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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Extraordinary Meeting of the Society was held at 6.15 p.m. on Wednesday, October 3rd, 1945, at the Chemical Society's Rooms, Burlington House, London, W.1, with the President, Dr. G. W. Monier-Williams, in the chair. The following Special Resolution was proposed by the President, seconded by the Honorary Treasurer and carried *nem. con.*:

"That Article 10 of the Articles of Association of the Society be amended by deleting therefrom the following words:

'Provided that no entrance fee shall be payable by any member who being eligible for membership of, is nominated by, any area section of the Society and pays the appropriate subscription for membership of that Section.' "

In proposing the Resolution the President explained that it had the approval of both the North of England Section and the Scottish Section.

An Ordinary Meeting followed at 6.30 p.m. After the reading of the Minutes the President referred to the Society's loss, by death, of one of its distinguished Past Presidents, Mr. John Evans, and the meeting paid honour to his memory by standing in silence.

A paper on "The Theory of Certain Analytical Procedures, with Special Reference to Microbiological Assays" was read by Eric C. Wood, B.Sc., A.R.C.S., F.R.I.C., and discussed.

NEW MEMBERS

Granville Broughton, B.Sc. (Lond.), A.R.C.S., A.R.I.C.*; Jack Davis, B.Sc. (Lond.); John William Gailer, B.Sc. (Lond.); Frederick William John Garton, B.Sc. (Lond.); Norman Theodore Gridgeman, B.Sc. (Lond.)*; Leonard Valentyn Collin Griffiths, B.Sc. (Liv.), A.R.I.C.; John King, O.B.E., F.R.I.C.; James Herbert Oliver, Ph.D. (Lond.), D.I.C., F.R.I.C.; Arthur Owen Pearson, B.Sc. (Lond.), A.R.I.C.†; Donald Ford Phillips; Roland Arthur Harrington Sutcliffe, B.Sc. (Manc.), A.M.I.E.E.*; Reginald Gerald Wallace Willcocks, B.Sc. (Lond.), A.R.I.C.

DEATH

WE regret to record the death of Sir George Christopher Clayton, an Honorary Member.

INAUGURAL MEETING OF THE BIOLOGICAL METHODS GROUP

THE Inaugural Meeting of the Biological Methods Group was held at 6 o'clock on Wednesday, October 17th, 1945, at the Chemical Society's Rooms, Burlington House, London, W.1. The President of the Society, Dr. G. W. Monier-Williams, opened the proceedings; on his motion the meeting elected Mr. A. L. Bacharach, M.A. (Cantab.), F.R.I.C., as the Chairman of the Group. Mr. Bacharach then took the chair and the following other officers and members of the Committee of the Group were elected.

Vice-Chairman: A. J. Amos, B.Sc., Ph.D., F.R.I.C.

Honorary Secretary: Eric C. Wood, B.Sc., A.R.C.S., F.R.I.C., Virol Ltd., Hanger Lane, Ealing, London, W.5.

Committee Members: E. C. Barton-Wright, B.Sc., F.R.I.C.; E. R. Dawson, M.Sc., Ph.D.; R. L. Edwards, B.Sc., Ph.D., F.R.I.C.; D. C. Garratt, B.Sc., Ph.D., F.R.I.C.; N. T. Gridgeman, B.Sc.; H. G. Rees, B.Sc., Ph.D., A.R.C.S., D.I.C., F.R.I.C.

Ex-officio members of the Committee are the President, the Honorary Treasurer and the Honorary Secretary of the Society and the Editor of THE ANALYST.

The meeting was opened to all members of the Society and to visitors at 6.45 p.m., when the Group Chairman, Mr. Bacharach, delivered an Inaugural Address on "Biological Assay and Chemical Analysis" (published on pp. 394–403); a short discussion followed.

* Through the North of England Section. † Through the Scottish Section.

Inaugural Address to the Biological Methods Group

(*Wednesday, October* 17, 1945)

Biological Assay and Chemical Analysis

By A. L. BACHARACH

For some time now it has become increasingly clear to analytical chemists that methods other than the purely chemical are useful, and may be essential, for carrying their analyses as far as available methods will permit. In this Society such a state of affairs has been publicly recognised in the introduction of the group system. True, the Microchemistry Group may rightly claim still to be concerned with chemical methods, though some of the physical considerations involved in micro-analysis are so far removed from the relatively simple stoichiometry underlying classical analytical chemistry as to make it possible that many of the learned fathers of our subject would have thought the filter stick and the micro-balance to be instruments of the devil. On the other hand, in the Physical Methods Group chemistry as a practical craft is almost completely eschewed. True, its subject-matter extends and supplements, as its Chairman cogently pointed out in his inaugural address, the study of physical methods that had already been accepted as legitimate for the analytical chemist. Yet by no manner of means can most of that subject-matter be brought within even the wide confines of modern chemistry.

The object of the analyst to-day has been clearly stated by one of our ex-Presidents. It was Dr. Hughes who, in the Jubilee Memoral Lecture of the Society of Chemical Industry in 1938,[1] defined analysis as ". . . the examination of a material to ascertain its composition, its properties and its qualities—in fact, any investigation necessary to give the requisite information about a substance."

This is an admirable summary of the position, but in fact it casts a wider net than I need for my purposes this evening. I am content to accept chemical analysis as coming within the general scope of analysis, but as being concerned only with the *composition* of a material, except in so far as knowledge of its properties and qualities may throw light on its composition. As such knowledge almost always does this, I do not really sacrifice much ground in apparently claiming less than Dr. Hughes.

In the course of the discussions of this Society on the introduction of the group system, I suggested that a more careful and dichotomous use of the phrases "analytical chemistry" and "chemical analysis" might help to clarify the position. If we use the word "analysis" in Dr. Hughes's sense, then clearly "analytical chemistry" must be chemistry of the particular kind that is devised for carrying out analysis as defined; on the other hand, "chemical analysis" need not necessarily imply any more than the determination or statement of the chemical composition and properties of a substance. And if we accept this comprehensive description, it is clear that analytical methods besides those of chemistry may legitimately—indeed, sometimes must—be used in order to carry out chemical analysis.

