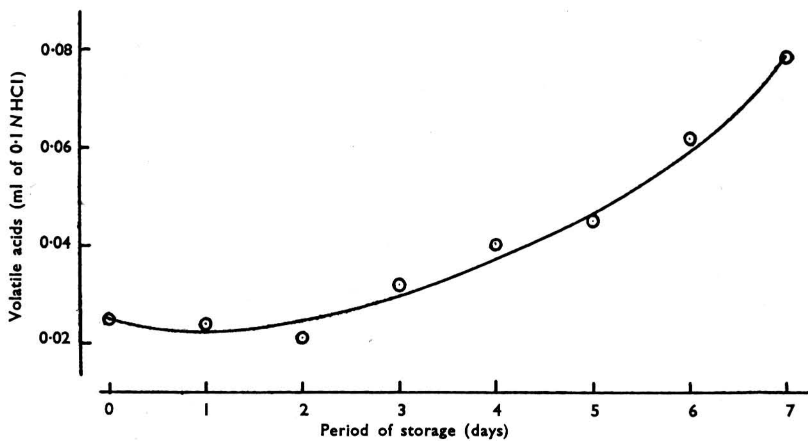

Fig. 1

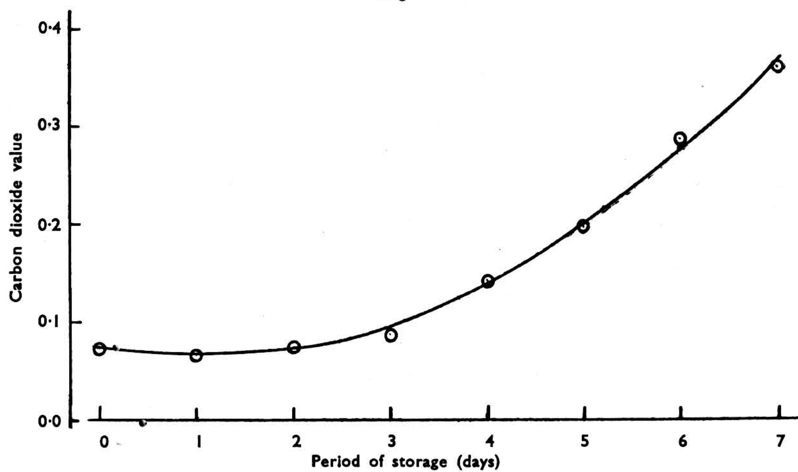

Fig. 2

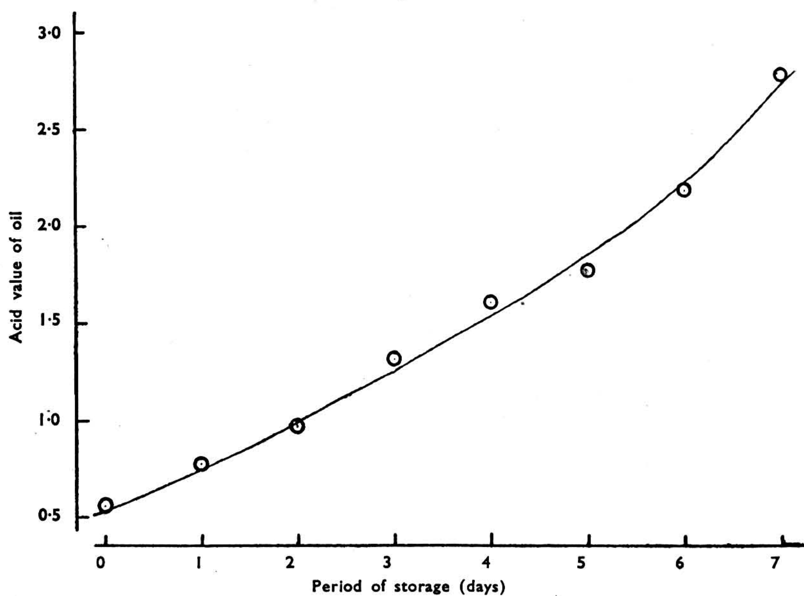

Fig. 3

in the table were taken about 10 a.m. each day, the time at which the samples were drawn for analysis.

TABLE I

RESULTS OF ANALYSES OF EXPERIMENTAL SAMPLES

Time (days)	Temp. of stored samples, °C.	Volatile acids of uncooked tissue (ml of 0·1 *N* HCl)	Carbon dioxide value of cooked samples (ml of CO_2 per g)	Acid value of oil	*p*H of aq. liquid of cooked samples
0	10·0	0·0248	0·072	0·554	6·70
1	2·5	0·0238	0·066	0·776	6·70
2	3·0	0·0206	0·073	0·966	6·71
3	5·5	0·0324	0·086	1·306*	6·81
4	3·5	0·0404	0·141	1·586	6·81
5	3·5	0·0449	0·197	1·756	6·98
6	3·5	0·0620	0·285	2·182	7·02
7	3·5	0·0788	0·360*	2·779	7·05

* Column 4, average of 2 results; column 5, average of 4 results.

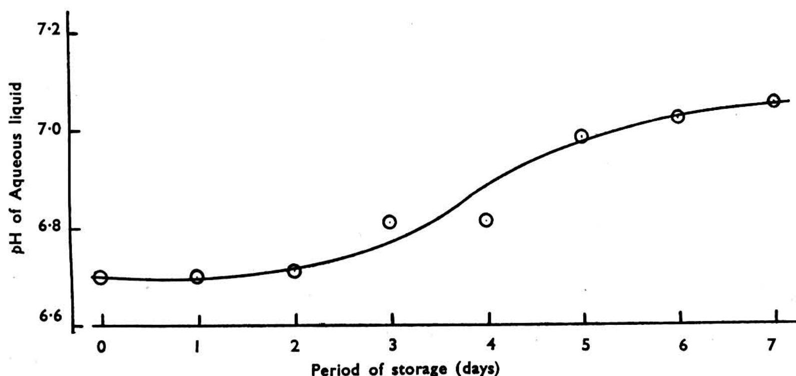

Fig. 4

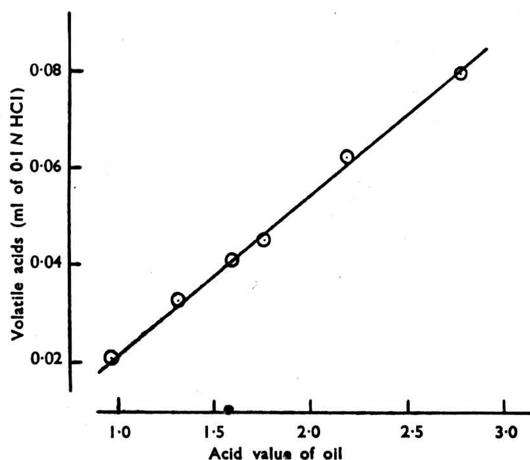

Fig. 5

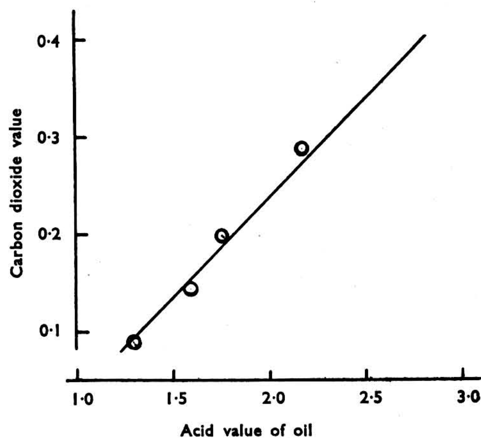

Fig. 6

Figs. 1 to 4 show, respectively, the volatile acids of the uncooked tissue, and the carbon dioxide value, the acid value of the oil and the *p*H of the aqueous liquid of the cooked tissue plotted as functions of the time of storage of the samples. Fig. 5 shows the relation between volatile acids of the uncooked tissue and the acid value of the oil, and Fig. 6 shows the corresponding relation between the carbon dioxide value of the cooked tissue and the acid value of the oil after the samples had been stored for 48 hours, the time at which under the above conditions the first signs of incipient spoilage, as judged organoleptically, appeared in the uncooked samples.

The curves illustrated in the figures confirm the deduction inferred above and also indicate that the volatile acids value of the uncooked muscle tissue afford a reliable measurement of the amount of post-mortem spoilage in unprocessed herring. In addition, the data illustrated in the figures strongly support the assumptions made in previous papers,[2] namely, that the relation connecting the acid value of the oil of canned herring and examiner's rating for freshness, and the similar relation between the carbon dioxide value or volatile acids of canned salmon and examiner's rating for freshness, are linear. As will be seen from Figs. 5 and 6, the relations connecting the volatile acids of the uncooked tissue and the corresponding carbon dioxide value of the cooked tissue with the acid value of the oil are very definitely linear over the range from incipient spoilage to when spoilage begins to be pronounced. Consequently, since examiners' ratings are largely influenced by the odour of the sample, which must obviously be derived from volatile compounds, it is reasonable to conclude that, over this range, these relations will be linear when the data are described by a proper statistical model which takes into account variations in both variates.

Finally, it is of interest to note that the fact that a test for spoilage is valid under controlled conditions is not a sufficient reason for concluding that the test will be valid under variable conditions. For example, as shown in Fig. 4, the *p*H of the aqueous liquid of canned herring packed under the foregoing conditions furnishes a valid test for the degree of post-mortem spoilage of the herring prior to canning, but under varying industrial conditions this characteristic is apparently of no value for this purpose. Similarly, further work has shown that the *p*H value, when applied to canned salmon,[3] is unsatisfactory owing to the fact that this characteristic, like examiners' ratings, is greatly influenced by the seasonal condition of the fish.

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CANNED FISH INSPECTION LABORATORY
BALLANTYNE PIER, VANCOUVER, B.C. *February*, 1944

Addendum to the Discussion on p. 124, following Dr. Houghton's paper on "Micro-Determination of Carbon by Wet Combustion":

To Dr. Griffiths, Dr. Houghton replied that nothing he had tried had eliminated the blank. If, after running a blank, more twice-recrystallised potassium dichromate was added to the flask a further blank was obtained. Owing to dust in the air, carbon-free reagents are very difficult to obtain, and the total blank was only about 5 parts of carbon per million of the reagents. Dr. Houghton told Miss Hadfield that titrating into the absorption tube was not satisfactory, as a foam of barium carbonate always formed on the surface.

Notes

OVER-RIPE APPLES

AN authoritative statement appeared in the daily press recently that over-ripe apples were suitable food for pigs and poultry, in reasonable amounts. When apples are allowed to lie about to get mouldy and rotten they suffer a definite loss of food value, the expressed juice is unsuitable for cider making owing to mustiness and acetification, and the residual pomace is unsuitable for pectin manufacture owing to loss of jellying power.

The following analyses of the juice from such "over-ripe" apples may therefore be of interest.

Sp.gr. at 15·5° C. ..	1·0295	1·0306	1·0264	1·0C80
Alcohol, % v/v ..	0·74	0·92	1·47	0·45
Extract, %	7·93	8·29	7·41	10·03
Acetic acid, % ..	0·51	0·52	0·61	0·22
Total acidity, % ..	1·24	1·28	1·39	1·09
Original gravity ..	1·0384	1·0408	1·0419	1·0430
Nitrogen, % ..	0·0133	0·0091	0·0182	0·0077
Sugars, %	—	5·46	4·35	—

In seasons like the last, when apple growers were short of labour for gathering in the apples and cider makers were short of labour for pressing, and the autumn rainfall was abnormally high, some spoilage of apples was likely to occur. The figures given above were for January juices from unsound apples attacked by moulds and other micro-organisms. Judged by the usual standards, these juices should have contained 8 g or more of sugar per 100 ml. They all contained acetic acid and alcohol, and in the two worst the sugar was only 5·46 and 4·35%. The juice tends to be lost from such apples by draining away. Also, obviously, there is a loss of food value.

Even with sound apples the juices, after reaching a maximum gravity in November, thereafter show a decrease which would be accelerated in the juice of unsound apples. The total acidity of apple juices decreases as the season advances, but juices from unsound apples show an increase due to acetic acid.

The following figures show the acidities and s.gr. of press juices as the season advances.

Pressings	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	All
Acidity, 1%	1·36	1·18	1·11	0·92	0·92	0·87	1·07
Sp.gr. at 15·5° C. ..	1·0365	1·0405	1·0446	1·0456	1·0422	1·0364	1·0428

These results show not only the effect of advancing maturity, but also the gradual change over from early to late varieties.

Warcollier in "*Cidrerie*" supplies tables connecting the sp.gr. and sugar content; according to his results these juices would contain 7·6 to 9·7 g per 100 ml; average 8·9 g. In 1934 the juice contained on the average 10% of sugar as against an average of 8% in 1936.

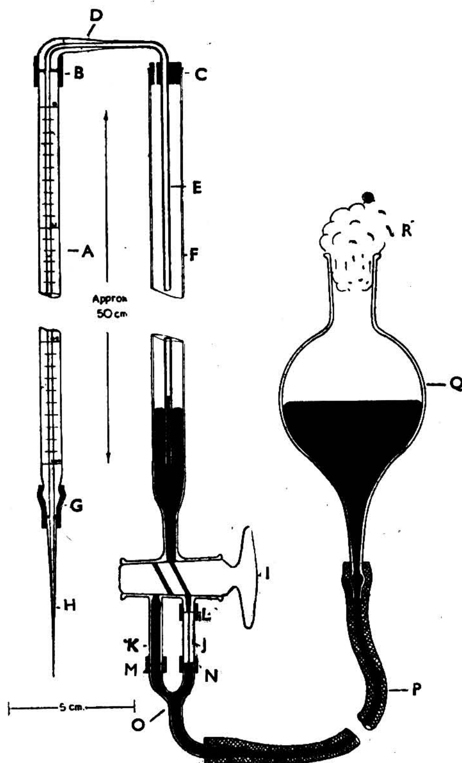
THE LABORATORY, WM. GAYMER & SON, LTD.
ATTLEBOROUGH, NORFOLK

D. W. STEUART
January, 1945

A COARSE OR FINE CONTROL MICRO-BURETTE

THE micro-burette described in this note has proved very useful in this laboratory for the thiocyanate back-titration in the micro-determination of bromides by Volhard's method, the added excess of silver nitrate solution being weighed. The burette might be useful to workers employing other micro-volumetric methods. It is similar to the Rehberg burette in that the solution is expressed from the burette by means of a mercury column.

The burette A is of 1 ml capacity and is graduated in 0·01 ml. The capillary tip H is attached to A at the butt connection G. A is similarly connected at B to the tube D–E, which has an internal diameter very slightly greater than that of A, and extends not quite to the bottom of F. F is a 50-ml syphon-filling type burette fitted with the two-way glass tap I. F communicates with the mercury reservoir Q either through the tube K or the inserted capillary J, and the rubber connecting tube P. M, N and L are butt connections. The rubber bung C holds D–E in position and is provided with a small hole so that the air in F is always at atmospheric pressure. A loose plug of cotton wool R protects the mercury in Q from dust.



PROCEDURE—Fill the burette by sucking the soln. in through H. This is accomplished by opening I to K when Q is lowered. Eject the soln. until the reading is just below the zero graduation mark. Touch the tip of H with filter-paper to remove any excess of liquid from the tip of the capillary. Read the burette to the nearest 0·001 ml by interpolation of the graduation marks. Raise the soln. for titration until H dips below the surface. Titrate by opening I to J when the mercury level in Q is higher than that in F. The sensitivity is adjusted by raising or lowering Q. In this laboratory the soln. is stirred during titration by a long glass shaft flattened at the lower end to a small blade and directly driven by a small electric motor. This avoids the tendency to splash or spray observed in the bubbling methods. At the end-point the burette is finally read before lowering the titrated solution from H. With practice volumes of soln. up to 1 ml can be delivered to within ± 0·002 ml. The "cushioning" effect due to the column of air in D–E is negligible, provided that the sensitivity of the burette is properly adjusted and the tip of H is not too fine. The temperature effect is likewise negligible in a room of reasonably constant temperature, and when the titration is not prolonged for more than a minute or two.

The burette is easily constructed from apparatus usually available in the laboratory, and has the advantage of rapid filling and emptying when required. It also avoids the disadvantages of screw plunger types which require special care to prevent leakage at the piston or error due to thread backlash.

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DEPARTMENT OF SCIENTIFIC AND
INDUSTRIAL RESEARCH
PEST INFESTATION LABORATORY, SLOUGH

F. P. W. WINTERINGHAM
October, 1944

THE RAPID DETERMINATION OF FAT IN POWDERS

EXPERIENCE shows that in the determination of fat in powders Bolton's apparatus[1] is to be greatly preferred to that of Soxhlet both for rate of extraction and for economy of solvent. (A similar apparatus to Bolton's is the Johnson extractor.[2]) Despite this fact, however, outside of Bolton's own treatise one rarely, if ever, finds any reference to it in the standard textbooks on food analysis.

For many years we have used Bolton's apparatus in connection with the analysis of foods and feeding stuffs in preference to Soxhlet's, which we now regard as definitely superseded, and of use only as a means of solvent recovery. For a finely divided powder such as cocoa, however, continuous extraction in a Bolton apparatus is not possible owing to the slow rate of percolation, as Bolton himself[3] points out,—a fact which we fully confirm. Recourse can naturally be had to the Soxhlet extractor, but, as both Liverseege[4] and Cox[5] point out, at least 12 hours are required for the completion of the process—an unduly long time, especially if a duplicate determination is subsequently found to be necessary.

In such circumstances we find that rapid results are still obtainable with the Bolton apparatus if the powder is placed, not in the inner glass tube, but in a thick paper extraction thimble resting on a perforated porcelain cone within this tube. Alternatively, the thimble and contents may rest within a tall glass collar on the three inward projecting points of the outer cylinder. In either procedure sufficient space must be left between the thimble and its glass jacket for the free passage of solvent and vapour.

Employing this modification, we find that, even with cocoa, complete extraction is obtainable within 1 hr., the bulk of the cocoa butter in fact being removable in 30 min. The temperature within the thimble was found to be 33° to 35° C., the m.p. range of cocoa butter being 28° to 33° C.