The reverse statement is also true. In, for example, the phosphatase test for efficient pasteurisation of milk, there are involved only chemical procedures, based upon certain biochemical considerations. It is an example of the practice of analytical chemistry, though, paradoxically enough, it is not carried out for purposes of chemical analysis! The object of the test is a biological analysis, namely, to determine whether or not certain heat-labile organisms have been destroyed, by testing for the presence of an enzyme that should be destroyed at the same time. It is, to my mind, quite proper for the biologist concerned with the safety of milk, even though he be not himself a chemist, let alone an analytical chemist, to use this method of chemical analysis as a means of getting biological information. Similarly we, as analytical chemists, are entitled to use methods of biological analysis to give us chemical information.

The position may be summarised in a single sentence; the analytical chemist, whose duty it is to carry out chemical analysis, may frequently find it necessary to make use of methods other than those of analytical chemistry.

It is in this sense that I claim many engaged in biological assay to be making contributions to chemical analysis: they are thereby qualified for membership of our Society and, by virtue of this right, charged, as are all of us, with the reciprocal duty of contributing to our common

pool of knowledge. After all, it is not only chemists who claim that biological assay has close connections with analytical chemistry. Sir Henry Dale, in his lecture to this Society in 1939,[2] was concerned to stress the similarities between the work of the analytical chemist and that of the biological assayist, and to emphasise their common aim. On that occasion, in seconding the vote of thanks to Sir Henry, I ventured to say that there was only one real difference between the analytical chemist and the biological assayist, for, whereas the latter used methods involving a large error, the extent of which he knew with considerable accuracy, the former used methods involving a small error, of whose very existence he was as a rule completely ignorant. This comment has, I think, still a certain bitter element of truth in it, but I wish to dwell to-day upon the bonds that unite the analytical chemist and the biological assayist rather than upon the methodological differences that separate them.

My proposed distinction between chemical analysis and analytical chemistry has some bearings on a matter that was much discussed again a few years ago in this Society, namely, its name. If I am right, this Society could legitimately be regarded as "a Society for Chemical Analysis," but would exclude from its ranks many who ought to be in them if it were described as "a Society for Analytical Chemistry." However that may be, I hope for assent to the view that, far from there being anything *a priori* improper in an association between biological methods and analytical chemistry, there is every reason to wish that the former shall be publicly acknowledged as a suitable mate for the latter. Indeed, I suggest that it may be high time for analytical chemistry to make an honest woman of biological assay.

It may pertinently be asked at this stage whether the Society and its new Group claim, as the Group's name might suggest, the whole of biological methods for its province. What of the highly complex methods used in genetical research? What of the fundamental researches of the physiologist? Are not anthropology and many branches of archaeology examples of the application of biological methods? The answer is, however, to be found by considering the full name of the Group, which is, after all, the Biological Methods Group of the Society of Public Analysts and Other Analytical Chemists. This, surely, should be sufficient to assure our scientific colleagues in the biological sciences that we are no poachers or body-snatchers,—or whatever term of opprobrium is considered suitable to apply to those who carry out raiding parties from their own scientific territory into that of their neighbours. Nevertheless, it may be useful to suggest the lines on which a division could best be made between work rightly coming under purview of the Group and that carried out by the biological scientists nearest to us—for example, the pharmacologists. These are already interested in getting quantitative results by using biological methods, methods, that is, involving the use of whole animals or plants, or of surviving tissues or organs from living animals or plants. Although I am not at all sure that it is going to be easy to codify in words the differences in method that arise logically from the different objectives pursued by the pharmacologists and ourselves, I think one or two illustrations may help to make clear the lines along which any issue can be judged and to ensure that no territorial disputes need arise. It may be that there will be certain zones that should be internationally controlled by both groups. I know of no better way of securing this in the scientific world than by the holding of joint meetings as occasions arise, and these are certainly to be encouraged.

Let us consider in this connection the determination of toxicity. When a pharmacologist is asked by those concerned with the chemical side of chemotherapeutic research to investigate the properties of a new drug or series of drugs, almost the first thing he does is to determine its median lethal dose, that is, the amount of the substance that will just kill half of the test animals in a group. When he has done this, he has useful information to guide him during subsequent investigations. He will know how much of the drug he can safely give any group of animals, when examining it for some specific pharmacological property, without running too serious a risk of killing all or any of them. He will be able to estimate its therapeutic index, that is, the ratio of the median lethan dose to the median effective dose, effective being used in the sense of exhibiting the therapeutic property for which it has been prepared. The higher the therapeutic index the safer the drug. He can compare its toxicity, and from this and its therapeutic efficacy he can also compare its therapeutic index with those of other drugs of the same group or having an entirely different chemical constitution.

All this information is of the utmost importance not only to the pharmacologist himself, but also to those who may subsequently be asked to make clinical trials of the new drug. Yet none of this information is likely to throw any light on the chemical composition of the drug itself. This is, in fact, *ex hypothesi* already known. What is in hand is an investigation

to determine the biological properties of a substance of known chemical constitution. I contend that such work falls outside the domain of chemical analysis and that the Group is not directly concerned with it at all.

Yet there are occasions, and they are not infrequent, when the determination of toxicity has an analytical bearing. A familiar example is that of the anti-syphilitic arsenical drugs. Some of these are substances of ill-defined chemical composition, so that the application to them of chemical and physical tests cannot give necessary information as to their chemical composition and therefore as to their biological properties, and in particular their toxicities. In arsphenamine and some of its analogues it is not possible to distinguish, by chemical or physical tests the arsenic that is organically combined and relatively non-toxic from the arsenic that is present in some other form and highly dangerous to man and animals. The presence of more than a certain very small amount of the latter renders the drug unfit for use. The chemist is impotent to establish the amount in any given sample. In deciding whether or not a sample is safe he has, therefore, to ask for the assistance of the pharmacologist who in this role becomes, not indeed an analytical chemist, but certainly a practitioner of chemical analysis, for his tests on mice establish whether or not an undesirable contaminant, arsenic in certain forms, is present in more than defined limiting quantities. He is helping to "ascertain its composition." It follows that the biological procedure adopted for determining the toxicity of neo-arsphenamine, as laid down in the British and other Pharmacopoeias, must rightly come under the purview of a Group such as ours; the use of the test gives information that certainly falls within the scope of Dr. Hughes's definition, and also of my more limited one.