Before having occasion to modify the Bolton extractor, we had for several years used along with it a simpler form of apparatus, particularly suitable for fine powders, consisting of a reflux condenser fitting into an adapter, the stem of which passes through the cork of a fat-flask containing 40 ml of solvent. The extraction thimble, having a plug of cotton wool on top of the powder, is kept clear of the lower outlet of the adapter by means of thick copper wire, which, after being coiled in the form of two rings round the thimble, passes into the stem of the adapter. Six such adapters were made for us this year to modified measurements at a cost of 2s. each by one of our laboratory furnishers. Apart from its speed of working and low cost, this type of adapter possesses the additional advantage of easy replacement in case of breakage. The dimensions for one capable of holding a thimble 26 × 60 mm are: body 35 × 120 mm; stem 10 × 40 mm; capacity 110 ml. The solvent is recovered by condensation in a Soxhlet fitted with a tap for convenience of withdrawal. The following table summarises the percentages of fat obtained from samples of cocoa.

			Time of Extraction, hours							
Sample	Solvent	Apparatus	¼	½	1	2	3	4	5	8
1	Ether	Modified Bolton	12·80	23·42	23·76	—	23·78	23·82	—	—
2	do.	Adapter	—	—	13·36	—	13·40	13·40	13·40	—
3	Light petroleum	do.	—	—	23·90	23·90	—	—	—	—
3	do.	Modified Bolton	—	—	23·86	23·86	—	—	—	—
2	do.	Soxhlet	—	—	—	—	—	—	—	11·92*

The results indicate that equally good results are obtainable after 1 hr. whether using the modified Bolton apparatus or the adapter. The latter can also be used with advantage for the determination of fat in milk by the Adams process, excellent agreement with either the Werner–Schmid or the Röse–Gottlieb figures being obtainable after 1½ hr. For this determination the Adams coil with a superimposed layer of cotton wool is used without the thimble. With a dried full-cream milk powder, however, results were 0·5 to 0·6% lower than those given by the S.P.A. Committee method (25·2 as against 25·8%). Accordingly, the adapter process is not recommended, even as a sorting test, for milk powders, as the fat percentage in these is rarely found to be greatly above the regulation 26%.

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March, 1945

ASSAY OF 5-AMINO-ACRIDINE HYDROCHLORIDE

The introduction of 5-amino-acridine hydrochloride into medicine has made a suitable assay process for this antiseptic desirable. The estimation from a determination of the chlorine by a suitable gravimetric or volumetric method is easily carried out but it is not entirely satisfactory, in that any halide or free hydrochloric acid would be estimated as amino-acridine hydrochloride. We consider an estimation of the base is to be preferred, and the following process, similar to the B.P.C. assay process for euflavine, has given satisfactory results in our hands.

* Increased to 13·34% by 3 hr. subsequent extraction with light petroleum in a modified Bolton apparatus. The sufficiency of 1 hr. in Bolton's apparatus had not been established at this stage. The product was a low grade cocoa, only 80% of which passed a 100-mesh sieve.

Dissolve 0·5 g, accurately weighed, in 100 ml of water. Add 0·1 *N* sodium hydroxide until a ppt. forms and then just sufficient 0·1 *N* hydrochloric acid to re-dissolve it. Add 5 g of sodium acetate and 10 ml of a 0·1 *M* soln. of potassium ferricyanide with vigorous mixing. After 5 min. filter through a Buchner funnel and wash the ppt. and filter with two 25-ml portions of water. Unite the filtrate and washings, add 2·5 ml of hydrochloric acid and 5 g of sodium chloride, 1 g of potassium iodide and 3 g of zinc sulphate, allowing each to dissolve before adding the next. Stand for 5 min. and titrate the liberated iodine with 0·1 *N* sodium thiosulphate, using starch mucilage as indicator.

Determine by means of a blank expt. the number of ml of 0·1 *N* sodium thiosulphate equiv. to 10 ml of 0·1 *M* potassium ferricyanide soln. and calculate the volume of 0·1 *M* potassium ferricyanide required by the amino-acridine hydrochloride; 1 ml ≡ 0·0691 g of $C_{13}H_{10}N_2$, HCl.

Most samples of 5-amino-acridine hydrochloride assayed by this method have given figures of 98 to 100% $C_{13}H_{10}N_2$,HCl, calculated with reference to the substance dried at 120° C. *in vacuo*, and no sample has given less than 95 %.

We wish to thank the Directors of the Wellcome Foundation for permission to publish this note.

WELLCOME CHEMICAL WORKS
DARTFORD, KENT

G. AMOR
G. E. FOSTER

March, 1945

THE WEBER COLOUR TEST FOR THE IDENTIFICATION OF NATURAL RUBBER

THE Weber colour reaction,[1] mentioned in a recent note,[2] has become of importance in detecting natural rubber in mixings in which it may be considerably diluted with synthetic rubber or non-rubber materials. The detailed instructions for carrying out the test are given elsewhere[2,3,4] and Stern[4] has published a table showing also the colours obtained when the test is applied to rubbers other than the natural product. It is clear from this table and is confirmed by our experience that the strong violet colour developed is distinctive for natural rubber and gutta-percha, provided that the material has been extracted with acetone. However, colour reactions in organic chemistry are rarely found to be as specific as earlier workers have claimed, and work is being carried out in these laboratories to establish the limitations of the reaction when applied to rubber-like materials.

With very few exceptions, synthetic rubbers and rubber-like materials at present available do not give a positive result with this test, although very faint violet colours, which cannot be confused with a positive result, are sometimes obtained. Those giving any violet colour are listed in Table I.

TABLE I

Material	Nature of the material	Result of test
Methyl-rubber	Polymer of 2, 3-dimethyl butadiene	Strong violet as for natural rubber
Neoprene FR ..	Co-polymer of chloroprene with second component	,, ,, ,,
Gutta-percha and balata	Polyisoprenes, isomeric with natural rubber	,, ,, ,,
Butyl B	Co-polymer of isobutene and 1½% isoprene	Very faint violet colour
Pliofilm	Natural rubber hydrochloride	,, ,, ,,
Buna 85	Polybutadiene	,, ,, ,, (some samples only)

Cyclised and vulcanised natural rubbers (including ebonite) give a positive reaction, but other modified rubbers, *e.g.*, chlorinated rubber, rubber nitrate and rubber hydrochloride, give a negative or faint result.

EXAMINATION OF ISOPRENE DERIVATIVES—The liquid obtained from the dry distillation of rubber contains considerable quantities of isoprene, but it gave no violet colour until it had been heated under reflux for an hour. The intensity of the colour increased on standing or after continued heating. Isoprene prepared in the laboratory from acetone *via* a Grignard reaction similarly showed a negative reaction until it had been heat-polymerised. Bay oil, containing myrcene, a linear dimer of isoprene, gave the violet colour, but only after the brominated compound had been left in contact with phenol overnight, whilst turpentine (containing cyclic isoprene derivatives) did not give the violet colour, even after heating.

NATURE OF THE REACTION—Brominated rubber was prepared by bromination below 0° C. in presence of a trace of alcohol. This treatment has been shown by Bloomfield[5] to give a compound in which all the bromine is taken up at the double bond. The bromination was carried out in carbon tetrachloride solution, and phenol, also dissolved in carbon tetrachloride, was added. No colour was obtained on warming. Another sample of brominated rubber was prepared by the use of N-bromosuccinimide, the bromination being thus restricted to substitution in the alpha position. This compound gave the usual colour on warming in carbon tetrachloride soln. with phenol. Bromination of the methylene group alpha to the double bond in a polyisoprene thus seems essential for the production of the violet colour on warming with phenol. In methyl rubber this essential structure exists, whilst modifications of natural rubber produced by vulcanisation or cyclisation involve only a proportion of the monomer units. Chlorination, hydrochlorination and nitration are more drastic and involve most, if not all, of the monomer units either by addition at the double bond, or substitution, or, most probably, in commercial samples a mixture of both forms of attack; these do not give the colour. The reaction is, therefore, probably specific for an activated alpha methylene group. In natural rubber this activation is achieved by means of the methyl group.

EXCEPTIONS—As already mentioned, a very faint colour has been obtained with some samples of Buna 85, some samples of a rubber hydrochloride (Pliofilm), and Butyl B, a co-polymer of isobutene with small amounts of isoprene. The colour with the Pliofilm can be accounted for by supposing that some monomer units of the original rubber remain unattacked, but that arising from Buna 85, is, at present, unexplained but may arise from the occurrence of 1·2 addition of butadiene. This faint colour cannot, however, be

mistaken for the positive result given by even very small amounts of natural rubber. Neoprene FR is a copolymer of chloroprene and an undisclosed second component. Wet oxidation of the material gives some acetic acid,[2] and it is reasonable to suppose that isoprene is a unit of the copolymer. The necessity for prior extraction with acetone in any doubtful test is stressed because positive indications are obtained with certain non-polymeric materials, of which linseed oil is an example. Here, the methylene group between the two double bonds in linoleic acid is known to be especially reactive. Cyclopentadiene, also containing methylene-interrupted double bonds, will react in the same way, with the formation of a coloured compound. We are indebted to Dr. G. H. Wyatt for drawing our attention to the fact that the Weber reaction is strikingly similar to the Halphen-Hicks reaction for colophony, and there can be no doubt that a very similar mechanism is responsible, since colophony will give a colour under the conditions of the Weber test, but the colour is produced immediately, is of a different shade, and rapidly turns brown. Confusion is, therefore, impossible and, in any event, rosin (which is frequently used in Neoprene compounds) is acetone-soluble and would be extracted.

Natural rubber reclaimed by the alkali process fails to give the reaction, nor could it be restored by treatments ranging from mild washing to autoclaving with hydrochloric acid. It is hoped to deal elsewhere with the implications of this.

CONCLUSIONS—The Weber colour reaction appears specific for an active methylene group next to a double bond, such as is in the grouping—$CH^2-C=C$ occurring in linear polyisoprenes, of which

CH^3

natural rubber is the best known example. The grouping occurs also in methyl-rubber and in co-polymers containing isoprene. In analytical practice it is useful for identifying natural rubber (except as alkali reclaim), although caution must be used where an isoprene co-polymer is likely. Further work is in progress with a view to substantiating these conclusions and to establish the mechanism of the reaction.

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RESEARCH ASSOCIATION OF BRITISH
RUBBER MANUFACTURERS, CROYDON

I. F. C. PARKER
W. C. WAKE

January, 1945

Ministry of Food

INTERDEPARTMENTAL COMMITTEE ON FOOD STANDARDS

REPORT ON CUSTARD POWDER (Précis)

1. The Committee have received a request from the Starch Division for advice as to an appropriate standard for custard powder with a view to the issue of an Order prescribing a standard under the Defence (Sale of Food) Regulations, 1943.

2. The following information was given to the Committee by members of the trade and by the Division.

(i) Before the war most if not all custard powders consisted of 100% starch with added colour and with or without added flavouring material.

(ii) The manufacture of custard powder is now controlled under the Starch Food Powders (Control) Order, 1944, which prohibits the manufacture of custard powders except in accordance with the terms of a licence granted by the Ministry of Food.

(iii) Custard powder is defined in that Order as: "Any product consisting of starch, with or without other farinaceous ingredients, and so blended with flavouring, colouring and other ingredients as to be suitable for use in the preparation of custard, custard pudding or of any similar product."

(iv) Starch is defined in the same Order as: "(i) starch made from maize, potatoes, rice, rye or wheat (including any wheat product); (ii) flour or starch made from sago or tapioca (including cassava, manioc, mandioca and any similar tapioca plant); (iii) arrowroot."

(v) At present it is a condition of the licences issued under the Starch Food Powders (Control) Order that custard powders shall consist of—maize starch, 75; white wheat flour, 20; rye flour, 5% colouring and flavouring, a trace. The proportions of ingredients used to supplement the starch may vary from time to time according to the availability of suitable farinaceous diluents, and the licences are altered as may be found necessary.

(vi) It is unlikely that the cereal diluents will include flours other than wheat or rye.

(vii) The larger manufacturers are likely to revert to the pre-war composition as soon as they are able, but since the price of starch is likely to be higher than that of flour there may be some inducement to retain the present composition or to use an even higher proportion of cereal diluent.

3. For analytical purposes the main distinction between cereal flours and starch is that the former contain nitrogen. Assuming that the maximum nitrogen content of white wheat flour is about 2·9% and of rye flour about 2·0%, both figures being calculated on the flours dried at 100° C., a custard powder conforming to the composition at present required would contain not more than 0·7% of nitrogen calculated on the dry material.

4. It is possible that the cereal diluents may on occasion contain a higher proportion of nitrogen than is suggested above, so that a product made from the prescribed formula would contain slightly more than the permitted percentage. To meet this possibility the Committee suggest that if the Ministry of Food

decide to issue a Standard Order for custard powder the standard should include a formula for the product, with a provision that if the nitrogen content exceeds the specified limit it should rest with the defendant to prove that the formula had in fact been followed.

5. In the opinion of the Committee the definition of starch in the Control Order is wide enough to include certain low grade products containing substantial amounts of fibre and mineral matter which are not suitable for use in custard powders. If a Standards Order is issued it should be drafted so as to exclude these products.

6. For many years "custard powder" has been the usual name of an appropriately coloured and flavoured starch product, and while doubtless the average purchaser is unaware of its nature, he is both familiar and satisfied with the results it gives. Although, therefore, if starch custard powder were about to be marketed for the first time the Committee would have preferred a name which clearly differentiated a custard made with starch from one made with eggs, they do not suggest that there is any occasion to take exception to the continued use of the established name.

7. On the other hand, the Committee would view with regret the issue of an Order which would prevent or even deter manufacturers from improving the nutritional value of custard powder. Although dried egg is not at present allocated for use in prepacked flour mixtures and similar products, there is no reason to suppose that the difficulties which have necessitated this ban will not eventually be overcome. Manufacturers might similarly desire to use such materials as dried milk or soya.

8. The Committee understand that if the standard embodied the formula now included in the licences issued under the Starch Food Powders (Control) Order, it would be an offence under the Food Standards (General Provisions) Order as amended to sell a product containing any other ingredient under the name custard powder or under any name so similar as to lead an intending purchaser to believe he is purchasing custard powder. A standard which included a maximum figure for the nitrogen content would similarly discourage the use of additional ingredients containing nitrogen.

9. The Committee have accordingly reached the conclusion that no standard can be framed which would provide satisfactory control of the composition of custard powders as at present licensed without unduly restricting the choice of ingredients. In these circumstances they feel unable to recommend any standard for custard powder.

10. If, however, the Ministry of Food take the view that it is unncessary to consider future possibilities and that a standard based on the composition at present licensed is desirable, it is suggested that:

(i) The standard should consist of the formula which is now specified in the licences, with a proviso that the product should be deemed not to have been made in accordance with that formula if the proportion of nitrogen exceeds 0·7% calculated on the powder dried at 100° C. (ii) It should be a defence to prove that the specified proportions of ingredients had been used even if the nitrogen limit were exceeded. (iii) Steps should be taken to prevent the use of unsuitable grades of starch.

FOOD STANDARDS AND LABELLING DIVISION,
NORFOLK HOUSE, COLWYN BAY *March,* 1945

STATUTORY RULES AND ORDERS*

1945—No. 250. Order, dated March 6, 1945, amending the Manufactured and Pre-Packed Foods (Control) Order, 1942, and prescribing an Appointed Day thereunder and revoking the Flour and Flour Mixtures (Current Prices) Order, 1942. Price 1d.

A change in labelling made solely in order to comply with the Labelling of Food (No. 2) Order, 1944, or any other change in labelling made with the Minister of Food's approval, shall not oblige a manufacturer or pre-packer to hold a licence which would not otherwise be necessary. This Order also brings into force Articles 5, 6, 7 and 8 of the Manufactured and Pre-Packed Foods (Control) Order, 1942, in respect of flour mixtures as from March 6, 1945, rendering obsolete the Flour and Flour Mixtures (Current Prices) Order, 1942, which is accordingly revoked as from the same date.

— No. 268. Order, dated March 8, 1945, amending the Flour Confectionery (Control and Maximum Prices) Order, 1944. Price 1d.

(1) Restrictions on the maximum amounts of oils or fats and sugar which may be incorporated in flour confectionery are removed. The requirement that uncooked pastry shall not contain less than 25% of oils and fats remains. (2) The addition of substances after the baking or cooking of flour confectionery is no longer prohibited. (3) It will no longer be an offence to buy (but will remain an offence to sell) flour confectionery above the maximum price. (4) The definition of flour confectionery is amended to make it clear that products having a cereal breakfast food basis (unless they are biscuits or have been classified as chocolate or sugar confectionery) are within the definitions. These amendments take effect from March 19, 1945.

— No. 296. Order, dated March 19, 1945 (**amending the Bread** (**Control and Maximum Prices**) **No. 2**) **Order, 1943.** Price 1d.

The permitted proportion of white flour which may be used for batch bread in Scotland is reduced to 12·5% to conform with the provisions of the Flour Order, 1945. The definition of "Vienna loaf" is deleted. Instead of listing by name certain breads exempted from the Order by the Third Schedule, a general exemption is given to all bread having a minimum protein content of 20%.

— No. 304. The Use of Milk (Restriction) Order, 1945. Dated March 22, 1945. Price 1d.

The restrictions imposed by the Order no longer apply to Northern Ireland. Catering establishments and institutions are excluded from the scope of the restrictions. Skimmed milk powder may be used for the preparation or manufacture of synthetic cream.

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* Obtainable from H.M. Stationery Office. Italics signify changed wording.

1945—No. 331. Order, dated March 23, 1945, amending the Coffee (Retail Prices) Order, 1943. Price 1d.

After April 1, 1945, the minimum quantity of pure coffee required to be present in coffee mixtures is reduced from 66⅔ to 51%. *There is an increase in price of pre-packed coffee in containers of* 1 *lb. or less.*

PHOTOELECTRIC COLORIMETER FOR VITAMIN A DETERMINATION

THE Labelling of Food (No. 2) Order, December 28th, 1944, of the Ministry of Food has focused the attention of food manufacturers, public analysts and others upon the need for reliable methods for the estimation of vitamins involving the minimum of expensive apparatus. The estimation of vitamin A is a case in point and, at the request of the Margarine Manufacturers, Professor R. A. Morton of Liverpool University has been trying to co-ordinate the efforts to reach a standardised procedure for the analysis.

The method in favour is based upon the antimony trichloride reaction and requires (*a*) detailed instructions for the preparation of unsaponifiable matter; (*b*) a photoelectric colorimeter for measuring the rather transient blue colour; (*c*) an agreed way of interpreting the results.

A photoelectric colorimeter consisting of a light source, a lens, an absorption cell, a suitable light-filter, photoelectric cell and galvanometer is being considered. An experimental model has been tested and found quite satisfactory for the purpose. Negotiations with instrument makers are nearing completion. If the manufacturers can be given an order for 40 instruments the price of each would be about £35.

Food manufacturers, public analysts and others who would be interested in the purchase of an instrument intended specially for the antimony trichloride test for vitamin A are invited to communicate as soon as possible with Professor R. A. Morton, Biochemistry Department, The University, Liverpool. The information thus gained concerning the probable sale will then be passed on to those responsible for accepting a final design and quotation.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Fluorine in Fish Pastes. W. Harvey (*Nature*, 1945, **155**, 175)—The calcium, phosphorus and fluorine content of 5 well-known brands of fish paste have been determined by ashing the samples at dull red heat, extracting the ash with dilute hydrochloric acid and determining the calcium by pptn. as oxalate followed by back-titration with standard hydrochloric acid, the phosphorus by pptn. as magnesium ammonium phosphate and ignition of the ppt. to the pyrophosphate, and the fluorine by the method described in the ANALYST (1944, **69**, 243). The results obtained were as follows.

Type of paste	Calcium % w/w	PO_4 % w/w	Fluorine p.p.m.
1. Bloater	0·17	0·39	5·8
2. Salmon, shrimp, etc.	0·22	0·43	8·2
3. Smoked herring ..	0·12	0·25	3·1
4. Salmon and shrimp	0·23	0·37	8·0
5. Salmon and shrimp	0·26	0·34	8·9

The suggested daily requirement of fluorine (*ca.* 1 mg) would be provided by 4 oz. of fish paste (types 4 or 5), compared with 2·5 quarts of milk or 3 g of beef bone. A short review of the possible effect of adequate fluorine intake on the incidence of dental caries is given. J. A.

Sources of Error in the Determination of the Protein Content of Bulk Wheat. F. C. Hildebrand and R. C. Keohn (*Cereal Chem.*, 1944, **21**, 370–374)—Sources of error examined were: (1) Heterogeneity of bulk wheat, (2) sampling error, (3) variation in cleaning procedure, (4) differences in grinding methods, (5) analytical error. The following standard errors in percentage protein were found (1) 0·078, (2) 0·189, (3) 0·182, (4) 0·165, (5) 0·110%. The overall standard error was found to be 0·307%. The best ways of improving the results would therefore appear to consist in better sampling, cleaning and grinding procedures. W. M.

Report of the 1943-44 Methods of Analysis Sub-Committee on the Determination of Iron in Cereal Products. M. Howe (*Cereal Chem.*, 1944, **21**, 412)—Erratic results, generally on the low side, were found to be due to the presence of pyrophosphate in the ash. This affects the colour development in the method used (αα′-dipyridyl procedure) and it is shown that the pyrophosphate must be converted into the orthophosphate to get correct results. This is accomplished by boiling the ash for at least 15 min. with diluted hydrochloric acid (1+1). The waveband used was 520 *mμ*. Sodium carbonate fusion of the ash, and also the wet ashing procedure (Jackson, *Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 302) were found to offer no advantage compared with the direct ashing in the dry state. W. M.

Composition of the Fatty Acids of the Seed Fat of *Lophira alata*. M. A. Saboor (*J. Indian Chem. Soc.*, 1944, **21**, 303–306)—Fat from decorticated seeds of *Lophira alata*, an African tropical tree, had the following characteristics, sp.gr. at 40/40° C. 0·9021; m.p. (open tube), 27·0° C.; sap. val., 191·6; iodine val. (Wijs), 73·4; unsap. matter, 0·7%; Reichert–Meissl val., 0·7. These constants are similar to those obtained by previous workers. Pickles and Hayworth (ANALYST, 1911, **36**, 493) also investigated the fat semi-quantitatively. The mixed fatty acids from the present sample were separated by the usual Twitchell–Hilditch lead salt method into solid saturated (41·9%) and liquid unsaturated acids (58·1%). These were calculated as palmitic (23·2%), stearic (0·3%), arachidic (4·8%), behenic (4·9%), lignoceric (6·9%) palmitoleic (11·7%), oleic (8·3%), linolic (31·5%), eicosenoic (2·1%), docosenoic (5·6%), and unsap. matter (0·7%). The equal percentages of solid and liquid acids obtained by Pickles and Hayworth are attributed to incomplete separation by the method available in 1911. Their argument, based on the iodine val. (139), that oleic and linolic acids occurred in equal proportions, is considered

fallacious, owing to the presence of other acids in the liquid acids. E. B. D.

Detection of Coumarin Derivatives in Drugs. P. Casparis and E. Manella (*Pharm. Acta Helv.*, 1943, **18**, 711; *Quart. J. Pharm.*, 1944, **17**, 333)—Practical details for the tests previously described (*Pharm. Acta Helv.*, 1943, **18**, 347) are given. To separate from tannins, which give similar reactions, the coumarin is sublimed *in vacuo* at 180° C. Place *ca.* 3 g of the powdered material, previously washed with light petroleum, in the subliming apparatus and dry by heating to 110° C. *in vacuo*. Heat to 180° C. to cause the coumarin to sublime. To purify the sublimate, dissolve it in 5% potassium hydroxide soln., wash with ether, acidify and extract with ether. Purify the ethereal extract by a rapid washing with potassium hydroxide soln., remove the solvent, and re-sublime the product. Coumarin derivatives are tested by fluorescence, iodine absorption, mercuric acetamide reaction, sulphite and ferric chloride reaction, and diazo reaction. J. A.

Determination of Hyoscyamine in *Duboisia* Species. L. A. Lean and C. S. Ralph (*J. Proc. Roy. Soc. N. S. Wales*, 1944, **77**, 96; *Quart. J. Pharm.*, 1944, **17**, 333)—The method of the British Pharmacopoeia for the alkaloidal assay of Belladonna leaf is not applicable to the leaf of *Duboisia* species owing to the strongly coloured extractions obtained. The following method is recommended—Extract 40 g of the dried leaf with an alcoholic soln. of ammonia, remove the alcohol *in vacuo* and dissolve the residue in 0·67% sulphuric acid. Wash the acid soln. with ether, make alkaline with ammonia, extract the alkaloids with chloroform and remove the solvent *in vacuo*. Dissolve the residue in an excess of standard acid and titrate with standard alkali, using methyl red as indicator. To follow the decomposition of hyoscyamine, determine tropic acid by extraction with ether from the aqueous soln. made acid to thymol blue. *Duboisia myoporoides* and *D. Leichhardtii* had alkaloidal contents ranging from 1·3 to 3·5% according to their habitats and the time of collection. J. A.

The Alkaloids of *Delphinium Ajacis*. M. V. Hunter (*Quart. J. Pharm.*, 1944, **17**, 302–308)—The seeds of the larkspur (*Delphinium Ajacis*) have been found to contain *ca.* 1% of total alkaloids and *ca.* 39% of a fixed oil. The oil was extracted by percolation of the seed with light petroleum and purified by washing a chloroform soln. with dilute sulphuric acid. The solvent was removed and the constants of the oil, which was deep yellow and had a characteristic rank odour, were determined by the methods of the British Pharmacopoeia: sp.gr., at 15·5° C., 0·905; sap. val., 192·9; acid val., 132·2; iodine val. (Wijs), 83·0; unsap. matter, 1·04%. Five alkaloids were isolated, one of which was amorphous; the presence of ajacine and ajaconine was confirmed (see Keller and Völker, *Arch. Pharm.*, 1913, **251**, 207); they had m.p. 140° C. and $[\alpha]_D^{16°C}$ + 53° (c = 0·66 in chloroform) and m.p. 167° C. and $[\alpha]_D^{17°C}$ − 133° (c = 1·61 in chloroform) respectively. The names ajacinine and ajacinoidine have been suggested for two new alkaloids, having m.p. 210/211° C. and $[\alpha]_D^{17}$ + 52° (c = 0·56 in chloroform) and m.p. 120/126° C. after sintering at 110° C. and $[\alpha]_D^{16°C}$ + 46° (c = 1·57 in chloroform) respectively. A fifth base, having properties closely resembling those of lycoctonine, has been isolated, but its identification is not complete. J. A.

Colorimetric Assay of Simple Solutions of Adrenaline. G. F. Somers and G. B. West (*Quart. J. Pharm.*, 1944, **17**, 308–314)—A method for the colorimetric assay of adrenaline involving the use of Folin and Ciocalteu's phenol reagent* (*J. Biol. Chem.*, 1927, **73**, 627) is described. *Method*—Add 0·1 ml of the soln. to be examined to a mixture of 16·9 ml of freshly distilled water and 1 ml of the phenol reagent contained in a boiling-tube, followed by 2 ml of a saturated soln. of sodium carbonate, and heat in a boiling water-bath for exactly 2 min. to develop the full colour. Allow to cool and compare the blue colour in a Klett colorimeter with that produced by 0·1 ml of a 0·1% w/v soln. of adrenaline in hydrochloric acid (final pH of this standard soln. is 2·7) treated in the same manner at the same time. It is important that the adrenaline be added directly to the diluted reagent to avoid oxidation and that a fresh standard be prepared for each expt., as the depth of colour is dependent on the amount of heating. Eight estimations are made on each sample to reduce the error. The accuracy of the method was checked by the assay of known dilutions of a standard adrenaline soln. From the results of these expts. the standard deviation was calculated to be 3·3%, whence the limits of error (P = 0·99) are 100 ± 8·5%. The proposed method was compared with that of Folin, Cannon and Dennis (*J. Biol. Chem.*, 1912, **13**, 477). Using essentially the same procedure for the latter as described above, 0·2 ml of sample had to be used and the colours produced were not so stable. On heat-treated adrenaline solns., however, the method of Folin, Cannon and Dennis gave lower results than the proposed method, and these were in better agreement with those obtained physiologically by the frog-heart method of West (*J. Physiol.*, 1943, **102**, 367). Metabisulphite, which has been proposed as an antioxidant for adrenaline solns. (Woolf, *Quart. J. Pharm.*, 1941, **14**, 234; Berry and West, *Quart. J. Pharm.*, 1944, **17**, 242), interferes with nearly all the published colorimetric methods and itself gives a blue colour with the phenol reagent. It has been found that aeration of an adrenaline soln. strongly acidified with 10% hydrochloric acid for 30 min. in a closed boiling-tube effectively removes all the sulphur dioxide and allows satisfactory colorimetric measurements to be made. It was not possible, however, to remove this source of interference with solns. containing metabisulphite which had been previously heated in closed ampoules and it is suggested that this may be due to the formation of a compound between the adrenaline and the metabisulphite. J. A.

Freezing-point of Artificially Induced Bovine Secretions. B. C. Veinoglou (*Nature*, 1945, **155**, 172–173)—Samples of the mammary secretions of

* Folin and Ciocalteu's phenol reagent is prepared as follows. Put 100 g of sodium tungstate ($Na_2WO_4,2H_2O$), 25 g of sodium molybdate ($Na_2MoO_4,2H_2O$) and 700 ml of water in a 1500 ml Florence flask. Add 50 ml of 85% phosphoric acid and 100 ml of conc. hydrochloric acid. Connect with a reflux condenser by means of a cork or rubber stopper wrapped in tin foil and boil gently for 10 hr. Then add 150 g of lithium sulphate, 50 ml of water and a few drops of liquid bromine. Cool, dilute to 1 litre and filter. The finished reagent should have no greenish tint, indicative of the presence of blue reduction products, and it should be well protected against dust, which may cause slight reduction—EDITOR.

3 maiden Shorthorn heifers, which had been treated by implantation of a total of 2·40 g of stilboestrol, 5·01 g and 2·54 g of hexoestrol respectively (see Folley and Malpress, *J. Endocrinol.*, 1944, **4**, 1), were examined for freezing-point depression by a modification of the apparatus of Temple (ANALYST, 1937, **62**, 709). The Hortvet type thermometers were standardised by using 3 sucrose solns. (Elsdon and Stubbs, ANALYST, 1936, **61**, 198). Three control animals from the same herd were tested, a total of 119 evening and 103 morning samples being examined, of which 59 morning and 46 evening samples were from the treated animals. The secretions from the treated heifers gave a persistently higher depression, the general mean being 0·559° C. (0·558° C. for morning samples; 0·560° C. for evening samples) compared with 0·550° C. (morning 0·547° C.; evening 0·553° C.) for other cows. Aschaffenburg and Veinoglou (*J. Dairy Res.*, 1944, **13**, 267) have shown that for their normal cows, the freezing-point depression is persistently higher for evening milk than for morning samples during the summer, whereas during the winter the reverse is true. Induced secretions were examined only in the summer but likewise persistently higher figures were obtained for evening samples. There were differences in age and parity of the animals from which the samples were taken, but Aschaffenburg and Veinoglou (*loc. cit.*) and many others have shown these factors to be without influence on the freezing-point depression of the milk. J. A.

Biochemical

Estimation of Iron in Tissue. E. M. Scott. (*Arch. Biochem.*, 1945, **6**, 27–32)—*Semi-micro method for total iron*—Digest until dry not more than 0·5 g of tissue with 0·5 ml of 60% perchloric acid and 0·5 ml of conc. sulphuric acid, and dissolve the residue in 2·5 ml of 5% nitric acid by boiling for 15 sec. Add 1·0 ml of conc. hydrochloric acid, 5·0 ml of water and 2·0 ml of 20% potassium thiocyanate soln. Measure the optical density in a spectrophotometer from 540 to 450 *mμ* at 10 *mμ* intervals, with a 1 cm cell, and divide the readings by the appropriate factors given in the table below. *Micro method for total iron*—Digest as before, not more than 0·1 g of tissue, and dissolve the residue in 1·0 ml of 5% nitric acid and 0·1 ml of conc. hydrochloric acid by boiling for 15 sec. Add 5·0 ml of water and 2·0 ml of 20% potassium thiocyanate soln. Cool below room temp., add 3·5 ml of *iso*amyl alcohol, and shake. Measure the absorption of the alcohol layer, which should be clear, from 540 to 450 *mμ*, and divide the values so obtained by the appropriate factors given in the table. *Free iron of tissue extracts*—Prepare a fine suspension of the tissue in a glass grinder, and make up to 6 ml with water. Add 0·1 ml of conc. hydrochloric acid, and extract with three 5-ml portions of ether to remove haematin, centrifuging to break the emulsion. To 5 ml of the extracted suspension add 2·0 ml of 20% potassium thiocyanate soln. and 3·5 ml of *iso*amyl alcohol. Shake and centrifuge. If the alcohol becomes cloudy in the absorption cells, warm gently. Record the absorption from 540 to 450 *mμ* as before, and divide the readings by the appropriate factors. The semi-micro method will estimate up to 70 *μ*g and the micro-method up to 20 *μ*g of iron with precisions of 0·2 and 0·05 *μ*g respectively. The error involved in the estimation of free iron is ±5%, and quantities up to 20 *μ*g can be estimated.

FACTORS FOR CALCULATION OF IRON IN *μ*g
(L = 1 cm.)

Wave length *mμ*	Log I_0/I Semi-micro Total Fe	Micro Total Fe	Free Fe of tissues
540	0·01094	0·0470	0·0342
530	0·01250	0·0527	0·0381
520	0·01400	0·0573	0·0414
510	0·01520	0·0611	0·0442
500	0·01615	0·0634	0·0459
490	0·01686	0·0641	0·0466
480	0·01701	0·0626	0·0450
470	0·01659	0·0590	0·0423
460	0·01589	0·0529	0·0379
450	0·01490	0·0459	0·0329

F. A. R.

Micro-determination of Potassium by Precipitation and Titration of the Phospho-12-tungstate. D. D. Van Slyke and W. K. Rieben (*J. Biol. Chem.*, 1944, **156**, 743–763)—Transfer 1 ml of serum or plasma to a small platinum crucible, add 0·5 ml of 4 *N* sulphuric acid and heat for 1 to 2 hr. on a boiling water-bath. Heat the residue on a hot plate and then ignite in a muffle furnace at 460° to 480° C. until a white ash remains. Rinse the walls of the crucible with 0·5 ml of 0·1 *N* hydrochloric acid to dissolve the ash, and add 0·5 ml of 4% phospho-12-tungstic acid soln. (Dissolve commercial phosphotungstic acid in an equal weight of water, add 1 vol. of ether and shake. The phospho-12-tungstic acid and ether form a heavy oil which settles to the bottom of the funnel. Wash this three times with an equal vol. of water, evaporate the ether, and dry the residue at room temp. in a vacuum desiccator protected from light. The purified acid should be white, and 1 g should dissolve without turbidity in 5 ml of water without heating.) Heat the crucible on a steam-bath until the contents are dry, and within 10 min. add 2 ml of water. Stir with a small rubber-tipped rod, and transfer the suspension to a 15-ml centrifuge tube. Add four more 2-ml portions of water, stir and transfer to the centrifuge tube. Rinse the stirring rod with 2 ml of water, bringing the total vol. to 12 ml, and centrifuge for 15 min. Remove all except 0·5 ml of the supernatant liquid by suction with a capillary tube dipping under the surface of the liquid. Rinse the tip of the capillary with a few drops and the walls of the tube with 5 ml of water, stir up the ppt. and rinse the stirring rod with 2 ml of water. Centrifuge for 2 min. and remove all except 0·2 ml of the supernatant liquid as before. Repeat the washing with another 7 ml of water. To the ppt. add a piece of alundum (to ensure smooth boiling) and 1 ml of 0·04 *N* sodium hydroxide from a burette. Heat to boiling over a micro-burner, and add more sodium hydroxide soln. until the ppt. is nearly dissolved. Add one drop of thymol blue phenolphthalein indicator and more alkali until a permanent blue-violet colour is formed. Add a further 3 to 5 ml of alkali and boil for at least 30 sec. If the colour of the soln. changes, add more alkali and repeat the boiling. Next add from a burette sufficient 0·04 *N* sulphuric acid to change the indicator to yellow, and a few drops of acid in excess, and boil for 30 sec. to expel carbon dioxide. Again add 0·04 *N* sodium hydroxide from the alkali burette until the soln. becomes violet, minimising absorption of atmospheric carbon dioxide either by closing the tube with a rubber cap through which the burette is inserted or by passing

a stream of carbon-dioxide-free air through the soln. The colour at the end-point should be the same as that of the same vol. of 0·1% disodium phosphate soln. containing the same amount of indicator. Make a blank estimation in which water replaces the serum. Calculate the amount of potassium (mg per 100 ml) from the expression: $16{\cdot}57\,(t - b) + 0{\cdot}90$, where t = ml of 0·04 N sodium hydroxide added from the alkali burette minus ml of 0·04 N sulphuric acid added from the acid burette, and b = ml of 0·04 N sodium hydroxide required to titrate the blank. By a suitable modification the method can be used for the analysis of 0·2-ml samples of serum or plasma. It is also applicable to the analysis of whole blood, urine, faeces and tissue. Standard potassium solns. were analysed with an error of less than 1%, and recoveries of potassium added to serum gave results within ±3% of the theoretical. F. A. R.