From consideration of this instance, a further consequence follows. I have already pointed out that the Group is not directly concerned with methods for comparing the toxicity of various drugs. I emphasise the word "directly," which I chose with care. But the Group and its members may have a very strong indirect interest in such a proceeding, the methods of which must have a direct bearing upon the method that we have to use in conducting a chemical analysis when such an analysis includes the estimation by biological means of toxic impurities, as it does in the instance of the organic medicinal arsenicals.

It should be noted that the particular kind of biological assay under consideration is concerned with the detection of unwanted impurities. In the given instance these impurities consist of all substances likely to produce lethal effects at doses much below toxic levels of the therapeutic material itself. But biological assays in this category may be much more specific in their nature. Thus they offer the only means of detecting products from the posterior lobe of the pituitary gland, that is, oxytocic and anti-diuretic substances, in extracts made from the anterior lobe and used for their gonadotrophic, thyrotrophic or other activities. Again, in the examination of liver extracts, especially the so-called "crude" extracts used for purposes rather more general than are the purified extracts designed for treatment of classical Addisonian pernicious anaemia only, it may be desirable to test for the presence of histamine or other substances having histamine-like action. This can only be done biologically, but it can now be conducted as a properly designed assay of comparative potency, using pure histamine as standard.

The type of pharmacological assay involving the use of an isolated tissue and the measurement of its contractions under the action of a test drug, by the procedure generally known as the "bracketing method," involves taking alternate readings with a dose of the standard and a dose of the test material until consecutive readings are obtained that are indistinguishable in extent. The activity of the middle dose is then related to that of the flanking doses. In the bracketing type of assay, although a large number of readings may be taken, in effect all are rejected except the last three. By a simple system of randomisation, Schild has eliminated in the histamine test the variation due to random changes in the sensitivity of the test tissue (a piece of guinea-pig ileum in Tyrode solution) and has thereby made it possible to carry out an assay for histamine-like activity that makes use of all the readings taken—a most useful application of modern bio-assay methods. When such an assay has been carried out, it is possible to calculate the potency of the test material in terms of pure histamine and also—equally important—to calculate the limits of error of the result at any required level of probability. Unfortunately, it has proved hitherto impossible to apply Schild's procedure to one of the most frequently used bracketing tests, namely, the official Pharmacopoeia and Therapeutic Substances Act procedure for measuring the oxytocic activity of posterior lobe pituitary extract. During this test there is a gradual drift in sensitivity of the tissue, virgin

guinea-pig uterine muscle, and this defies the randomisation of Schild's procedure. In the histamine test we have another example of a method for estimating, with a reasonable degree of accuracy, the amount of an undesirable contaminant in a therapeutic product—a measurement that cannot at present be carried out by chemical or physical means, if indeed, it ever could be made in that way. For the histamine-like activity of such products may in fact be a balance of several pharmacological actions, of which only one, probably a small portion, possibly none at all, is due to histamine itself. We are not, in point of fact, really interested in the total amounts by weight of substances with histamine-like activity or with anti-histamine properties so much as with an estimate, in terms of some single substance, like histamine itself, of the overall undesired activity. A similar procedure, using the anaesthetised cat, can be applied to estimating the over-all depressor effect of depressor and pressor substances present together. The making of such measurements must surely be considered to be part of a chemical analysis, as defined, and therefore to fall within the purview of the Group.

There is another and even more important kind of chemical analysis that has to be carried out biologically. For the estimation of many biologically active substances, in particular hormones and vitamins, no suitable chemical or physical procedure is available, especially when they are present at low concentrations in natural sources. Biological methods certainly lack the precision that is invariably demanded of chemical and physical methods, but they have the counter-balancing advantage of a much higher degree of specificity, which is, indeed, a particular form of accuracy. (I use the words precision and accuracy in the sense laid down by the American Chemical Society and increasingly adopted in this Journal.) Take as instance of this type of assay the estimation of vitamin D by the line-test or the radiographic technique. Here the biological method has to be applied to the assay of substances to which pure artificial vitamin D_2 (calciferol) has been added, although the biological activity of calciferol does not itself ever now need to be determined, because this is highly correlated with the purity of the specimen, as indicated by such determinations as melting-point, optical rotation and absorption coefficient in the ultra-violet region. Calciferol, however, is a compound of extremely high potency, and the amount that has to be incorporated in, for example, margarine, is so small that none of these methods are available for its determination therein. The melting-point is obviously ruled out, the optical activity is not detectable and the ultra-violet absorption is completely masked by that of other substances present in the solvent oils. A biological test is the only means available for checking the accuracy with which calciferol has been added to margarine and the intermediate concentrates and the leniency with which it has been treated during processing. There are, as will be well known to you, other naturally occurring biologically active materials that have not even been isolated; they are recognised, and must therefore be estimated, by means of their biological properties. The lactational hormone of the anterior pituitary has to be tested by its stimulating effect on the size of the crop-gland of pigeons; gonadotrophic hormones of either sex, of pituitary or of placental origin, may be estimated by a variety of animal tests, involving the weight of ovaries or testes, the examination of vaginal smears, the measurement of comb-height in capons and so on. For the estimation of thyrotrophic or thyroxine-like activity, we have tests based on the thyroid-weight in guinea-pigs or chicks and also now the elegant procedure of Parkes and Deanesley, involving the stimulation of early metamorphosis in tadpoles of the South African clawed toad, Xenopus. This list could be extended, but the instances given should be sufficient to make clear the second type of biological procedure with which the Group may legitimately concern itself, namely, the measurement for purposes of chemical analysis, where procedures of analytical chemistry and physics are not available, of substances having physiological activity and intended for therapeutic use.

Both types of biological assay so far considered have one important feature in common. They involve tests on animals or their tissues for estimating substances that have, or are likely to have, a physiological effect, desirable or undesirable, on the human organism. The analyst is also concerned with assays of this type when the substances to be estimated are physiologically active against other animals than man or even against plants. The assessment both of insecticides and of fungicides, when these are not chemical compounds capable of standardisation by chemical and physical means, may fall, I submit, within the sphere of the analyst.

In his interesting Hunter Memorial Lecture given on 8th March of this year to the Liverpool Section of the Society of Chemical Industry, Dr. R. E. Slade[3] showed that different isomers of benzene hexachloride show markedly different toxicities to, for example, the

grain weevil or the yellow fever mosquito or the house fly. There are several references in his paper to "crude benzene hexachloride 666," which contains from 10% to 12% of the active isomer, "Gammexane." This at once raises the question of how one would best determine the proportions of the different isomers in a crude mixture, supposing that the method of preparation led to the presence of more than one. After a proper chemical determination one could, from a knowledge of their different toxicities, estimate the over-all toxicity of the mixture. Surely this would be a very roundabout method of procedure. It would seem much more expeditious to carry out a straightforward toxicity determination by using an appropriate species of insect, thereby achieving in one step, perhaps somewhat less accurately but much less laboriously, what could possibly only otherwise be achieved by several intricate applications of analytical chemistry and an estimation of the weighted toxicity of the mixture. In such instances biological assay can cut through the Gordian knot of what may well be irksome chemical manipulations applied to previous determinations of toxicity that, by the way, are not necessarily any longer valid at a later time, and may furnish directly information that can only be deduced indirectly from chemical methods. In these circumstances the chemical analysis is itself a means to the end, namely, a knowledge of the mixture's toxicity; why not then determine the toxicity directly?

Again, the estimation of certain plant hormones by methods such as the Went pea test clearly fall within the definition of biological assay and equally clearly only enable us to state the over-all activity of the substance assayed, be it in soil, sewage or plant tissue. With all the disadvantages attaching to biological assay, such an integrated analytical procedure must be more economical than even the most rapid methods of determining singly each of the active substances present, especially when it must be remembered that anti-hormones may be present at the same time, and would have to be separately determined. The biological test, in short, gives a less accurate but much more rapid and complete picture of the total amount of active substances and their antagonists present, expressed not as parts of each per cent. or per million, but as the net total activity of all relevant substances. Such tests contribute to knowledge of the composition of the products in question and are therefore a part of chemical analysis, as I have defined it. We may, therefore, define the biological assays with which the analyst is rightly concerned as comprising the determination of the amounts of substances having, or likely to have, physiological effects, salutary or adverse, on animals and plants.

Even so, we have not exhausted the list of purposes for which the analyst may require to use biological methods. I am indebted to Dr. J. R. Nicholls for reminding me of another application, namely, the use of specific yeasts for the differential fermentation of sugars in mixtures. Even if this analysis were required because the sugars were to be used for the feeding of animals or plants, the procedure would not fall within the definition of biological assay already put forward, for the biological processes involved are not used for the *estimation*, except in an indirect way; the final steps may very well be one of the classical chemical methods for determining sugars. Yet a biological process is here an integral part of an analytical procedure. Intermediate between this kind of biological method, in which vegetable organisms are used to remove quantitatively constituents of a mixture, prior to a chemical determination of other constituents, and the direct assay, in which a biological response is measured, there is an important type of procedure so far, unless I am mistaken, little exploited in general chemical analysis. I refer to the manometric methods, using the well-known Warburg or Barcroft apparatus, in which cultures of living bacteria, fungi or yeasts, are made use of to liberate carbon dioxide and the volume of carbon dioxide evolved is measured by a suitable micro technique. A most ingenious application of this technique has recently been proposed by Gale, who analyses mixtures of amino acids by treating them in turn with individual specific amino-acid decarboxylases of bacterial origin. There is no *prima facie* reason why the bacterial cells should not themselves be used, rather than enzyme extracts prepared from them; it is presumably merely a matter of convenience in manipulation to use cell-free extracts. There would seem to be possibilities of very wide application for procedures of this type and I do not see how they can be excluded from the category of biological procedures having a place in chemical analysis. They will certainly present problems of their own and should afford a valuable opportunity for collaboration between the analytical chemist and the bacteriologist.

It will be found that microbiological methods of analysis generally fall only into two of the three groups to which I have alluded. The first group includes biological methods for

assessing the amount of an undesired contaminant in a product intended for therapeutic use, but I cannot recall any instance of a microbiological procedure having this aim. There is certainly no *a priori* reason why microbiological methods should not be available for this kind of work. In the second group we have those biological methods involving the estimation of an active principle, which may be required either for therapeutic or for destructive purposes. In this field microbiological techniques are becoming increasingly important; they have so far been mainly applied to the estimation of certain water-soluble vitamins, but there have been a number of papers describing their use for estimating individual amino acids and even some metals. The estimation of penicillin in metabolism fluid, in concentrates, or in the product as made finally available for clinical use, is no less a biological method of analytical chemistry because it happens to involve the use of a strain of staphylococcus than is the estimation of vitamin K on newly hatched chicks or of vitamin P on guinea-pigs. In the third group of biological methods involving the use of micro-organisms are those in which they form the basis not for the estimation but for preparing the solution on which the estimation is to be carried out.

It is true to say that, for example, estimations of riboflavin carried out with *Lactobacillus casei* and those involving *Rattus norvegicus albinus* are equally methods of biological assay, even though the first uses millions of unicellular plants while the second uses only thirty or forty small mammals, yet it is clear that the statistical problems involved are liable to be different, not only because of the difference in relationship between dose and response that appears to distinguish some microbiological methods from most assays that use whole animals, but also because the problem of sampling is quite different. The errors of microbiological assay can certainly not be to any significant extent due to the different behaviour of the collective cells of test organism in the different tubes. The differences between tubes that are not due to what the statistician calls treatment—in biological assay the doses of test material or standard—may, however, involve a number of factors that are of little importance in macrobiological assays. For example, errors in estimating turbidity or in *p*H measurement may require different statistical handling from those arising when a number of rats are weighed on a balance at various times. Thus it is of the greatest importance for the assayist, when he is planning a new or a modified type of biological test, to consider carefully the design of his procedure and to seek appropriate statistical assistance. If this is done, it is often possible to arrange the first tentative assays in such a way that they will furnish information on which may be based an improved procedure for subsequent assays. On other occasions it will be found necessary to carry out investigations that have for their only object to establish the factors influencing the dose response relationship, the extent to which these factors may be controlled and how to devise an assay procedure involving the minimum number of observations for a given degree of certainty in the result. In such experiments, clearly, the statistician's advice is at least as important as in the design of tentative assay methods.