Gravimetric Determination of Potassium as Phospho-12-tungstate. W. K. Rieben and D. D. Van Slyke (*J. Biol. Chem.*, 1944, **156**, 765–776)—Plasma or serum is treated as previously described (*cf.* preceding abstract) up to the point at which the ppt. is washed in the centrifuge tube, but the ppt. is dried and weighed instead of being dissolved and titrated. For the gravimetric analysis use 1 to 4 ml of serum or plasma, and ash with 0·5 ml of 4 N sulphuric acid per ml of plasma taken for analysis. Similarly, add 0·5 ml of phosphotungstic acid reagent per ml of plasma. The centrifuge tubes should be thoroughly washed, left in chromic acid mixture and finally rinsed several times with water and dried in an oven. The tubes should not be handled, and a similar empty tube should be used as a counterpoise in weighing them with and without the potassium phosphotungstate. The potassium content of the sample (mg per 100 ml) is given by the expression:

$$\frac{3{\cdot}32\,(\text{wt. of ppt. from sample—wt. of ppt. from blank})}{\text{vol. of plasma used for analysis}}$$

The agreement between the results obtained by titration and gravimetrically was excellent. F. A. R.

Estimation of Bromide in Body Fluids. J. S. Sharpe (*Brit. Med. J.*, 1945, i, 263)—The method of Barbour, Pilkington and Sargant (*Brit. Med. J.*, 1936, ii, 957) has been found unsatisfactory and the following procedure is recommended. *Urine*—Collect and measure a 24-hr. sample. Make 100 ml slightly alkaline with 5% sodium hydroxide soln. and evaporate to dryness in a silica basin on a water-bath. Ignite slowly until all the organic matter is decomposed, taking care not to burn off all the carbon, cool, add 10 ml of water to the residue, followed by 5% hydrochloric acid until slightly acid, heat almost to boiling and filter. Wash the filter with at least 3 portions of warm water. Cool the mixed filtrate and washings, transfer to a separator, add 5 ml of freshly prepared chlorine water, and shake vigorously with 10 ml of chloroform. Transfer the chloroform to a second separator containing 10 ml of water, extract the original soln. with a little more chloroform, wash the mixed chloroform extracts rapidly with water and transfer to a third separator. Add 10 ml of water and, with constant shaking, just sufficient anhydrous sodium carbonate to disperse the red colour of the bromine in the chloroform, shake vigorously and allow to separate. Discard the chloroform, add to the aqueous soln. 1·5 ml of a 1% soln. of gold chloride ($HAuCl_4$) followed by 5% hydrochloric acid dropwise until the orange-red colour is fully developed. Compare this in a colorimeter with the colour produced by diluting an appropriate quantity of a standard soln. of sodium bromide to the same vol. as the final aqueous soln. obtained above, slightly acidifying with 5% hydrochloric acid and adding 1·5 ml of the gold chloride soln. *Blood*—Incinerate a small measured quantity slowly until most of the carbon is burned off. Complete the procedure as described above. It is not claimed that the process is 100% efficient, but comparative results are obtainable. It is stressed that the volume of the aqueous extract of the ash should be kept as low as possible. J. A.

Estimation of Thiocyanate in Blood Serum. R. G. Bowler (*Biochem. J.*, 1944, **38**, 385–388)—In the method of Crandall and Anderson (*Amer. J. Digest. Dis. Nutr.*, 1934, **1**, 126), ferric nitrate soln. is added to a trichloroacetic acid filtrate of serum, and the resulting colour is compared with that given by a known amount of thiocyanate added to serum and treated in the same way. When an aqueous thiocyanate soln. was used as standard, it was found that the results were 7·5% low. This was due to the trichloroacetic acid interfering with colour development, and attempts to remedy this by the use of other protein precipitants were unsuccessful. The difficulty was overcome by increasing the concentration of the reagent, this being far below the optimum in the original method. Dilute various amounts of a standard soln. of sodium thiocyanate, containing 0·01 mg per ml, to 5 ml with water, add 5 ml of ferric nitrate reagent (80 g of $Fe(NO_3)_3 9H_2O$ in 250 ml of 2 N nitric acid diluted to 500 ml with water and filtered) in absence of daylight, and measure the colour against a control consisting of 5 ml of reagent and 5 ml of water. Use a 2-cm cell and a blue filter transmitting maximally at 470 $m\mu$. From the result plot a standard curve. For the estimation of thiocyanate in blood, to 1 ml of serum add 6·5 ml of water and 2·5 ml of 20% trichloroacetic acid. Mix, leave for 10 min. and filter through a Whatman No. 40 paper. To 5 ml of the filtrate add 5 ml of ferric nitrate reagent, and measure the colour within 15 min., using 1 ml of thiocyanate-free serum as control. In a series of 25 sera the recovery of added thiocyanate was quantitative, the maximum deviation from the mean being 3%. F. A. R.

Estimation of Choline. D. Glick (*J. Biol. Chem.*, 1944, **156**, 643–651)—By a critical examination of the reineckate method for estimating choline the following improved method was devised. Put a weighed sample, containing 2 to 5 mg of choline chloride, into an alundum thimble and extract for 24 hr. with 100 ml of methanol in a Soxhlet apparatus. With finely divided material, such as flour, mix with a little powdered pumice (from 2 to 8 times the amount of the sample is required according to the nature of the latter). Evaporate the methanol extract on the steam-bath until only a few ml remain, add 30 ml of saturated barium hydroxide soln. and heat for a further 90 min. Cool, add a drop of 1% alcoholic thymolphthalein soln. and just sufficient glacial acetic acid to discharge the blue colour. Filter through a sintered glass filter and rinse out the flask and filter with 15 ml of water added in several portions. To the combined filtrate and washings add 6 ml of a 2% soln. of Reinecke salt in methanol (freshly prepared each week) and leave at about 5° C. for

2 hr. Filter through a sintered glass filter, wash the ppt. with three 2·5-ml portions of *n*-propanol and dry by suction. Dissolve the ppt. in a few ml of acetone, collecting the filtrate in a tube graduated at 10 ml. Wash the filtrate with acetone until the vol. is 10 ml and transfer a portion of the soln. to the cuvette of a photoelectric colorimeter. Measure the absorption at 526 $m\mu$, and calculate the amount of choline present from a standard curve prepared with solns. of choline chloride of known concn. Standardise the instrument against a soln. of methyl red prepared by mixing 40 ml of aqueous methyl red soln. (6·25 mg per 100 ml) and 460 ml of 0·1 *M* citrate buffer, *p*H 3·7. F. A. R.

Estimation of Lysine in Protein Hydrolysates by a Microbiological Method. M. S. Dunn, S. Shankman, M. N. Camien, W. Frankl and L. B. Rockland (*J. Biol. Chem.*, 1944, **156**, 703–714, 715–724)—Prepare cultures of *Leuconostoc mesenteroides* P–60 on yeast-dextrose-agar and make sub-cultures at weekly intervals, incubating for 24 hr. at 37° C. and then storing in the refrigerator until required. Prepare inocula with the basal medium in which 50 mg of hydrochloric acid-hydrolysed casein, supplemented with cystine and tryptophan, are substituted for the amino acids and ammonium chloride. After 24 hr. centrifuge and re-suspend in 10 ml of sterile saline. Use one drop of this suspension to inoculate each tube. The basal medium is as follows (expressed in mg per litre unless otherwise stated): *dl*-alanine 2000, *l*(+)-arginine-HCl 80, asparagine (natural*) 400, *l*(−)-cystine 120, *l*(+)-glutamic acid* 150, glycine 100, *l*(−)-histidine.HCl.H_2O 20, *l*(−)-hydroxyproline 100, *dl*-isoleucine 150, *l*(−)-leucine* 75, *dl*-lysine.HCl* 160, *dl*-methionine 40, *dl*-norleucine 100, *dl*-norvaline 100, *dl*-phenylalanine 60, *l*(−)-proline 25, *dl*-serine 80, *dl*-threonine 450, *l*(−)-tryptophan 10, *l*(−)-tyrosine 30, *dl*-valine 150, glucose 20 g, adenine sulphate 12, guanine hydrochloride 12, uracil 12, sodium acetate 12 g, NH_4Cl 6 g, KH_2PO_4 500, K_2HPO_4 500, $MgSO_47H_2O$ 200, NaCl 10, $FeSO_46H_2O$ 10, $MnSO_44H_2O$ 10, aneurine hydrochloride 1, pyridoxine 1·6, *dl*-calcium pantothenate 2, riboflavin 2, nicotinic acid 2, biotin 5 μg, folic acid 2 μg, *p*-aminobenzoic acid 0·1 μg. Put 5 ml of the medium, prepared at twice the concn. given above, into each of the test-tubes. To one series add various amounts of the substance to be tested, containing from 30 to 200 μg of lysine, and to another series solns. containing similar amounts of pure lysine. The tests should preferably be run in duplicate or quadruplicate. Dilute the contents of each tube to 10 ml, autoclave for 10 min. at 15 lb. and incubate for 72 hr. at 35–37° C. Titrate with standard alkali, using bromothymol blue as indicator. Calculate the lysine content of the unknown from the values obtained with the lysine standards. The recovery of lysine added to casein hydrolysate ranged from 100 to 116% of the theoretical, with an average value of 106%. The amount of lysine in moisture-free, ash-free casein was found to be 8·3% and in silk fibroin 0·6%. F. A. R.

Cystine Estimation in Proteins and Foods. F. A. Csonka, H. Lichtenstein and C. A. Denton (*J. Biol. Chem.*, 1944, **156**, 571–576)—Hydrolyse the protein in 20% hydrochloric acid for 24 hr., filter and wash the residue with hot dil. hydrochloric acid. Transfer a quantity of the combined hydrolysate and washings, containing 4 to 5 mg of cystine, to a long Pyrex test-tube containing 1 or 2 glass beads, and evaporate to a syrup. Repeat the evaporation twice after addition of 5 ml of water, and then make up to 25 ml. Put 2·5 ml of glacial acetic acid, 3·5 ml of 5 *N* sodium hydroxide, and a quantity of distilled water sufficient to make the final volume to 40 ml into a centrifuge tube, cool to room temp., and add an aliquot of the test soln. containing 2–4 mg of cystine. Prepare a soln. of cuprous chloride by shaking about 0·5 g of the salt with 1% hydrochloric acid to remove cupric chloride, discard the supernatant liquid, and dissolve the residue in the minimum quantity of 25% potassium chloride soln. containing 0·2% of hydrochloric acid. Add 10 drops of this soln., dropwise with constant stirring, and after 40 min. centrifuge and discard the supernatant layer. Suspend the ppt. in 20 ml of abs. alcohol, stir for 10 min., again centrifuge and discard the supernatant soln. Dissolve the ppt. in 5 ml of 1% hydrochloric acid, and transfer to a 10-ml graduated flask. Add 0·5 ml of pyridine and 1 ml of 10% potassium thiocyanate soln. and dilute to 10 ml. Transfer 5 ml of this soln. to a 16-ml graduated flask, add 2 ml of 5% sodium cyanide soln. in 0·5 *N* sodium hydroxide and, after 10 min., 1 ml of an aqueous soln. containing 3 mg of sodium β-naphthaquinone-4-sulphonate, followed after 10 sec. by 5 ml of 10% sodium sulphite soln. in 0·5 *N* sodium hydroxide. Allow the colour to develop for 25 min., and add 2 ml of 5 *N* sodium hydroxide and 1 ml of a 2% soln. of sodium hydrosulphite in 0·5 *N* sodium hydroxide. Make up to volume and within 2 min. measure the colour in a spectrophotometer at 505 $m\mu$, using distilled water as blank. Calculate the results from a calibration curve, using pure cystine. F. A. R.

Estimation of Histidine with 3:4-Dichlorobenzene Sulphonic Acid. H. B. Vickery and J. K. Winternitz (*J. Biol. Chem.*, 1944, **156**, 211–229)—Heat a 5-g sample of protein, containing 1–3% of histidine, on the steam-bath with *ca.* 700 ml of 20% hydrochloric acid until there is no further frothing and then heat under reflux on a hot plate for 24 hr. Remove the excess of acid by repeated concentration *in vacuo*, dilute to 250 ml and estimate the nitrogen content in 1-ml aliquots. Dilute the remainder and decolorise by boiling with *ca.* 3 g of Norit. Remove the Norit and boil three times with water, concentrate the combined filtrate and washings, and adjust to 250 ml. Transfer 50-ml aliquots to 500-ml centrifuge bottles and add 20% silver nitrate soln. from a burette, with continuous stirring, until excess is present (about 70 ml). Adjust to *p*H 7·4 by addition of *N* sodium hydroxide (a little over 70 ml) with stirring, avoiding the production of local alkalinity and consequent pptn. of arginine. Centrifuge and decant the supernatant soln. through a thin layer of paper pulp. Stir the ppt. with 300 ml of water, again centrifuge, and repeat the washing twice more, filtering each washing through the filter. Again suspend the ppt. in water, add 3 ml of conc. hydrochloric acid and shake vigorously. Centrifuge and decant the clear soln. through the same funnel, taking care that all solid particles on the paper pulp are moistened with the acid soln. Wash the silver chloride ppt. with three 250-ml portions of water containing 1 drop of hydrochloric acid, using hot water for the last washings. Decant the washings through the same filter. The extract contains the histidine,

* The natural and *dl*-forms were employed interchangeably, the latter at twice the concentration of the former.

together with small amounts of other substances, *e.g.*, cystine and cysteine, that form insoluble silver compounds. Concentrate the combined extract and washings *in vacuo* to a few ml, but not to dryness, and filter the soln. through paper pulp to remove traces of silver chloride. Collect the filtrate in a 500-ml flask and again concentrate to a small volume. Transfer the concentrate, with the aid of a little hot water, to a 50-ml beaker and adjust to 20 ml. Add 3 g of the dihydrate of 3 : 4-dichlorobenzenesulphonic acid and warm until all pptd. histidine salt is in solution. Leave at room temp. for a few hours until crystallisation has begun and then stand in the refrigerator with occasional stirring for at least 3 days. Filter through a weighed sintered glass crucible in a cold room and wash the crystals with 3 to 5 ml of a cold 4% soln. of the reagent. Dry the crystals for a few hr. in a vacuum desiccator over sulphuric acid and then wash the crystals with three 10-ml portions of ether to remove traces of reagent. Dry at 105° C., cool in a desiccator and weigh. Calculate the weight of histidine by multiplying by the factor 0·2548. In order to ensure that the histidine salt is pure, recrystallisation is necessary. Remove as much as possible with a spatula to a 50-ml beaker and rinse the rest of the material in the crucible into the beaker with boiling water, keeping the volume of liquid below 20 ml. Dilute to 20 ml, add 2 g of the reagent and warm until the crystals are dissolved. Crystallise as described above. The loss in weight on recrystallisation was found to range from 10 to 15 mg, depending on the purity. In most instances a second recrystallisation was carried out, the average loss in this instance being about 11 mg; a similar loss in weight occurred on subsequent recrystallisation. For identification, the decomposition-point and nitrogen content were determined after successive recrystallisations. A correction of 10·2 mg should be applied to the weight of recrystallised preparations to allow for the solubility loss in the mother liquors. With pure solns. of histidine monohydrochloride (about 0·1 g) recoveries averaged 98·3% without correction for solubility and 99·4% when corrected. With larger quantities (0·3 to 0·4 g) recoveries were quantitative. Recoveries of histidine added to casein hydrolysate averaged 103% and to edestin hydrolysates, 101%.

F. A. R.

Determination of Plasma Mepacrine. A Note on the Anticoagulant. Anon. (from the Army Malaria Research Unit) (*Lancet*, 1935, **248**, 144)—In several recent methods for the determination of mepacrine in blood plasma (Masen, *J. Biol. Chem.*, 1943, **148**, 529; Brodie and Udenfriend, *Ibid.*, 1943, **151**, 299) the use of "oxalate" is recommended as anticoagulant. It has been found that if ammonium oxalate or any mixture containing ammonium compounds (*e.g.*, Wintrobe's salt mixture) is used, high figures for the plasma mepacrine level are obtained. It is suggested that the ammonia displaces mepacrine from the white corpuscles which have a high concn. of the drug (Brodie and Udenfriend, *loc. cit.*), and it is stated that potassium oxalate, sodium citrate and heparin are satisfactory anticoagulants. J. A.

Chemical Estimation of Tocopherols in Blood Plasma. M. L. Quaife and P. L. Harris (*J. Biol. Chem.*, 1944, **156**, 499–505)—The following method, which can be completed in about 80 min., involves lipid extraction, mild hydrogenation to reduce interference due to vitamin A, carotenoids and other unsaturated substances, and the use of the reagents of Emmerie and Engel. Put 5 ml of plasma from oxalated blood into a stoppered centrifuge tube, add 5 ml of abs. alcohol and, after mixing, exactly 12 ml of purified Skellysolve B (light petroleum). Shake for 10 min. and centrifuge. Transfer the upper layer to a 25-ml conical flask and evaporate just to dryness under nitrogen on the steam-bath. Dissolve the residue in 10 ml of abs. alcohol, ensuring that complete soln. takes place, transfer the soln. to a hydrogenation bottle, and add 0·1–0·2 g of palladised calcium carbonate. Hydrogenate at 30 lb. for 20 min. at room temp., and centrifuge. Transfer a suitable quantity of the supernatant liquid to an Evelyn photometer tube and dilute to 8 ml with abs. alcohol. Adjust the Evelyn colorimeter to the correct centre setting, using a blank consisting of 8 ml of abs. alcohol, 1 ml of αα′-dipyridyl (0·25 g in 50 ml of abs. alcohol) and 1 ml of ferric chloride soln. (0·01 g of $FeCl_3 6H_2O$ in 50 ml of abs. alcohol). Using filter 520, set the galvanometer so that it reads 100 exactly 15 sec. after addition of the last drop of ferric chloride soln. Add to the test soln. 1 ml each of the αα′-dipyridyl and ferric chloride reagents. Mix and measure the intensity of the colour 15 sec. after addition of the ferric chloride soln. Calculate the tocopherol content from a calibration curve prepared with pure α-tocopherol. The recovery of tocopherol added to blood plasma was theoretical. F. A. R.

Chemical Estimation of α-Tocopherol and Total Tocopherol in Mixtures of the α-, β- and γ- Forms. E. L. Hove and Z. Hove (*J. Biol. Chem.*, 1944, **156**, 601–610)—Emmerie and Engel devised a method for the estimation of tocopherol based on the quantitative reduction of ferric ions and estimation of the ferrous ions thus formed by means of the colour reaction with αα′dipyridyl. This reaction was investigated with pure α- β- and γ-tocopherols, and it was found that the colour developed most rapidly with α-tocopherol and more slowly and at an equal rate with β- and γ- tocopherols; with the latter a more intense colour was eventually formed. A method of estimating α- in presence of β- and γ- tocopherols was based on the intensity of the colour formed at 35° and 15° C. after 15 min. At 35° C. the colour intensity of the three tocopherols was the same, whilst at 15° C. the colour of the α- was more intense than that of the β- and γ- tocopherol solns. The method could be used for the estimation of α- and total tocopherols in vegetable oils.

Prepare a soln. of the material to be tested in Skellysolve B so that it contains approx. 40 μg per ml. Add 8 ml of reagent (250 mg of αα′-dipyridyl and 125 mg of $FeCl_3 6H_2O$ in 1 litre of glacial acetic acid) to 2 ml of the test soln., and measure the rate at which the colour develops at 35° and 15° C. in an Evelyn photoelectric colorimeter. Prepare standard curves showing the relationship at 35° and 15° C. between log I_0/I after 15 min. reaction and the amount (μg) of tocopherol, using pure specimens of the three tocopherols. The curves for α-tocopherol at these two temperatures are practically identical, whilst β- and γ- tocopherols give similar curves at each temperature but those at 35° C. are steeper than those at 15° C. Calculate the α-tocopherol content of the mixture from the expression: $L_\alpha = \dfrac{Z.L_{15} - L_{35}}{Z - 1}$

where L_{15} and L_{35} are the optical densities (log I_0/I) of the mixture at 15° and 35° C. respectively, and Z is the value obtained by dividing the L value of

the 35° standard by that of the 15° standard for β- and γ- tocopherol. From the value of L_α thus obtained, calculate the amount of α-tocopherol in the mixture from the standard α-tocopherol curve. Somewhat variable values were obtained by this method for the α- and total tocopherol contents of mixtures of known composition, the total ranging from 90 to 102% and the α-tocopherol from 69 to 115% of the theoretical values. In assaying vegetable oils preliminary treatment of a 1% Skellysolve soln. with 85% sulphuric acid, followed by a dilute alkali wash to remove carotenoids, as proposed by Parker and Macfarlane (*Canadian J. Res.*, 1940, **18**, 405) is recommended. By this method a quantitative recovery of added α-tocopherol was obtained. F. A. R.

Estimation of Tocopherols with Iron Dipyridyl Reagent in the Presence of Fats. H. Kaunitz and J. J. Beaver (*J. Biol. Chem.*, 1944, **156**, 653–660)—Emmerie and Engel and subsequent workers observed that fats interfere with the iron-dipyridyl reaction, and elaborate procedures have therefore been described to separate the tocopherol from the fat. It has now been established that many of these methods result in large losses of tocopherol, and a modification has therefore been devised for the determination of tocopherols, without attempting to remove fat. To 2 ml of a light petroleum soln. of the sample, containing 50–100 μg of tocopherol, add 10 ml of reagent (250 mg of ferric chloride and 500 mg of αα'-dipyridyl in 1 litre of glacial acetic acid) and note the colour developed after 10 min. To the original soln. add a measured quantity (about 100 μg) of α-tocopherol and again note the colour developed with the ferric chloride reagent after 10 min. Plot a curve relating the amount of added tocopherol to the colour intensity (log I_0/I) and calculate the amount of tocopherol (x) in the original sample either by extrapolation or from the expression: $x = \frac{a \times e_1}{e_2 - e_1}$ where e_1 and e_2 are the values of log I_0/I for the original soln. and the original soln. with added tocopherol respectively, and a is the amount of added tocopherol. This method gives better results than the removal of the fat by any of the published methods. When vitamin A and carotenoids are present, the amounts of these substances should be estimated by standard methods and a correction applied. F. A. R.

An Inositol-less Mutant Strain of Neurospora and its Use in Bioassays. G. W. Beadle (*J. Biol. Chem.*, 1944, **156**, 683–689)—A new strain of *Neurospora crassa* was developed by treatment with X-rays or ultra-violet light; it requires inositol for normal growth and can be used for estimating inositol. The basal medium is as follows (g per litre): ammonium tartrate, 5; ammonium nitrate, 1; potassium dihydrogen phosphate, 1; $MgSO_4 7H_2O$, 0·5; sodium chloride, 0·1; calcium chloride, 0·1; sucrose, 20·0; biotin, 5×10^{-6}. Add to the basal medium trace elements in the form of salts (mg per litre) as follows: B, 0·01; Cu, 0·1; Fe, 0·2; Mn, 0·02; Mo, 0·02; Zn, 2·0. Inoculate a series of test solns. and of solns. containing known amounts of inositol in 125-ml conical flasks by adding 1 drop of a conidial suspension to each flask. As the amount of growth varies with the size of inoculum, this should be standardised as far as possible and conidia taken from stock cultures of a uniform age of 4, 5 or 6 days. Incubate the flasks at 25° C. for 72 hr., disturbing the flasks as little as possible. Remove the mycelia, press out any free liquor, dry to constant weight at 80–90° C. and weigh the mycelia. Natural products should be hydrolysed by autoclaving in 3% sulphuric acid for 2 hr. at 15 lb., or by heating under reflux with 18% hydrochloric acid for 6 hr. With some materials more inositol is released by the second procedure than by the first. Recoveries of inositol added to various natural products showed excellent agreement with the calculated values. F. A. R.

New Method for the Oxidation of Aneurine to Thiochrome and a Procedure for the Estimation of Aneurine in Oats. W. I. M. Holman (*Biochem. J.*, 1944, **38**, 388–394)—The oxidation of aneurine to thiochrome by means of ferricyanide is not altogether satisfactory, and a new oxidising agent has been discovered which makes the oxidation easier to control and renders the extraction with *iso*-butanol unnecessary. The oxidising agent consists of a saturated soln. of mercuric oxide in 25% potassium chloride soln. as described below. The method gives reproducible results and a much greater yield of thiochrome than the ferricyanide method. The use of Decalso for adsorption of aneurine was also investigated and incorporated in the following method of estimating aneurine in oats.

Transfer 1·5 g of ground oats to a Pyrex boiling-tube, add 20 ml of 0·05 *M* potassium acid phthalate soln. (10·21 g per litre), mix, and immerse in a boiling water-bath for 5 min. Cool, add 0·1 g of papain and 0·1 g of takadiastase, both free from aneurine, and three drops of chloroform. Stopper the tube and incubate for 16 hr. at 37° C., mixing occasionally during the first few hours. Centrifuge for 5 min. and transfer the supernatant liquid to a tube calibrated at 30 ml. Wash the residue from the boiling-tube into the centrifuge tube with 10 ml of phthalate soln., mix and centrifuge for 5 min. Transfer the supernatant liquid to the calibrated tube and adjust the combined extract to *p*H 4·0 by adding *N* hydrochloric acid drop by drop. Make up to 30 ml with phthalate soln. and centrifuge for 5 min. Transfer 10 ml of the extract to a centrifuge tube containing 0·1 g of Decalso (wash each batch of Decalso with distilled water, centrifuge for 30 sec., pour off the liquid and repeat the washing. Dry the Decalso for several hours in an oven at 100° C. and pass through a 200-mesh sieve. The material is suitable if 0·1 g adsorbs over 90% of the aneurine from a soln. of 3 μg in 10 ml of 0·05 *M* phthalate soln.). Mix, centrifuge for 2 min., transfer the supernatant liquid to a second centrifuge tube containing 0·1 g of Decalso, and repeat until 5 successive adsorptions have been made. Discard the supernatant liquid. Put 5 ml of water in the first tube and transfer the Decalso from the second to the first tube with the aid of 5 ml of water. Similarly, put 5 ml of water in the third tube, and transfer the Decalso from the fourth and fifth tubes to the third tube. Centrifuge the first and third tubes for 5 min., discard the supernatant liquids, and transfer the Decalso from the third to the first tube with 10 ml of 25% potassium chloride soln. Heat in a boiling water-bath for several mins., cool, centrifuge for 5 min., and transfer the supernatant liquid to a Pyrex boiling-tube calibrated at 12 and 15 ml. Add 5 ml of 25% potassium chloride soln. to the residue, heat in boiling water, cool and centrifuge for 5 min. Transfer the supernatant liquid to the calibrated tube, and dilute to 15 ml with 25% potassium chloride soln.

Transfer 3 ml of the extract to a boiling-tube and add 2 ml of 25% potassium chloride soln. This

is the test soln. To the remaining 12 ml of extract, add 0·05 ml of 0·5 *N* sodium hydroxide and 0·2 ml of 12·5% $Na_2SO_3 7H_2O$ soln., and heat in a boiling water-bath for 1 hr. Cool, acidify with 0·2 ml of *N* hydrochloric acid and remove sulphur dioxide by aspiration through a fine capillary tube. Almost neutralise the soln. with 0·1 ml of 0·5 *N* sodium hydroxide and dilute to 12 ml. Transfer 3 ml to each of three boiling-tubes, add to each different amounts of a standard soln. of aneurine containing 1 μg per ml in 25% potassium chloride soln., and make up to 5 ml with 25% potassium chloride soln. To these three standards, and to the test soln., add 1 ml of 1% mercuric chloride soln., mixing thoroughly with the potassium chloride extract, and then add 4 ml of 0·06 *N* sodium hydroxide. Heat in a water-bath at 40° C. for 15 min., cool, and add 10 ml of acetone. Transfer to the glass cell of a Spekker fluorimeter and match against a quinine sulphate standard (1 μg per ml of 0·1 *N* sulphuric acid), using filter No. 7 in front of the right photocell and two strong neutral filters in front of the left photocell. Plot the readings given by the standards against the amounts of the added aneurine and calculate the aneurine content of the test soln. from the resulting curve. The recovery of added aneurine was 100 ± 5%, and duplicates agreed within 2%. F. A. R.

Rapid Method for Aneurine. W. A. Perlzweig, H. Kamin, I. Gue and J. V. Blalock (*Arch. Biochem.*, 1945, **6**, 97–103)—The following method was developed for the estimation of aneurine in urine for use in human nutrition surveys. It comprises adsorption on Superfiltrol, elution with an acid pyridine methanol soln., and oxidation, extraction and fluorimetric estimation by the method of Mason and Williams (*J. Biol. Chem.*, 1942, **146**, 589). Into each of three 15-ml graduated centrifuge tubes, put 1 to 5 ml of urine, containing 0·5 to 2 μg of aneurine, adjusted to *p*H 4·0–4·5. In tube A (the blank) put 0·5 ml of a freshly prepared 5% sodium sulphite soln., and dilute to 5 ml. Immerse in a boiling water-bath for 20 min. and cool. In tube B put 1·00 ml of a standard soln. containing 1 μg of aneurine and dilute to 5 ml. Into tube C introduce water to the 5-ml mark. Then introduce into all three tubes 1 ml of 0·5 *M* buffer soln., *p*H 4·6 (mix equal vols. of 0·5 *N* acetic acid and 0·5 *N* sodium acetate soln.) and 0·2 g of Superfiltrol. Close the tubes with rubber stoppers and shake gently for 3 min. Remove the stoppers and rinse these and the sides of the tube with water, diluting to 11 ml, and centrifuge for 3 min. Discard the supernatant liquids. Shake the Superfiltrol as before with 6 ml of water, rinse down the sides of the tube, again bringing the vol. to 11 ml, centrifuge and decant. Introduce, up to the 6·1-ml mark, elution mixture prepared by adding 800 ml of water to 200 ml of pyridine followed by 200 to 210 ml of conc. hydrochloric acid to bring the soln. to *p*H 1, and 600 ml of pure methanol. Shake the tubes, centrifuge, and decant the eluates. Into each of three 125-ml funnels pipette 9 ml of 25% potassium chloride soln., 3·00 ml of the three eluates and 12 ml of normal butanol. Stir the solns. with a current of air and add 2 ml of freshly prepared alkaline ferricyanide soln. (150 mg of powdered potassium ferricyanide in 0·5 ml of water mixed with 30 ml of 10 *N* sodium hydroxide). Aerate for a further 60 sec., and allow the two layers to separate. Remove the aqueous layer and add 1 g of sodium sulphate to the butanol layer. Measure the fluorescence of the latter in a fluorimeter in the usual way. If *a*, *b* and *c* are the readings given by the solns. in tubes A, B and C respectively, then the amount of aneurine in the aliquot taken for oxidation $= \dfrac{c - a}{2\,(b - c)}$. F. A. R.

Report of the 1943-44 Methods of Analysis Sub-Committee on Thiamine Assay. J. S. Andrews (*Cereal Chem.*, 1944, **21**, 388–397)—This report deals with the variation of results due to methods and procedures used in the thiochrome method, but it does not deal with the analytical results on specific samples by various laboratories. Points dealt with are: (1) efficiency of zeolite for removing interfering substances; (2) order of adding ferricyanide and alkali; (3) time of shaking; (4) stability of extracted thiochrome. The results showed that the use of zeolite presents no appreciable source of error, since it is equally effective with pure thiamine and with thiamine contained in cereal extracts. The concentration of ferricyanide and the order of adding ferricyanide and alkali have some effect upon the assay. It was shown that when a considerable excess of ferricyanide is used it is preferable to add the alkali first. It was also shown that by adding the alkali first a slightly greater fluorescence was obtained. Shaking for 30 sec. gave the maximum fluorescence; further shaking produced no increase. *Iso*butanol extracts of thiochrome were found to be stable up to one hour after extraction. W. M.

Agricultural

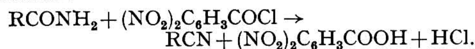
Factors Affecting Determination of Potash in Fertilisers. H. L. Mitchell and O. W. Ford (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 115–116)—Presence of phosphate causes low results in the determination of potash by the official Lindo–Gladding method of the A.O.A.C. Methods proposed for the removal of phosphate include pptn. with magnesium chloride, magnesium oxide or calcium carbonate. Another source of error is the occurrence of insol. matter in the potassium chloroplatinate ppt. Expts. were made to examine the extent of these errors. To 25 ml aliquots of a potassium chloride soln. in platinum dishes quantities of a sodium phosphate soln. corresponding with 0, 5, 25 and 50 mg of phosphorus pentoxide were added. Potash was determined by the official method, except that the potassium chloroplatinate was dissolved out of the crucibles and determined by re-weighing the crucibles. The potash content of the phosphate soln. added was determined both by the official method and by the colorimetric method of Sideris (*Ind. Eng. Chem., Anal. Ed.*, 1937, **9**, 145) and was applied as a correction to the total potassium chloroplatinate found. Similar expts. were made with solns. of 20% superphosphate prepared according to the official method for potash, aliquots of these solns. being mixed with aliquots of the potassium chloride soln. and the potash contents being determined as before. To investigate the effect of removing phosphate from the soln. the following materials were placed in four 250-ml flasks—(1), 100 ml of a potassium chloride soln.; (2), 100 ml of a potassium chloride soln. and 2·5 g of superphosphate; (3), 100 ml of potassium chloride soln., 2·5 g of superphosphate and 2·5 g of magnesium chloride hexahydrate; (4), 2·5 g of superphosphate and 2·5 g of magnesium chloride hexahydrate. The contents of flask (1) were made up to vol. with water. The contents of flasks (2),

(3) and (4) were prepared for potash determination by adding ammonium oxalate, boiling for 30 min., adding ammonium hydroxide and filtering. The insol. material from flasks (2) and (3) was dried and suspended in volumetric flasks which had been filled to the mark with water; the increase of vol. indicated that the potassium ions were dispersed in 249·3 and 248·5 ml of soln. in flasks (2) and (3) respectively, and the results were calculated for these corrected vols. Potash was determined in the four solns. by the official method, and, in addition, the correction value (flask 4) was determined colorimetrically as before. In a final expt. it was found that when the amount of sodium hydroxide soln. that is added to the aliquot before evaporation is reduced from 2 ml to 1 ml there is a decrease in the amount of residue left after dissolving the chloroplatinate. The amount of residue yielded by sodium hydroxide solns. of varying age was determined. The series of expts. showed that if the gravimetric correction for the potash in the phosphate source is used, the loss of potash is small (0·12% of potassium oxide in the original sample), but if the colorimetric correction is used the loss is appreciable (0·30%). Since 80% ethanol causes some loss of potassium chloroplatinate in the official method, the gravimetric correction is undoubtedly low, and if there is loss due to presence of phosphate at the time of ignition all the potash in the phosphate source will not be represented in the gravimetric correction value. It appears therefore that the colorimetric method more accurately measures the amount of potash in the phosphate source and that the loss of potash in the official method is greater than has been previously reported. Removal of the phosphate by means of magnesium chloride yields slightly higher potash values in spite of the slight occlusion of potassium ions by the magnesium ammonium phosphate ppt. Treatment of the insol. residue left after dissolving the potassium chloroplatinate with hydrofluoric acid showed it to contain 91·4% of silica. The sodium hydroxide soln. on long standing reacts with the glass of the container, and the sodium silicate formed becomes insoluble on ignition and is not removed in subsequent operations. It would thus seem desirable to modify the official method by specifying the use of a freshly prepared, silicate-free sodium hydroxide soln. or by recommending the dissolving out of the potassium chloroplatinate and the re-weighing of the crucible.