I have so far confined myself to considering the *types* of chemical analysis in which biological methods can or must be used. It would be possible now to offer a classification of these methods according to the organisms used, but I doubt whether such a classification would help to throw light on the scope of the Group's activities and its prospects of a useful future. I prefer rather, therefore, to touch briefly on a few examples of the practical problems that arise in the course of biological assays of various kinds.

There is a sense in which the animal or the plant or the living tissue to be used in a biological assay may be regarded as a chemical reagent, and in that sense there is an increasing tendency for the biological worker to demand that these reagents shall be of analytical standard. Very often they are far from this. The Group could do worse than consider in some detail the sources from which laboratories may draw experimental animals and the problems of organisation that arise when a bio-assayist needs a supply of animals uniform in genetic make-up and in environmental history. The demand for "standard" animals is a genuine and a legitimate one, but a word of warning is, in my opinion, necessary, lest we take too non-biological a view of what is essentially a biological problem. I remember several years ago how a chemical colleague expressed to me the view that it was time for those concerned with the estimation of vitamins by animal assay to devise methods of procedure standardised with that meticulous care that has characterised the work of the Analytical Methods Committee and its Sub-Committees. I think there is a fallacy here.

The animal stock in any given laboratory is maintained in the good health and condition

essential to its use for assay purposes as a result of a very delicate balance between the animals' internal and external environments. In both of these there are many unknown factors and some that are known but uncontrollable; indeed, there is almost as much art in the maintenance of a healthy stock of experimental animals as there is science. This does not, however, mean that scientific attention to the fundamental principles of genetics, nutrition and epidemiology can be ignored with impunity. Consider, for example, an instance that is perhaps a little fanciful, but not very far removed from something that has recently happened. In a certain laboratory there exist two pure lines of mice, that is to say, strains maintained for many generations by brother-sister mating, the second strictest form of inbreeding possible. In this particular laboratory these two strains of mice are submitted to identical conditions of animal husbandry. They are raised in the same stock room at the same temperature and humidity, on the same diet and in similar cages and, by no means the least important factor, they are handled by the same staff. At the request of a colleague in another laboratory the director of the first laboratory makes available to him some breeding stock of both strains. After a year or so notes are compared. It appears that in the second laboratory the two strains are also raised under identical conditions, but not, of course, under the same conditions as in the first. Yet in the first laboratory strain A proves itself to have high fertility and viability, while strain B seems to be hanging on to existence by a somewhat tenuous thread. Precisely the reverse has happened in the second laboratory. Strain B flourishes; strain A pines. Clearly the proper procedure, if both strains are of use to both laboratories, is for the first laboratory to concentrate on producing strain A and the second strain B.

Just as the different conditions in the two laboratories affect differentially the two stock strains of mice, so it may also be found that in using one strain or the other for the same assay in the two different laboratories markedly different conditions will be necessary for maximum accuracy. These conditions can only be worked out in each laboratory for itself. To attempt to standardise the test would, in my submission, be to fly in the face of biological facts, even though many of these facts be unknown.

How then can the bio-assayist, or more generally the analytical chemist using biological methods, resolve this difficulty? The answer is, of course, to be found in the use of a common stable standard preparation for biological assays, and of rigid control tests in the other types of analysis involving living organisms. I do not propose here to enter into a discussion of International Standard Preparations and the subsidiary laboratory standards that must in most instances be brought into use. The general principles underlying the use of these standards, and the procedures followed to secure their adoption, were admirably stated by Sir Henry Dale before this Society in the lecture[2] to which I have already referred. It is, however, perhaps permissible to emphasise one point that he made.

The ultimate object of biological assays, and therefore of the creation of Standard Preparations, is to encompass their own disuse. We can see this happening before our very eyes. Thus, there exist International and British Standard Preparations for vitamin C. Already it is only in comparatively rare instances that a biological assay for vitamin C may be necessary; even when it is, I cannot imagine anyone troubling to make use of the International Standard Preparation, still less to prepare by biological means a subsidiary laboratory standard for himself. He will simply use as standard any sample of ascorbic acid that satisfied rigid pharmacopoeial standards for chemical purity. Again, in riboflavin we have an instance of a vitamin that was isolated, analysed and synthesised so rapidly that there was no time to create an International Standard, and biological assays of riboflavin, whether with whole animals or with micro-flora, are simply carried out against chemically verified specimens of the pure compound. Similarly, it has not been thought necessary to introduce an International Standard Preparation of vitamin K, because pure methyl-naphthoquinone, although different in chemical constitution from the two naturally occurring forms of the vitamin, has been found to be a valid standard and to be capable of verification by chemical means. What applies to certain vitamins is also true of the steroid hormones and the oestrogens. International Standards exist for several of these compounds, but it is unlikely that many workers use them.

The course of events that lead to the disappearance of a biological assay, and the accompanying paraphernalia of standard preparations, would seem to be broadly as follows. The biological technique is improved in accuracy, as experience of it grows. This assists the search for chemical and physical tests which, always more precise, cannot be established as accurate, that is to say, specific, until the specific biological method becomes sufficiently

precise to act as a check on the specificity of the proposed non-biological procedure. This kind of reciprocal advance is frequently characteristic of scientific progress.

There is another aspect of standard preparations to which the Group will almost inevitably be compelled to pay attention. There are certain standards that are theoretically unsound, although they were the best that could be devised at the time of their introduction. But is it any longer justifiable to use β-carotene as the standard preparation for vitamin A, when a pure stable vitamin A ester is available and its use for the purpose would avoid certain difficulties that inevitably accompany the use of one substance as a standard preparation for another The same is true of the International Standard Preparation for vitamin D, for which the present mixture of calciferol with ergosterol and certain of its other irradiation products should surely be replaced by a solution of either pure calciferol or pure vitamin D_3. The need for changes of this kind is well-known to bio-assayists, some of whom have indeed expressed themselves forcibly on the subject. Yet there is no organisation in which they meet sufficiently frequently and are represented sufficiently completely for it to be able to make representations on their behalf in official quarters to the end that overdue changes shall be made. Is it too much to hope that such action could be regarded as the right and the duty of the Group?