A. O. J.

Organic

Determination of Unsubstituted Acid Amides. J. Mitchell, Jr., and C. E. Ashby (*J. Amer. Chem. Soc.*, 1945, **67**, 161–164)—Unsubstituted amides are determined by reaction with 3.5-dinitrobenzoyl chloride, in presence of pyridine, according to the reaction:



$$RCONH_2 + (NO_2)_2C_6H_3COCl \rightarrow RCN + (NO_2)_2C_6H_3COOH + HCl.$$

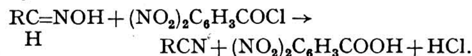
Excess of dinitrobenzoyl chloride is decomposed with methanol, and a blank test is made:

$$CH_3OH + (NO_2)_2C_6H_3COCl \rightarrow (NO_2)_2C_6H_3COOCH_3 + HCl.$$

The increase of acidity in the sample over the blank is equivalent to the amide originally present. *Procedure*—Weigh the sample, containing about 10 mg-equivalents of amide, into a 250-ml glass-stoppered volumetric flask containing 5 ml of pyridine and 15 ml of 3.5-dinitrobenzoyl chloride reagent (prepared by dissolving 461 g of Eastman Kodak 3.5-dinitrobenzoyl chloride in sufficient purified anhydrous 1.4-dioxan to make 1 litre of soln., treating with activated carbon and filtering rapidly without exposure to excessive moisture). Place the flask and a blank in a water-bath at 60° C. for 30 min. (70° C. for 1 hr. when analysing amides of dibasic acids, which require a higher temp., a fine state of division and frequent shaking; 4-mm glass beads in the flask aid in dispersing the solid). Remove the flasks and cool in ice. Add 2 ml of dry methanol, and, after 5 min., add a further 25 ml of dry methanol. Titrate the soln. with 0·5 *N* sodium methylate, using as indicator phenolphthalein or ethyl bis-2.4-dinitrophenyl acetate (Fehnel and Amstutz, *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 53; this indicator is sold by Eastman Kodak Co. and by R. P. Cargille, 118, Liberty Street, New York 6, N.Y., as "Clearol Blue"). With dark-coloured solns., as obtained with formamide, potentiometric titration is necessary. The net increase of acidity of sample over the blank, after correction for free acid and water originally in the sample, is equivalent to the primary amide. Some data are given in the table.

Substance	Condition	Recovery weight %
Formamide (*b*) ..	0·5 hr. at 60° C.	101
Acetamide (*c*) ..	0·5 ,, ,, ,,	100·0 ± 0·2
Diacetamide hydrochloride (*d*) ..	0·5 ,, ,, ,,	100·0 ± 0·0
Propionamide (*c*) ..	0·5 ,, ,, ,,	99·5 ± 0·2
Butyramide (*c*) ..	0·5 ,, ,, ,,	99·6 ± 0·3
Isobutyramide ..	0·5 ,, ,, ,,	100·4 ± 0·2
n-Valeramide ..	0·5 ,, ,, ,,	100·4 ± 0·1
Heptamide ..	0·5 ,, ,, ,,	100·8 ± 0·2
Succinamide (*e*) ..	1 ,, ,, 70° C.	98·9 ± 0·4
Glutarimide (*e*) ..	1 ,, ,, ,,	100·3 ± 0·1
Adipamide ..	1 ,, ,, ,,	99·4 ± 0·2
Benzamide (*c*) ..	0·5 ,, ,, 60° C.	95·5 ± 0·3
Salicylamide ..	0·5 ,, ,, ,,	100·2 ± 0·5
p-Nitrobenzamide	0·5 ,, ,, 65° C.	94·4 ± 0·2
Phthalamide ..	1 ,, ,, ,,	95·8 ± 0·3
Furoamide ..	0·5 ,, ,, ,,	101·5 ± 0·2

Results obtained with formamide, adipamide, salicylamide, urea, biuret, acetyl urea, malonamide and *l*-asparagine are discussed. Allyl urea failed to react, and oxamide reacted only partially, apparently because of its insolubility. No interference was encountered with N-substituted amides, urethanes and anilides. Negative results were obtained on urethane, amyl carbamate, melamine, dimethylformamide, acetanilide and propionanilide. Amides and alcohols do not interfere, beyond using up some of the reagent. Water and free acids increase the hydrogen ion concn. of the final soln., but as both reactions are stoichiometric the results can be corrected directly. Aldoximes presumably are dehydrated thus—



$$\underset{H}{RC{=}NOH} + (NO_2)_2C_6H_3COCl \rightarrow RCN + (NO_2)_2C_6H_3COOH + HCl.$$

The reactions of acetamide with acetylpyridinium chloride and benzoyl chloride are also discussed.

E. M. P.

Analysis of Wood Sugars. J. F. Saeman, E. E. Harris and A. A. Kline (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 95–99)—For experimental work in wood saccharification two methods were developed for determination of reducing sugars, *viz.*, an electrometric titration method for obtaining analytical results quickly and the Shaffer and Somogyi method (*J. Biol. Chem.*, 1933, **100**, 695) for use

when a large number of determinations have to be made simultaneously. In the electrometric method standard Fehling's soln. is titrated with reducing sugar solns. as in Lane and Eynon's method (*J. Soc. Chem. Ind.*, 1923, **42**, 143T; ANALYST, 1923, **48**, 220) but the difficult end-point caused by presence of alkaline wood hydrolysates is avoided. Britton and Phillips (ANALYST, 1940, **65**, 18) have shown that the electromotive force across a normal calomel cell and a platinum electrode can be used to determine the end-point in the titration of glucose with Fehling's soln. Expts. showed that when all but 1 or 2 ml of the sugar soln. is added to the Fehling's soln., the mixture boiled for 2 min and the titration completed within the next min., the point of max. inflection of the titration curve (−550 millivolts) agrees with the end-point found with methylene blue. An apparatus is described in which it is not necessary to clean and refill the burette for each determination nor to make an accurate dilution of the sample soln. if the concn. is below 12 g per 100 ml. The burette is arranged for automatic filling with a 5-mg per ml soln. of glucose and after each determination the ground-glass plug in the calomel cell is momentarily loosened for rinsing. Titrations are made in tall-form beakers, and a motor effects rapid stirring. Make a blank titration with 25 ml of Soxhlet's modification of Fehling's soln. (≡ *ca.* 120 mg of glucose). Estimate the reducing sugar content of the wood sugar sample by multiplying °Brix by 0·7. Dilute a vol. of the unknown soln. containing less than 120 mg of reducing sugar in the titration beaker with water to *ca.* 5 mg of reducing sugar per ml. The dilution need not be precise, since an error of ± 5 ml will result in an error of only 0·3% in the apparent sugar concn. Add Fehling's soln. and complete the titration with the standard glucose soln. as described by Lane and Eynon (*loc. cit.*) but determining the end-point electrometrically. Formic acid alters the shape of the titration curve but does not alter the titre. Calcium, which causes low sugar values, must be removed by shaking the neutralised wort with 0·5% of its wt. of sodium or potassium oxalate and filtering or centrifuging, a moderate excess of alkali oxalate having no effect upon the titre. The other method for reducing sugars is that of Shaffer and Somogyi (*loc. cit.*) with their reagent 50 and a boiling time of 30 min. To determine fermentable sugars in wood hydrolysates advantage is taken of the fact that high concns. of yeast will remove sugars rapidly from dil. solns. Dilute the sample to *ca.* 1·5 mg of reducing sugar per ml and determine the reducing sugar content by the Shaffer and Somogyi method. To the diluted sample add sulphuric acid from a burette in the proportion of 0·1 ml per 100 ml. To 20 ml of the acidified soln. in a 30-ml vial add enough pptd. chalk to leave a small insol. excess. This procedure adjusts the *p*H to 6·3 ± 0·2. Add *ca.* 1 g of compressed baker's yeast and shake the stoppered vial for 1 hr. at 30° C. Centrifuge and analyse an aliquot of the supernatant liquid for sugar by the Shaffer and Somogyi method. The difference between the sugar content before and after treatment with yeast indicates the fermentable sugar content. If unwashed yeast is used a blank determination is necessary. To determine the alcohol content of fermented wood sugar solns., convert an analytical balance into a Westphal balance by replacing one pan by a mercury-filled glass bob. Distil 100 ml of the liquid neutralised to bromothymol blue used externally, collecting 48 ml of distillate in a 50-ml flask. Add 1 ml of a suitable wetting agent (*e.g.*, 0·8% Nacconol NR) and adjust the vol. to 50 ml. Determine the sp.gr. of the distillate under thermostatic conditions by weighing the bob in air, in the distillate, and in water to which the same amount of wetting agent has been added, taking care to ensure that the container is always filled to the same level so that the suspending wire of the bob displaces the same vol. of liquid. The concn. of alcohol in the distillate is double that in the sample. The alcohol content may be checked by means of an immersion refractometer. A. O. J.

Colorimetric Estimation of Pentoses and Pentosans. W. L. McRary and M. C. Slattery (*Arch. Biochem.*, 1945, **6**, 151–156)—In this method soluble sugars are first removed from the dry material by alcoholic extraction, the residue is then hydrolysed to render the pentosans soluble, interfering substances are removed by fermentation, if necessary, and the resulting soln. is assayed fluorimetrically.

Extract 1-g samples of the dry, ground material continuously with 80% ethanol for 6 hr. and dry the residues in an oven at 70–80° C. for a short period. Transfer the dry material quantitatively to 200-ml graduated flasks, add 25 ml of *N*-sulphuric acid, and immerse in a boiling water-bath for 10 to 15 min. Add another 75 ml of acid and heat at 100° C. for a total of 3 hr. or until maximum pentosan values are obtained (as determined by preliminary expts.). Cool, make up to volume and filter a portion of the suspension. Put 5 ml of the filtrate, containing not more than 3 mg of pentose per 100 ml, 5 ml of xylose standard containing 2 mg per 100 ml, and 5 ml of water, into 3 test-tubes. Add to each 15 ml of orcinol reagent (dissolve 2 g of orcinol in 50 ml of 1·5% $FeCl_3 6H_2O$ soln. and make up to 1 litre with 30% hydrochloric acid). Mix and immerse the tubes in a boiling water-bath for 20 min. Cool, and measure the colours in a photoelectric colorimeter with a red (660 $m\mu$) filter, with the blank soln. as reference. Calculate the pentosan content of the unknown soln. from the readings given by the standard, expressing the result as xylose. Removal of hexose sugars by fermentation is rarely required, but where this must be done, neutralise 20 ml of the filtrate, using phenolphthalein as indicator, and add 4 ml of a suspension of pressed baker's yeast in an equal vol. of phosphate buffer, *p*H 6·8. Incubate at 38° C. for 2 to 3 hr., dilute to 50 ml, centrifuge, and use a portion of the supernatant liquid for colorimetric determination, as described above. The recovery of added pentose was 98–102% of the theoretical. F. A. R.

Interference with the Colorimetric Estimation of Lactic Acid by Nitrate and Nitrite Ions. J. A. Russell (*J. Biol. Chem.*, 1944, **156**, 463–465)—The estimation of lactic acid by the method of Barker and Summersen (*J. Biol. Chem.*, 1941, **138**, 535; ANALYST, 1941, **66**, 384) occasionally gives wide variations in the amount of colour developed in the reaction. This has been shown to be due to the use of different batches of sulphuric acid, some of which contain traces of nitrates or nitrites. It has been shown that addition of small amounts of sodium nitrate or nitrite interferes to a considerable extent with colour development in the lactic acid reaction. Only sulphuric acid free from nitrite or nitrate should be used. F. A. R.

Colour Test for Oils and Resins with Hirschsohn Reagent for Cholesterol. H. C. Brinker (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 130)—The

Hirschsohn reagent for cholesterol (9 g of trichloroacetic acid and 1 ml of water) provides a simple colour test for some types of oil, especially when the colour produced is compared with that produced by authentic specimens of the oil. The reagent also gives colour reactions with some resins. To 1 or 2 drops of oil, or a corresponding amount of resinous material, on a porcelain spot plate add 3 or 4 drops of the reagent and allow the colour to develop for 1 to 5 min., noting any changes that occur within that period. To induce more rapid development of the colour the plate may be warmed to 35° C. The following selections from the list given indicate the value of the test.—Linseed oil, blue to blue-purple; heavy-bodied linseed oil, dark brown; maize oil, light blue to purple; neat's-foot oil, light pink; soya oil, dirty blue to purple; cold-pressed castor oil, practically no colour; cottonseed oil, light to medium purple; blown castor oil, orange to brown; fish oil, dirty red to blue; rosin, blue-green to dark blue; cumar, light red; linseed type varnish film, brown to brown-red; dehydrated castor type varnish film, light yellow. A. O. J.

Detection and Estimation of Steam-distilled Wood Turpentine in Gum Spirits of Turpentine. S. R. Snider (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 107–109)—A reliable test is required for detecting the adulteration of gum spirits of turpentine with steam-distilled turpentine. The presence of benzaldehyde in steam-distilled turpentine and its absence in gum spirits has been demonstrated by several workers, and a means of detecting and determining the minute amount of benzaldehyde present would serve to identify the adulterated product. An analytical procedure was suggested by the ready oxidation of benzaldehyde to benzoic acid. The Mohler test for benzoic acid ("*Methods of Analysis of the A.O.A.C.*," 1935, p. 217) consists in nitration of the benzoic acid and its subsequent reduction to 3,5-diaminobenzoic acid, the ammonium salt of which is red. By the following procedure all constituents of turpentine affecting the Mohler test adversely are removed. Distil the sample, preferably through a 30-cm Vigreux column, and collect the fraction distilling below 170° C. Extract at least 300 ml four times with 10-ml portions of 30% sodium bisulphite soln. Remove all traces of turpentine from the combined extracts by washing with two 25-ml portions of ether. Filter the liquid into a separating funnel, cautiously add sat. sodium carbonate soln. until effervescence ceases and the soln. is alkaline to litmus, and extract the regenerated benzaldehyde with 25 ml of ether, allowing ample time for a sharp separation of the layers. Reserve the aq. layer and wash the ethereal layer with two 25-ml portions of water. Shake the ethereal layer vigorously with 2 ml of *ca.* 2 *N* sodium hydroxide and a few drops of 30% hydrogen peroxide in a test-tube, suspend a cotton thread in the tube and remove the ether by evaporation. Re-extract the reserved aq. layer with ether and treat the extract in the same manner. Heat the tube containing the combined residues in an oil-bath at 120° C., adding drops of hydrogen peroxide until the liquid is colourless or nearly so, and adding a drop of water when evaporation becomes too rapid. A few grains of alundum or white sand or asbestos fibre may then be added to prevent caking during the subsequent nitration. Evaporate to complete dryness and heat in a vacuum oven at 100° C. for 30 min. or in a drying oven at 130° C. for 1 hr. Cool the tube *in vacuo* over phosphorus pentoxide, add 0·3 g of potassium nitrate crystals and 3 ml of conc. sulphuric acid and heat in boiling water for 20 min., breaking up the salt cake with a rod to ensure complete soln. To the cold product add 5 ml of water and then, cautiously, 30 ml of 15% ammonium hydroxide, cooling the tube in running water. Add 2 ml of fresh 6% hydroxylamine hydrochloride solution, mix thoroughly and heat in a water-bath at 65° C. for 5 to 6 min. and avoid overheating. Filter the liquid. A red colour in the filtrate indicates benzaldehyde in the original sample. For the quantitative application of the method, prepare colour standards by dissolving 104 mg of benzoic acid in 100 ml of ether, and pipette 0·2 ml, 0·4 ml, *etc.*, to 2·0 ml of the soln. (corresponding with 1, 2, . . . 10 p.p.m. of benzaldehyde in 300 ml of the original sample) into test-tubes. Add 2 ml of 2 *N* sodium hydroxide and a few drops of hydrogen peroxide and proceed as already described. On a basis of 70% recovery of added benzaldehyde the benzaldehyde content of the sample in p.p.m. is given by

$$W \times \frac{1000}{SV} \times \frac{C_6H_5CHO}{C_6H_5COOH} \times \frac{100}{70}$$

where W is the wt. in mg of benzoic acid in the comparison tube, S is the sp.gr. of the sample and V its vol. Assuming an average sp.gr. of 0·860 and a vol. of 300 ml, this expression is reduced to 4·81 W. A table giving the combinations of red and yellow Lovibond glasses that may be used instead of the prepared colour standards is given. A. O. J.

Microscope Tests for Cottonised Bast Fibres in Presence of Cotton and "Zellwolle." Haller (*Melliand Textilber.*, 1943, **24**, 6–8, Jan.)—When native bast fibres are treated with zinc chloride soln., washed well, and stained with a dilute soln. of auric chloride, the middle lamella develops a deep brown-red shade, but the cell wall remains unstained. Immersion of the fibres in a soln. of Ciba Violet B, followed by heat treatment in a glycerin bath above 100° C., deposits the adsorbed dye in the lumen and middle lamella of the fibres. These structures are then rendered visible under the microscope by the use of dark-ground illumination or of polarised light. Bast fibres (*e.g.*, hemp) may be "cottonised" on the commercial scale, *i.e.*, disintegrated so as to separate the middle lamella from the remainder of the fibre without damaging the characteristic fibre structure. If a mixture of such material with cotton and "zellwolle" is treated on a microscope slide with copper ethylenediamine and warmed so as to evaporate the water, the cotton swells irregularly along the length of the fibre, whilst the "zellwolle" and cottonised hemp fibres swell uniformly, although only the "zellwolle" dissolves subsequently. [The preparation of the copper ethylene diamine reagent is not described. It is usually prepared by saturating a soln. of ethylene diamine with cupric hydroxide.—ABSTRACTOR.] J. G.

Microscope Stain for Mixtures of Unbleached Paper Fibres. A. Noll. (*Papier-Fabrikant*, 1943, **41**, 261–265)—The stain is prepared by dissolving 0·1 g of pure Lauth's violet or methylene blue in a mixture containing 25 ml of glycol monoacetate and 25 ml of a 4% soln. of aniline sulphate in water. Unbleached pulps are stained as follows: soda pulp, deep blue; mechanical wood pulp, yellow to brown; sulphite pulp, practically no staining reaction. Sizing agents, loadings and dyestuffs which may interfere, should be absent.

The reagent is stable for several months if impurities are absent, and if it is stored in the dark. J. G.

Inorganic

Colorimetric Determination of Bismuth with Dimercaptothiobiazole. A. K. Majumdar (*J. Indian Chem. Soc.*, 1944, **21**, 240–244)—Dimercaptothiobiazole gives a red ppt. with bismuth nitrate soln. If the reaction is conducted in presence of gum acacia a colour is produced by which bismuth may be detected at a limiting dilution of 1 in 6×10^6, and which obeys Beer's law up to a concn. of 650 p.p.m. The peak of the absorption curve occurs at 470 $m\mu$. *Reagents*—Dimercaptothiobiazole (prepared and recrystallised according to Losanitsch, *J. Chem. Soc.*, 1922, **121**, 2544): 0·5% aqueous soln. Fresh solns. of freshly prepared reagent are more sensitive than fresh solns. of reagent a few months old or solns. a few days old. Gum acacia: 0·5% aqueous soln. *Method*—Add to a suitable vol. of bismuth soln., faintly acid with nitric acid, 5 ml of *N* nitric acid, 5 ml of gum soln., water, and a few drops of reagent soln. (sufficient to develop the colour fully) so that the final vol. is 20 ml. After 5 min. compare with a similarly treated standard soln. in a colorimeter, *e.g.*, Duboscq. Excess of reagent or of gum soln. does not affect the colour. Extra gum soln. is sometimes necessary when the soln. contains large amounts of electrolytes. The following minimum quantities of other ions interfere with the determination of 0·26 mg of bismuth—Cu^{++}, 0·07 mg; Co^{++}, 2·5 mg; Ni^{++}, 9 mg; Cd^{++}, 3 mg; Pb^{++}, 5 mg; Hg^{++}, 1 mg; Ag^{+}, 0·5 mg; As^{+++}, 5 mg; Sb^{+++}, 0·2 mg; Sn^{++}, 1 mg. At least 50 mg of Zn^{++}, Mn^{++}, Fe^{++}, sulphate, oxalate, tartrate, chloride, sulphite and phosphate do not interfere. The method may be applied to bismuth after separation as phenylarsonate. Notes are given of the solubility of dimercaptothiobiazole compounds of several metals in organic liquids. Separation of the bismuth by solvent extraction of the coloured compound is being studied. L. A. D.

Titration of Nickel. S. Sen (*J. Indian Chem. Soc.*, 1944, **21**, 311–312)—A volumetric procedure, useful for the analysis of non-ferrous alloys, depends on titration of an ammoniacal soln., free from copper, tin and lead, with 1% alcoholic dimethylglyoxime soln. *Method*—Dissolve 0·2 g of the sample in 50 ml of nitric acid, evaporate almost to dryness, add 50 ml of water and filter off the tin ppt. Remove copper and lead either with hydrogen sulphide or by electrolysis with platinum electrodes. Boil out hydrogen sulphide, if used, dilute to 200 ml, add 20 ml of 25% ammonium chloride soln., 10 ml of 20% citric acid soln. and 50 ml of ammonia soln. Warm to 55–60° C. for 5 min., add 5 ml of ammonia soln. and titrate with dimethylglyoxime soln. Use a filter-paper soaked in dimethylglyoxime soln. as external indicator. While any nickel remains in solution a red colour is produced which near the end-point can only be seen by transmitted light. After 3 or 4 drops more this colour is not produced, and this is taken to indicate the end-point. Standardise the dimethylglyoxime soln. against a known nickel soln. Use an automatic burette to avoid loss of alcohol by evaporation. Iron is prevented from interfering by the citric acid, although the end-point is sharper when iron is absent. Up to 40% of zinc in the alloy does not interfere. L. A. D.

Recovery of Dimethylglyoxime from Nickel Precipitate. S. Sen (*J. Indian Chem. Soc.*, 1944, **21**, 312)—Suspend the ppt. in water, or preferably alcohol, warm and pass hydrogen sulphide until cold, stirring if the gas stream is not sufficient to break up the mass. Digest under pressure for 1 hr., filter, and concentrate the filtrate to recover the dimethylglyoxime, which may then be recrystallised. L. A. D.

Rapid Volumetric Determination of Aluminium. L. T. Snyder (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 37–38)—In neutralised tartrate soln. the reaction $Al(OH)_3 + 6KF \rightarrow K_3AlF_6 + 3KOH$ can be made the basis of a reliable and rapid acidimetric method. To the chloride soln. free from ammonium ions (0·025 to 0·13 g Al) add standard barium hydroxide soln. until there is a slight turbidity, then add 30 ml of 30% sodium potassium tartrate soln. and continue the titration to the phenolphthalein end-point. Add 30 ml of 30% potassium fluoride soln. (neutral to phenolphthalein) and titrate with 0·3 *N* hydrochloric acid until the colour does not return after 30 sec. An accuracy of ±0·1 mg is claimed. The acid must be standardised against a soln. obtained from pure aluminium wire dissolved in carbonate-free *N* sodium hydroxide; the alkaline soln. is slightly acidified with hydrochloric acid and diluted to known volume. If primary standards are used a consistent negative error of 0·8% results. For an unexplained reason, titration with sodium hydroxide gives lower results than with barium hydroxide. Phosphate, silicate, sulphate, ammonium, and chromium interfere, whilst carbonate, chloride, potassium and sodium do not. Small amounts of copper, iron, manganese, magnesium and zinc (in a molar ratio to Al of 1 : 100) are harmless. W. R. S.

Specific Spot Test for Vanadium. G. Ashburn and J. H. Reedy (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 63)—Mix a drop of the test soln. with one of 85% phosphoric acid and, after a few seconds, one of 10% sodium tungstate soln.; vanadium gives a yellow to orange colour. Sensitivity 4 μg, or 8 μg in presence of 1000 parts of other metals unless the soln. is strongly coloured. The vanadium must be present as vanadate, and may be oxidised by warming with bromine if necessary. Reducing agents and strong acids interfere. The reaction may be due to the formation of a labile heteropoly acid; phosphoric acid is not essential, although it intensifies the colour while bleaching that of ferric salt. W. R. S.

Volumetric Determination of Calcium. J. J. Lingane (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 39–41)—The procedure consists in a direct single oxalate pptn. at controlled acidity without separation of other metals and subsequent permanganate titration. If a limestone contains organic matter, ignite 0·4 g in a platinum crucible over a blast burner. Dissolve the residue, or the original material (dolomite) in 5 ml of water and 10 ml of strong hydrochloric acid; heat to dissolve, dilute to *ca.* 50 ml, heat almost to boiling, and filter 100 ml of hot 5% ammonium oxalate soln. into the assay soln. Add methyl orange and ppt. by dropwise addition of diluted ammonia (1+1), with continuous stirring over a period of 5 to 10 min. until the tint is pinkish-yellow (*p*H 4±0·3). Set aside to settle for 20 to 30 min., collect the ppt. in a Gooch crucible, and wash thoroughly with 8 to 10

small portions of ice-cold water (not over 100 ml). Return the crucible to the pptn. vessel, add *ca.* 100 ml of water and 5 to 6 ml of strong sulphuric acid, and titrate at 90° C. with permanganate standardised against 0·3 g of sodium oxalate dried at 110° C. A duplicate determination can be made within 2 hr. Ferric oxide and alumina (0·1 g) do not interfere, but 0·2 g causes a positive error of 0·5%. There is no interference from 0·5 g of phosphorus pentoxide. If the amount of manganese exceeds 2 mg, it is co-pptd. and causes a positive error which is not proportional to the total amount present. Titanium gives slightly high results and the error is not eliminated by its pptn. as phosphate, but the normal small amount of titanium present in calcareous materials will not cause a material error. W. R. S.

Fractionations in the Rare-Earth Group by Means of Sodium Sulphate. T. Moeller and H. E. Kremers (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 44–45)—Sodium sulphate, a familiar group-reagent, is capable of effecting a convenient concentration of the terbium and yttrium earths by systematic fractionation. The authors heat the filtrate from double-sulphate ppts. produced in the cold, whereby a further fraction is obtained. In the last stages, however, when the cerium group is eliminated and the fractions become small, only one pptn., from hot soln., is carried out. Monazite residues (1710 g; 5% of yttrium earths) were added to hot strong nitric acid and the soln. was filtered, which eliminated *ca.* one-third of the ceria as an insoluble basic salt. The cold filtrate, diluted to contain 8·5% of oxides, was pptd. by slow addition of powdered sodium sulphate until the neodymium absorption bands in the mother-liquor became almost invisible. The ppt., containing 1230 g of earths almost free from yttria, was discarded. The filtrate was heated, yielding 208 g of earths in a secondary ppt., and a filtrate from which 55 g of earths were recovered by means of oxalic acid. These oxides were separated into 5 main fractions by pptn. with sodium sulphate from cold 10% nitrate soln. and heating of the mother liquors, each fraction being re-treated. In this way 65 fractionations were made in series of 2 to 5 fractions, those from the insoluble end being discarded when the yttria dropped below 15%, and those from the soluble end when the resulting oxides were almost colourless. Serial re-pptn. of the first main fraction gave 2 fractions and a mother-liquor containing the terbium earths with a few % of cerium earths and less than 15% of yttria; total weight 35 g, average atomic weight *ca.* 150. The 3rd, 4th and 5th main fractions yielded 6 fractions free from cerium earths, containing 95 to 99% of yttrium earths; balance terbium earths; total weight 66 g, average atomic weight 95. The oxides were recovered from the double-sulphate ppts. by solution in 10% ammonium acetate soln. and pptn. as oxalates. Fractions were combined on the basis of analyses. The latter involved determination of the average atomic weight (ANALYST, 1944, **69**, 30) and absorption spectrophotometry in the range 400 to 700 $m\mu$ of nitrate solns., with an instrument calibrated against the pure oxides. Praseodymia, neodymia, samaria, erbia and holmia were estimated from absorption data, and yttria from the average atomic weight after estimation of the coloured earths. The terbium earths with dysprosia were estimated like yttria and reported together; lanthana by difference. W. R. S.

Microchemical

Convenient Titrimetric Ultra-Micro Method for the Estimation of Urea and Kjeldahl Nitrogen. A. E. Sobel, A. M. Mayer and S. P. Gottfried (*J. Biol. Chem.*, 1944, **156**, 355–363)—Set up an aeration apparatus consisting of pairs of Pyrex test-tubes with side arms, each fitted with a one-hole rubber stopper carrying a glass tube with a drawn-out tip. Connect the side-arm of one tube—the digestion tube—by means of rubber tubing with the tube passing through the stopper of the other test-tube—the receiver. Put the soln. to be tested, containing 10 or 100 μg of ammoniacal nitrogen, and 1 ml of water into the digestion tube, and 1·5 ml of 2% boric acid soln. and 0·015 ml of indicator (8 parts of 0·1% alcoholic bromocresol green soln. and 1 part of 0·1% alcoholic methyl red soln.) into the receiver. Introduce 0·5 ml of alkali soln. (12 parts of water, 6 parts of sodium hydroxide and 1 part of sodium thiosulphate) into the digestion tube, and immediately stopper both tubes and begin to aerate slowly. Stop the aeration after 20–30 min. by rapidly disconnecting the tubes from the pump. Remove the glass tube from the receiving tube, wash with a little water, and titrate the boric acid soln. back to its original *p*H (4·2) with the aid of a capillary micro-burette, the tip of which is kept beneath the surface of the liquid; this is stirred by means of a stream of air. A receiver containing the boric acid and indicator mixture only, serves for matching the end-point. In analysing a sample of protein, add 0·2 ml of digestion mixture (500 ml of water, 30 g of potassium sulphate, 5 g of copper sulphate, 480 ml of conc. sulphuric acid, and 0·5 g of selenium in 20 ml of conc. sulphuric acid) to the sample in a digestion tube and digest on a hot plate. After the tube has cooled for a few moments, rinse down its walls with 1 ml of water and proceed as described above. Include with each batch of samples 0·1 ml of a standard soln. (0·1 mg per ml) of ammonium sulphate. *Non-protein nitrogen*—Precipitate the protein in 0·1 ml of serum with 2·5 ml of trichloroacetic acid, centrifuge and digest 1·0 ml of the supernatant with 0·2 ml of digestion mixture. *Total protein*—Dilute 0·1 ml of serum to 1 ml with water and digest 0·1 ml of the mixture with 0·2 ml of digestion mixture on a hot plate, supporting the tubes at an angle of about 45° to prevent the liquid from bumping out. Cool and add 1 ml of water. *Albumin*—Precipitate the globulin from 0·2 ml of serum, diluted 10-fold, with 0·3 ml of 23% sodium sulphate soln., and then shake with 1 ml of ether for 1 to 2 min. Centrifuge for 10 min. and digest 0·2 ml of the aqueous layer with 0·2 ml of digestion mixture. Excellent agreement was obtained between the results by this method and by the standard micro-Kjeldahl method. *Urea*—Add 0·1 ml of serum, spinal fluid or urine, containing 10 to 200 μg of urea nitrogen, to a digestion tube containing 1 drop of phosphate buffer *p*H 7·0 (15 g of sodium pyrophosphate, 1·4 ml of 85% phosphoric acid and 100 ml of water), 1 drop of urease extract,* and 3 drops of anti-foam reagent (capryl alcohol saturated with thymol is preferred to capryl alcohol only). Incubate for 10 min., add

* Wash 10 g of permutit with three 50-ml portions of 2% acetic acid and add 100 ml of 2% acetic acid, 150 ml of 0·001 *N* sulphuric acid and 50 g of jack bean meal. Shake for 15 min., add 200 ml of glycerin, shake for a further 10 min., and leave overnight in the refrigerator. Centrifuge the supernatant liquid.

0·5 ml of 56% potassium carbonate soln. and aerate for 20 to 30 min. The recoveries of urea added to serum were practically theoretical. F. A. R.

Microdetermination of Calcium. C. D. Kochakian and R. P. Fox (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 762–764)—Calcium is pptd. as oxalate, which is dissolved in perchloric acid and titrated at room temp. with ammonium hexanitratocerate soln., Setopaline C being used as indicator. Ceric sulphate and ceric ammonium sulphate solns. do not always give sharp end-points and heating is required. Pipette the sample into a 15-ml conical centrifuge tube, add 2 drops of methyl red soln. and 1 ml of 4% ammonium oxalate soln. Add dropwise 1 *N* ammonia until the liquid is just yellow, then 1 *N* hydrochloric acid until the re-appearance of pink. Stir with a glass rod, remove the latter and hang it on a rack. After 1 hr., centrifuge, pour off the supernatant fluid and invert the tube for 2 min. on a towel. Wipe the rim, replace the stirring rod, pipette 5 ml of wash soln. (mix 2 ml of 28–29% ammonia, 98 ml of distilled water, 100 ml of redistilled ether and 100 ml of redistilled ethyl alcohol) down the sides of the tube, and disperse the ppt. Centrifuge and repeat the washing. Dissolve the ppt. in 4 ml of 1 *N* perchloric acid and add 6 drops of *warm* indicator soln. (Warm 50 mg of Setopaline C with 100 ml of distilled water. The soln. is always used warm, as the dye ppts. on cooling.) Titrate with 0·01 *N* ammonium hexanitratocerate soln.* until the colour changes from yellow to salmon pink. Use a micro-burette graduated at 0·02 ml and keep the tip above the surface of the soln. The colour-change is extremely sharp and distinct, but persists only 15 sec. at the end-point. With 1–2-mg portions of calcium, the error is about 1%, results tending to be low. As little as 0·5 mg of calcium may be determined. J. T. S.

Modification of the Rast Method of Micro-Cryoscopic Molecular Weight Determination. H. Keller and H. v. Halban (*Helv. Chim. Acta*, 1944, **27**, 1439–1443)—By making the observation with polarised light, the difficulty of determining the exact temp. at which the last crystals of solvent disappear may be overcome. The apparatus consists of a beaker-like bath, into the walls of which are sealed two V-shaped pieces of glass tubing (similar to a "P-tube" used for detn. of m.p.). Heating coils are wound on the lower arms of the V-tubes. Two variable resistors provide coarse and fine adjustment of the rate of heating. The heating coils may be switched either in series or in parallel, permitting rapid attainment of high temps. without loss of control. The bath liquid is 25% calcium chloride soln. for temps. up to 100° C., while conc. sulphuric acid is used up to 210° C. The thermo-siphon method of heating promotes mixing of the bath liquid, but additional agitation is provided by an electric stirrer. A brass plate, attached to the Plexiglass lid of the bath, carried the stirrer-spindle, the thermometer, and a glass rod supporting a perforated gold plate with recesses for the thermometer bulb and the melting-point tube. Light from a small electric lamp passes through a converging lens and a Bernauer (Zeiss) polarisation filter placed behind the bath, and a similar filter in front serves as analyser. Both filters may be moved out of position when required. J. T. S.

* Dissolve 6 g in *ca.* 200 ml of 1 *N* perchloric acid without warming and dilute to 1 litre with acid. Store in a black bottle in the dark, as the titre rapidly falls on exposure to diffuse daylight. To standardise, add 0·5 ml of 60% perchloric acid to 4 ml of 0·01 *N* sodium oxalate soln. and titrate as above.

Physical Methods, Apparatus, etc.