When it has been decided what particular response and what particular animal or plant shall be used, there will remain a number of questions that the bio-assayist must settle. In a recent paper[4] the Honorary Secretary of this Group discussed with great clarity the nature of the dosage-response relationship on which all biological assay depends. He showed how in certain types of assay the response was a linear function of the dose and how in others it showed a linear relationship to the logarithm of the dose. There is a further group of assays in which the response is of what is called a "quantal" or "all or nothing" nature; here the relationship between dosage and response is again different, and it is found that the normal equivalent deviation of the percentage response is a linear function of the logarithm of the dose. Even if the results are to be evaluated only by graphical methods, such linear relationships have an obvious advantage over all others. When statistical methods are to be applied the advantage, though perhaps less obvious, is no less great. Yet there are certain occasions when this linear relationship is unsuitable for treatment by accepted statistical methods, at any rate of the kind that the practising chemist is competent to grasp and apply. I cite one example of this.

In computing the standard error of an assay, that is, of the estimated relative potency of the test material and standard, it is necessary to calculate the standard deviation of a single experimental observation. This is done by calculating the standard deviations for each of the groups receiving doses whether of test preparation or of standard and then averaging them, obviously on the assumption that variance, or standard deviation, is independent of level of response. Inspection of the figures generally reveals that this is so and statistical analysis may confirm it. There are, however, certain assays for which this turns out not to be true. We have ourselves found, for example, in the pigeon crop-gland assay of prolactin, that there appears to be a positive correlation between variance and level of response. Such a finding, if proved true, would invalidate the pooling of variances and the subsequent calculation of the error of the assay. Mr. E. C. Fieller[5] has been kind enough recently to send me some interesting suggestions for curing this ill. It is necessary to find some transformation of the response that will, so to speak, smooth out the variances and make them independent of the level of response. Such a transformation might, for example, be the logarithm of the response or the square root of the response or the square of the response and so on, and Mr. Fieller has suggested a technique for deciding which transformation is most suitable. It is, however, important to realise that the transformation of the response, though it gives normally distributed variances, may no longer show the original linear relationship to dose or log dose. I hope that at some future date Mr. Fieller may give this Group the benefit of his views on the whole subject.

Some of the more chemically minded may at this point be inclined to ask what kind of validity can attach to methods, and what kind of honesty to those who use them, when they involve "juggling with the results" until a linear relationship is established. In judging both the methods and the honesty of their users, it must, however, be remembered that what we are considering is an analytical procedure. In using this we have to find an effect, or a function of that effect, that shows a simple mathematical relationship with the amount of the substance producing it. The simplicity of the relationship is necessary for purely practical

reasons of calculation. I see no fundamental difference between the spectroscopist whose analytical results are got by using such expressions as log I_o/I and the bio-assayist who gets his by plotting, say, the square root of an area on the skin of a rabbit against the logarithm of the dose producing an effect over that area. Surely the test is a pragmatic one. What works? Or, rather, what works best? I believe that the Group can contribute valuably to the discovery of what works best in various types of biological assay and what are therefore the right procedures.

I do not propose to attempt consideration or justification of the statistical methods used in biological assay. The inherent variability of the test object, be it animal or plant, even when the most rigorous inbreeding and other steps have been pursued to reduce this variability to a minimum, makes it essential to be able to answer with some degree of exactness how far the differences between groups of animals or plants receiving different doses are due to the differences in dose or might have happened if all had received the same dose. There are accepted devices available for answering this question, perhaps none more useful than the simpler applications of Fisher's method of variance analysis. Without any previous understanding of the mathematical aspects of probability theory, it is easy for any chemist who is a reasonably accurate arithmetician to use these procedures and to calculate for himself, and for some of his sceptical colleagues, just how unsatisfactory his assays are. The first essential preliminary to reducing error is to know its extent.

I think, further, that in the matter of statistical evaluation the Group has another, if somewhat humble, duty to perform, and could well perform it in close consultation with our statistical colleagues, of whom I hope at least some will see fit to join the Group. I refer to nomenclature and symbols.

As an example, one may consider the following passage in a recent journal: "the degree of variability within each group is indicated by the magnitude of the standard deviation." The table of results gave "the mean and standard deviation for 25 observations," and the authors stated that they gave these statistics and not the individual observations to save space, a most laudable object. Yet it is impossible to discover whether what they have given are the standard deviations for the individual observations in each group of 25, or the standard deviations, that is the standard errors, of the means of each group. As one of these would be five times the other, the matter is of some interest! Surely it ought to be possible to restrict use of the term "standard deviation" to refer to a single observation and the term "standard error" to the mean of a number of observations. Again the use of Roman and Greek letters for statistical symbols, though most statisticians appear now to be following a uniform policy, seems for many bio-assayists to be the occasion for the exercise of individual whim rather than self-imposed discipline.

While I am discussing nomenclature and symbols, I would like to be permitted a further observation. It has been my aim on two or three occasions to introduce, almost surreptitiously, terms or phrases into scientific literature to express ideas that could otherwise only be expressed by clumsy periphrasis. The first occasion was when I wished to explain that an investigation had been carried out on groups of animals of the same sex from the same litter. In the particular instance it did not matter whether the animals were male or female, provided that all the animals in any one group were of the same sex. After discussion with terminological experts, including Dr. Lancelot Hogben and Dr. Joseph Needham, we hit on the term "isogenic" and I persuaded the then Editor of the *Biochemical Journal* to let me use it, provided I defined it. Since then others have found it convenient, and it generally appears, when it appears at all, without any explanation. True, this term was not introduced strictly in connection with statistics, but it would have had an easier passage and a more extended use with the backing of a Group such as this. Some years ago I inserted the phrase "four-point assay" into a paper, and this passed without query, because it consisted of simple and well-known English words. It appears to have come to stay, but this was more through my luck than my cunning. Mr. Wood, in the paper to which I have already referred, has also coined a number of useful short phrases to describe the different methods for the statistical evaluation of assays in which response is a linear function of dose. More recently I wished to find a term to describe the particular transformation of the response that was being used in calculating the dosage-response curve, or regression line, and in the statistical evaluation of the standard error. You might consider that it would have been sufficient to talk of the function of the response, but this would have been clumsy, because I should have been compelled to discuss what function of the dose this function of the response manifested, and perhaps

ambiguous, in the event of my wishing also to use the term "function" in the physiological sense. I wanted a word X that would enable me to say "the X of a response is the logarithm, or the square root, or the square, of the measurement actually made." I again had recourse to my friend Professor Hogben, who suggested the word "metameter," derived by contrast from the mathematical term parameter. This word I was again permitted to use, with an accompanying explanation, and I have used it once since, on condition that I inserted a reference to its original use!