Qualitative Spectrographic Analysis. G. W. Standen (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 675–680)—Three of the principal applications of qualitative spectrography are: (1) As a preliminary to quantitative analysis, to indicate what elements should be determined and what separations may be required to avoid interferences. (2) As an aid to qualitative X-ray diffraction analysis to limit the search for an X-ray pattern match to compounds containing the principal elements found. (3) To detect the presence of beneficial or deleterious trace elements in raw materials or finished products. The direct current arc with graphite electrodes is probably the best for general detection of metallic constituents from the standpoint of sensitivity, general applicability and convenience. For a reliable analysis, complete volatilisation of the sample is necessary, and this may involve a photographic exposure time of several minutes. A much more rapid technique is the Hasler high streaming velocity arc, whereby the sample, in the form of a fine powder, is intimately mixed with carbon powder obtained by the charring of sucrose (graphite is not suitable). This material is packed into the crater of a special graphite electrode and volatilised in the d.c. arc. Under these conditions the gases developed by the burning sucrose carbon powder blow the sample rapidly into the arc flame. Most samples are completely removed in 30 sec. The transfer of the sample from the electrode to the arc is much more uniform than with the fractional distillation taking place in the conventional arc. In addition, this method is somewhat more sensitive than the ordinary arc. Another method, using a moving plate, sometimes gives greater sensitivity in detecting elements which volatilise over a short period at some stage of the arcing period. In this method a spectrum is photographed for each 30 sec. of exposure over the entire 5 to 15 min. arcing period of an ordinary d.c. arc burn, the plate being moved down one spectrum width each 30 sec. Results indicate that this method is more likely to detect all the elements present than the high streaming velocity arc method. A number of useful tables are included in the article, among which is one listing, for all the detectable elements, the concn. of all interfering elements which may prevent identification of traces of the element sought. B. S. C.

Spectrophotometric Study of the Oxidation of Quenching Oils. G. L. Clark and W. I. Kaye (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 740–745)—The residual strain in aluminium alloy castings quenched in a large tank of oil was found to increase considerably as the oil was used. A spectrophotometric study of the changes occurring in the oil, and also in other varieties of quenching oil, was made and the results are given in this paper. The spectrophotometric data are believed to be related to the light scattered by colloidal particles or "precipitables" suspended in the oil. The methods used to interpret the results are described and the oxidation of oils and the action of various inhibitors or antioxidants are discussed. Some comments are given on the nature of the material precipitated from oxidised oils, and it is reported that the used oil which had been completely freed from suspended

matter by careful filtration showed a great improvement in performance and had a much longer useful life than new oil. Oxidation stability and absence of precipitable polymeric particles are correlated with the quality of the quenched castings measured in terms of residual strain. L. A. D.

Photometric Determination of Silica in Aluminous Materials. J. A. Brabson, I. W. Harvey, G. E. Maxwell and O. A. Schaeffer (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 705–707)—Silica in sodium aluminate soln. and in calcined alumina, and silicon in aluminium, may be determined photometrically by means of the molybdenum blue reaction. *Procedure for calcined alumina*—Transfer a sample of from 0·1 to 0·4 g of alumina (depending upon the silica content) to a 125-ml platinum dish, cover with 4 g of anhydrous sodium carbonate and 0·7 g of boric oxide and mix inmately. Fuse at 1000° C. in a muffle furnace to obtain a perfectly clear melt (about 15 min.). Cool, add 50 ml of water and digest on a steam-bath until the melt dissolves. Cool, transfer the soln. to a 250-ml beaker containing 50 ml of water and dilute to 170 ml. Add 8 drops of thymol blue indicator and conc. hydrochloric acid, drop by drop, until the aluminium hydroxide, which first precipitates, is nearly dissolved and the soln. is yellow (not red). Then add drops of 10% hydrochloric acid, stirring constantly, until the aluminium hydroxide completely dissolves and the indicator is pink. This operation is critical and the adjustment of *p*H may take 5 min. with samples of higher aluminium salt concn. Add 5 ml of 10% hydrochloric acid, 5 ml of acetic acid (1 part of glacial acid to 2 parts of water) and 5 ml of ammonium molybdate soln. (25 g in 250 ml of water) in that order, stirring vigorously after each addition. Wait 5 min., transfer to a 250-ml volumetric flask and reduce, by adding slowly from a pipette while shaking vigorously, 20 ml of 17% sodium sulphite soln. Wait 8 min., add 5 ml more of the acetic acid, dilute to the mark and mix thoroughly. Then, 30 min. after adding the sulphite, determine the optical absorption of this soln. in a 2-cm. cell, using a suitable photometer fitted with a 650 $m\mu$ red filter. In a similar manner determine the absorption of a blank on the reagents. For aluminium metal samples, place 0·05 to 0·20 g (depending upon the silica content) in a 125-ml platinum dish, add 50 ml of water and *ca.* 0·6 g of sodium hydroxide. Wait until the sample disintegrates, add a few drops of hydrogen peroxide and digest for 15 min. on a steam-bath. Cool, transfer the soln. to a 250-ml beaker containing 50 ml of water and dilute to 170 ml. Then proceed as for calcined alumina. Prepare two calibration curves for the photometer, based respectively on 0·2 and 0·4 g of alumina, using suitable aliquots of aluminium chloride soln. (10 ml corresponding to 0·4 g of alumina) and standard sodium metasilicate soln. (10 ml corresponding to 0·1 g of silica). The use of the appropriate curve minimises the effect of aluminium salt concn. on the colour development. The method may be used for the estimation of the silica content of calcined alumina in the range 0·01 to 1·0%. When it is applied to homogeneous samples a precision of 4% may be expected.

B. S. C.

Determination of Small Amounts of Zinc by Measurement of Fluorescent Turbidities. L. L. Merrit, Jr. (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 758–760)—Stable reproducible turbidities of zinc 8-hydroxyquinolinate may be produced and then accurately determined by measuring the fluorescence of the compound. The method covers a range from 0·02 to 0·6 mg of zinc in 50 ml of the final test soln., and the accuracy is within 0·02 mg. It is subject to all the interference usually associated with procedures employing 8-hydroxyquinoline. *Method*—Place amounts of zinc soln. (faintly acid with hydrochloric acid and containing between 0·05 and 0·5 mg of zinc) in 50-ml graduated flasks, and add to each 5 ml of 2 *N* ammonium acetate and 2 ml of 2% gum arabic soln. Dilute to *ca.* 45 ml, add from a pipette 0·20 ml of 8-hydroxyquinoline soln. (5 g dissolved in 12 ml of glacial acetic acid and diluted to 100 ml), adjust to 50 ml and mix. Pour the turbid soln. into the 25-ml cell of a fluorescence meter (Lumetron 402EF) and measure after 2 or 3 min. The fluorescence is best excited by light of wavelength between 460 and 350 $m\mu$, but, as the absorption by the excess reagent becomes increasingly serious as the wavelength is shortened in this range, a narrow band filter with max. transmission at 420 $m\mu$ is used in the primary beam. Yellow filters (as used with the same instrument for riboflavin determination) are used in the path of the secondary light. The instrument is calibrated by measuring known zinc solns. and a dil. soln. of dichlorofluorescein (about 0·35 ml of 0·1% alcoholic soln. in 1 litre of water) may be measured and used for several weeks to reset the instrument. Some variations in the amounts and order of addition of the reagents can be tolerated, but it is recommended that the procedure be rigidly standardised. In particular, the temp. of the soln. when the turbidity is developed should be within ± 2° C. of that used when making the calibration curve. L. A. D.

Determination of the Cuprammonium Viscosity of Cellulose, using the Hoeppler Viscosimeter. W. O. Hisey and C. E. Brandon (*Paper Trade J.*, 1945, **120**, *T.A.P.P.I. Sect.*, 11th Jan., 11–20; 18th Jan., 21–24)—The Hoeppler (falling ball-type) viscometer gives results on an average 7·5 and 34·3% lower than those obtained by the standard *T.A.P.P.I.* method (based on that of Clibbens and Geake, *cf.* ANALYST, 1943, **68**, 96) according as a glass or steel sphere is used in the former method, respectively. Advantages of the Hoeppler method are that the reproducibility is approx. 50% higher than that of the *T.A.P.P.I.* method; a lower pulp concn. may be used; the large ball enables the falling time to be determined easily; the same soln. may be tested several times. An increase in viscosity was noted, however, when the successive tests were made with the glass sphere; the first test is the more accurate. A disadvantage is that the dispersion must be prepared separately and transferred to the viscometer. The sp.gr. of the *T.A.P.P.I.* dispersion was found to be 0·945, and not 0·96 as stated in the standard method, and the specified *T.A.P.P.I.* tolerance for the ammonia concn. is too large for accurate work. The dispersion of the pulp in the *T.A.P.P.I.* cuprammonium soln. may be accelerated by using a wheel speed of 29·4 r.p.m., and a pulp concn. of 0·25%. The results are examined statistically. J. G.

Note on the Blow-Lamp Method of Detecting Organic Halogen Compounds in the Air. L. B. Timmis (*J. Soc. Chem. Ind.*, 1945, **64**, 32)—An ordinary petrol blow-lamp ("Monitor" ½-pint size) is enclosed in a well-ventilated metal box so that the flame is shielded from strong daylight, but may be viewed from above and behind. Light

petroleum (b.p. 80–100° C.) is used as fuel. Trichloroethylene (1 : 40,000), carbon tetrachloride (1 : 100,000), and "Freon" (CCl_2F_2) (1 : 30,000) all produce a trace of colour at the tip of the flame. As the concns. are increased the entire flame becomes bright green. The flame rapidly clears on removal to a pure atmosphere. The sensitivity is comparable to that of the special alcohol lamp ("*Methods for the Detection of Toxic Gases in Industry*, D.S.I.R. Leaflet, No. 12, 1940, H.M.S.O.), which now appears to be unobtainable. J. T. S.

Apparatus for the Detection and Estimation of Chlorinated Hydrocarbon Vapours in Air.* L. B. Timmis (*J. Soc. Chem. Ind.*, 1944, **63**, 380)—The air to be tested is passed over an electrically-heated nichrome wire. The decomposition products of the chlorinated compound are detected and estimated by the colour produced on a *p*-dimethylaminobenzaldehyde - diphenylamine test paper (D.S.I.R. Leaflet, "*Methods for the Detection of Toxic Gases in Industry*," No. 8, 1939, H.M.S.O.). Two forms of apparatus are described. One is a sensitive detector, but is unsuitable for quantitative work. A test-paper is supported at the upper end of a vertical Pyrex tube within which the heated wire is mounted. Convection causes a stream of air to impinge on the underside of the test-paper; carbon tetrachloride (1 : 40,000) gives a strong colour in 1 min. In the other form, a constant-flow aspirator draws the sample at 60 ml per min. over the heated wire and through the test-paper. The latter is held in position by a spring-loaded platform operated by a trigger. The volume aspirated is adjusted to produce a colour which matches one of the printed standards in the D.S.I.R. Leaflet. Since the colour develops somewhat after exposure, matching is carried out 5 min. after removing the test paper from the apparatus. Increase in temp. of the wire generally increases the sensitivity, but eventually pure air gives an appreciable stain. A very dull red heat produced by a current of 1·84 amp. is suitable for trichloroethylene, carbon tetrachloride, tetrachloroethane, ethylene dichloride, chloroform and other compounds. "Freon" (CCl_2F_2) requires a current of 2·00 amp. Accuracy is not high, but is sufficient to ensure safe working conditions. J. T. S.

* Patent Application No. 8073/44. Made by Messrs. J. W. Towers & Co., Ltd., Victoria House, Widnes.

Reviews

COLORIMETRIC ANALYSIS. By NOEL L. ALLPORT, F.R.I.C. Pp. 452 + xii. London: Chapman & Hall, Ltd. 1945. Price 32s. net.

Colorimetric methods occupy an important place in analytical practice. In many instances they are the only methods applicable to the determination of small quantities of substances; in some they enable results to be obtained in a fraction of the time demanded by other methods. Visual assessment of colour is subject to appreciable errors, which, however, are generally calculable; physical measurements can be much more precise, but non-calculable errors may be caused by interfering substances. The application of colorimetric methods to other than pure substances requires caution and the experience of those familiar with such applications is invaluable. The present volume embodies the experience of one who has wide knowledge of colorimetric methods as applied in pharmaceutical chemistry. Some may regard this field as limited; the term can, however, be accepted in the sense in which the universe is limited, *i.e.*, finite but unbounded.

This book is divided into five sections as follows (the number indicating the individual items:—Metals (25). Acid Radicles (15). Substances of Clinical and Biochemical Significance. (30). Alkaloids, Hormones and Vitamins (17). Miscellaneous Substances (20). With each Section there is an introduction and with each item there are an introduction, the method, its applications, a discussion and notes on its applications. The methods selected are, in general, chosen on considerations of wide interest and reliability, but a few processes of more restricted scope are given.

An attempt to cover such a wide subject within the compass of a moderate sized volume necessarily involves selection and omission. In the reviewer's opinion it was wiser to restrict the number of methods included than to curtail the valuable discussions and notes. The selections can be heartily approved, and it is to be hoped that some of the omissions will be made good in a supplementary volume. J. R. NICHOLLS

THE ESTIMATION OF VITAMIN A. By N. T. GRIDGEMAN. Pp. 76. London: Lever Brothers & Unilever Limited. 1944. Available on application to the Author

This trenchant commentary is worthy of the close attention of all concerned either directly or indirectly with the evaluation of materials containing vitamin A. The body of the work is divided under nine main headings as follows: The Variability of Conversion Factors. The Estimation of $E^{1\%}_{1\,cm}$ 325 mμ. The Biological Assay. Aspects of Biological Standards for Vitamin A. Carotene as Provitamin A. The Potency of Pure Vitamin A. The International Unit. Aspects of "Natural" Vitamin A. Towards the Future. There follows an Appendix consisting of a closely reasoned statistical study of the results attainable by the method of biological assay and the book concludes with a bibliography of 175 references to the scientific literature.

The main theme of the discourse is the conversion factor relating the spectrophotometric reading with the biological International Unit or, for America, the United States Pharmacopoeia Unit, which is officially, but not in fact, identical with the International Unit. It is pointed out that "the literature records experimentally observed conversion factors ranging from <1000 to >3000" and some of these discrepancies are due to differences in the technique adopted for determining the value of $E^{1\%}_{1\,cm}$ 325 mμ which, for example, in the "feeding solution of vitamin A β-naphthoate used in the last British co-operative assay was enhanced $11\frac{1}{2}\%$ when read in *cyclo*hexane." However, the author, after presenting a statistical analysis of published figures, concludes that the spectrophotometric method is capable of giving results to within $\pm 5\%$ by "direct" examination of oil and within $\pm 10\%$ for estimations conducted on unsaponifiable fractions; but, even so, the universal attainment of this degree of precision will necessitate more rigid control of working conditions than exists at present.

As to the biological assay, Mr. Gridgeman shows that even when 80 rats are used and two dosage levels employed over a test period of 5 weeks a result may be expected to fall between 77% and 130% of the true figure. After the presentation of extensive available data he concludes that the method of bioassay, quite apart from the unsatisfactory official descriptions of the technique, "is to all intents and purposes valueless as a method of routine estimation," and it is asserted that this "applies to techniques of unimpugnable biometrical rigour—the point being that normal animal variations prohibit, in a test of average size, a result of reasonable accuracy."

Turning to the question of standards, after discussing the conversion of carotene into vitamin A *in vivo* and the possible synergistic action of vitamin E the author invokes the judgment of Hickman, Harris and Woodside (*Nature*, 1942, **150**, 91) that retention of β-carotene as the International Standard of vitamin A is becoming something of a farce. Clearly, if the bioassay is abandoned the present Standard will go with it, and in this connection Mr. Gridgeman marshals cogent experimental evidence to condemn cod liver oil as a reference material. As the outcome of his incisive discourse the author suggests an international unit of the same order of magnitude as the one now in use based on that fraction of $E^{1\%}_{1\,cm}$ 325 mμ due to vitamin A alone which might be expressed provisionally as "that quantity which, when made up to 100 ml with a specified organic solvent, gives a solution having an extinction coefficient at 325 mμ of 0·0005," while qualifying clauses would have to be settled and "the way left open for a revision of details as the unravelling of analytical problems advanced." Is it possible that even this proposal would not quite fill the bill now that Robeson and Baxter (*Nature*, 1945, **155**, 300) have characterised a new form of vitamin A existing in soupfin shark and dog fish liver oils having approximately equal biological potency to the vitamin we have known but with an extinction coefficient of $E^{1\%}_{1\,cm}$ 328 mμ = 1675 as compared with $E^{1\%}_{1\,cm}$ 325 mμ = 1750 for the ordinary form?

In a Foreword the Publishers state that: "While, pending the further development of the spectroscopic method, the use of the new unit may be attended with difficulty, it at any rate provides the manufacturer with an official method of assay by means of which the claims he makes for his products may be tested," and they also suggest that it may perhaps become the basis of an agreed international standard. Apart from possible dissent in respect of details, or even principles, surely all concerned will welcome this book as a firm step in the right direction.

N. L. ALLPORT

ISSUE OF NEW LIST OF MEMBERS

THE last printed List of Members of the Society with addresses was issued in 1943 and it is proposed to issue a new List this year.

For the new List, Public Analysts and Official Agricultural Analysts are asked kindly to inform the Secretary of any changes necessary in the description of their appointments as given in the 1943 List.

Regarding the addresses of members in the new List, these will normally be the addresses to which THE ANALYST and other communications from the Society are now being sent. If a member prefers to have a different address in the List there is no objection; and when the current address of a member is subject to frequent change there is an advantage to the member in having a more permanent one in the List.

Changes in F.R.I.C. and A.R.I.C. qualifications will be inserted without notification, but members are asked to notify any other changes in degrees, etc.

Any notifications on the points mentioned above should be sent as soon as possible to the Secretary, "Society of Public Analysts," 7–8, Idol Lane, London, E.C.3.

THE PRACTICAL SIDE OF FINE TEMPERATURE CONTROL

Lecture delivered to the Society of Chemical Industry at Burlington House on May 1st, 1944 by L. T. Townson and R. Barrington Brock

We have had so many requests for reprints as a result of the very short summary in "Chemistry & Industry" that we have felt it necessary to print it in full (with illustrations) as

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