I give these examples in order to illustrate the point that the bio-assayist may frequently find himself at a loss for a suitable term to describe a concept or a measurement, and may be conscious that other bio-assayists are in the same difficulty. So far there has been no appropriate place where he might decide with them collectively on the introduction of a suitable new term or phrase. In the Group there should now be opportunities for these simple but useful exchanges of idea.

I fear that I have very inadequately covered the ground that an address of this kind might suggest. I have refused to be drawn into a non-mathematician's justification for the use of mathematical procedure that he does not fully understand, if only because I have already expressed my views on this subject more than once at meetings of the Society. I have also avoided discussion of the proposition that microbiological assay and methods can never be satisfactory, because unicellular organisms are so labile as to make it impossible to be certain that they will respond to the same stimulus in the same way on any two consecutive occasions. I have said nothing about the sordid but essential questions involved in the maintenance of an animal colony without disease or nutritional deficiency, nor have I attempted to describe the dull but necessary steps that must be taken by way of record keeping if a stock of animals is to be maintained in a state of maximum genetic uniformity. Such practical matters will, no doubt, receive attention from others at subsequent meetings of the Group, and it seemed wrong at its inaugural meeting to dwell upon details except in so far as they bore upon certain central themes. My themes, as I hope has been clear, have been, first, that analytical chemists may and must concern themselves with some biological methods; secondly, that there is no question of poaching as between them and, say, pharmacologists and bacteriologists, but rather of agreeing to the common territory over which they may jointly meet and operate; thirdly, that there are in biological assay many problems, both of a methodological and of a mathematical nature, yet remaining to be solved and requiring for their solution frank and unrestricted discussion between scientific workers in a number of fields, biological and non-biological. The Group may well afford the only opportunity for bringing this about; I believe it will certainly afford the first.

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Determination of the Peroxide Value of Oils and Fats

By C. B. STUFFINS and H. WEATHERALL

(*Read at the Meeting of the North of England Section on April* 14, 1945)

THE iodimetric method, based upon the liberation of iodine from potassium iodide, is the most reliable procedure for the determination of peroxide-oxygen in oils and fats. In this country the Lea[1] methods carried out at the boiling point of the solvent are commonly used, while in the U.S.A. room temperature methods such as that of Wheeler[2] are favoured.

It is a generally accepted fact that there are two main sources of error in the iodimetric methods, namely

(1) Oxidation of the reagents by air, accelerated by light.
(2) Re-absorption of the liberated iodine by the fat.

The purposes of this paper are to describe an investigation into the causes of these sources of error and to suggest a modified method for the determination of peroxide values which eliminates them.

The procedure proposed by Lea[1] is essentially as follows: Weigh 1 g of the oil or fat into a pyrex test tube (6 in. by 1 in.), add 20 ml of a mixture of glacial acetic acid and chloroform or carbon tetrachloride (2 : 1 by vol.), and about 1 g of powdered potassium iodide. Fit the tube with a rubber stopper bored with two holes and pass inert gas into the air space above the reaction mixture. Heat the tube over a small flame until the contents are bubbling freely, and then transfer the tube to a boiling water-bath. When vapours begin to issue from the hole in the stopper plug it with a glass rod. Cool the tube under a tap and pour the contents into a flask containing 30 ml of water. Titrate the free iodine with 0·002 *N* sodium thiosulphate, and express the result as millilitres of 0·002 *N* sodium thiosulphate per gram of sample.

Lea also suggested a simplified method in which the procedure is similar to that described above except that an inert gas is not used and the tube is not sealed before cooling.

Experiments on the Lea Methods

The conditions of experiment laid down in the Lea methods were varied and the effects of these variations are illustrated in Tables I–VI.

Table I

Effect of Time taken to bring the Reaction Mixture to the Boil

(*a*) When inert gas is passed into the air-space above the reaction mixture.
(*b*) ,, ,, ,, bubbled through the reaction mixture.
(*c*) ,, ,, ,, not used.

Material	Total time of heating secs.	Time taken to bring to the boil* secs.	Peroxide values: Inert gas (CO_2) passed into the air-space above the reaction mixture (*a*)	Peroxide values: Inert gas (CO_2) bubbled through the reaction mixture (*b*)	Peroxide values: No inert gas used (*c*)
Crude groundnut oil (1)	60	30	12·40	9·90	12·55
,, ,, ,, ,,	190	180	16·95	11·90	19·15
,, ,, ,, ,,	310	300	16·80	12·10	20·15
,, ,, ,, (2)	60	30	—	0·70 0·75	1·30 1·25
,, ,, ,, ,,	60	60	4·40 4·55	1·10 1·20	4·75 4·95
,, ,, ,, ,,	190	180	5·05	0·95	—
Edible groundnut oil	60	60	0·90 0·65	0·10 0·10	1·00 1·05
,, ,, ,,	180	170	1·30 1·65	0·30 0·25	1·95
Hardened linseed oil	60	40	—	0·40 0·35	0·35
,, ,, ,,	110	95	—	—	0·50
,, ,, ,,	190	180	—	0·40	—

* Samples taking over 40 secs. to boil were heated in a water-bath only.

Results shown in Table I indicate the following points:

(1) When inert gas is not used (see Lea's simplified method) the time taken, from the commencement of heating, to bring to the boil is very important. With increase in this time there is increase in the values obtained, eventually leading to erratic results. These observations are confirmed by Sabalitschka and Maas,[3] who also used a water-bath only for heating the reaction mixture.

(2) When inert gas is used simply to displace the air in the space above the reaction mixture oxidation is retarded but not prevented.

(3) The time taken, from the commencement of heating, to bring to the boil is not important if the inert gas is bubbled through the reaction mixture. The values obtained for comparatively fresh oils and fats are approximately constant even with large increases in the time taken to bring to the boil. On the other hand the values on more highly oxidised oils and fats vary a little, but the increases are only slight compared with those obtained when inert gas is not used.

Further to the above the following results indicate that:

(i) Unless inert gas is bubbled through before and during the heating period, even a minimum time (30–40 secs.) taken to bring to the boil gives high values, *e.g.*, a sample of crude groundnut oil gave peroxide values as follows:

	Peroxide value
Inert gas bubbled through before and during the heating period ..	14·65 15·95
,, ,, ,, ,, during the heating period	17·4 17·6

(ii) The total time of heating is not important when inert gas is bubbled through the reaction mixture. Similar values were obtained on edible groundnut oil after a one-minute reaction time (1·45 P.V.) and after a twenty minutes' reaction (1·50 P.V.).

(iii) If, after the addition of potassium iodide, the bubbling of inert gas through the reaction mixture is delayed more than two minutes, high results are obtained; *e.g.*, the following results were obtained on a sample of edible groundnut oil:

	Peroxide value
No delay	0·95
Two minutes' delay	1·05 1·00
Five ,, ,, ...	1·20 1·33

This becomes an important point if a method be adopted in which several tests are started simultaneously.

TABLE II

RELATIVE EFFECTIVENESS OF CARBON DIOXIDE AND NITROGEN IN PREVENTING OXIDATION

Material	Total time of heating secs.	Time taken to bring to the boil secs.	Peroxide values: CO_2 bubbled through the reaction mixture	Peroxide values: Nitrogen bubbled through the reaction mixture	Peroxide values: No inert gas used
Crude groundnut oil ..	60	30	1·30 1·15	1·40	1·25 1·30
,, ,, ,, ..	60	60	1·30	1·35 1·35	—
Edible groundnut oil ..	60	30	0·75 0·75	1·15	0·95 1·00
,, ,, ,, ..	60	60	0·80 0·75	1·20	1·85 1·55

Table II indicates that both gases behave similarly in preventing oxidation during the reaction. Slightly lower results are obtained when carbon dioxide is used, but the differences are not great enough to be important.

TABLE III

EFFECT OF THE COMPOSITION OF THE SOLVENT

Material	Total time of heating secs.	Time taken to bring to the boil secs.	Peroxide values. Solvent mixtures as stated: 2 Acetic acid 1 Chloroform (b.p. 87° C.)	1 Acetic acid 2 Chloroform (b.p. 72° C.)	3 Acetic acid 1 Chloroform (b.p. 94° C.)
Edible groundnut oil (1)	60	50	0·30 0·30	0·25 0·25	—
,, ,, ,, ,,	140	130	1·85	0·55	—
,, ,, ,, (2)	60	50	1·55 1·80	0·75 0·75	—
,, ,, ,, (3)	60	50	20·6 20·4	13·2 13·25	—
Edible palm oil	60	50	23·4 24·5	15·0 14·5	—
Crude soya oil	60	40–50	32·0 31·4	21·7 20·10	33·6 34·10
Cod liver oil	60	40–50	5·50 5·60	4·20 4·50	6·10 6·00
Crude groundnut oil	60	40–50	5·80 5·40	3·50 3·70	6·20 6·50

Table III shows that:

(*a*) when the mixed solvent contains more chloroform than acetic acid results as low as 50–60% of the normal values are obtained;

(*b*) slightly high values are obtained when the mixed solvent contains a lower proportion of chloroform than in the Lea methods.

TABLE IV

EFFECT OF AMOUNT OF POTASSIUM IODIDE USED FOR THE TEST

Material	Time of reaction secs.	Peroxide values: Sat. soln. of KI in water 1 ml	Solid KI *ca.* 1·5 g	Solid KI 3–4 g	Solid KI 4–5 g
Crude groundnut oil ..	60 (bunsen and bath)	22·2 23·2 22·6	29·2 29·3		
,, ,, ,, ..	60 (bath only)	22·6 22·0 23·4	33·1	30·9	29·2

The results in Table IV do not confirm the suggestion of Sabalitschka and Maas[3] that an insufficient amount of solid potassium iodide is used in the Lea method. In fact, lower results were obtained with *ca.* 5 g than with *ca.* 1·5 g of solid potassium iodide.

TABLE V

RELATIVE EFFECTS OF SOLID POTASSIUM IODIDE AND ITS SATURATED SOLUTION

Material	Total time of reaction secs.	Peroxide values: Solid KI No inert gas	Solid KI Inert gas bubbled *through* the reaction mixture	Sat. KI soln. No inert gas	Sat. KI soln. Inert gas bubbled *through* the reaction mixture
Deodorised groundnut oil (1)	60	35·6	31·5 29·6 30·2	31·2 30·8	31·2 30·2
Hydrogenated linseed oil	60	0·80	0·75	0·95	0·70
Cooking fat (old sample)	60	—	276 273	—	278 273

If inert gas is not used a saturated solution of potassium iodide produces lower but more consistent results than solid potassium iodide. But when inert gas is bubbled *through* the reaction mixture during the experiment similar results are obtained with both solid and a saturated solution of potassium iodide.

TABLE VI

EFFECT OF ADDING POTASSIUM IODIDE TO:

(*a*) A cold solution of the sample (ordinary method).
(*b*) A boiling solution of the sample.

EFFECT OF REDUCING THE WEIGHT OF SAMPLE TAKEN WHEN THE PEROXIDE VALUE EXCEEDS 10

Material	Time when KI was added to the reaction mixture	Total time of reaction with KI secs.	Peroxide values: Solid KI Oil sample taken 1 g	Solid KI Oil sample taken 0·5 g	Sat. soln. of KI Oil sample taken 1 g
Edible groundnut oil ..	before heating	60	0·80 0·90	—	0·95 0·80
,, ,, ,, ..	when boiling	60	0·45 0·50	—	0·55 0·75
*Deodorised groundnut oil (1)	before heating	60	16·95 16·9	20·9	13·05 13·7
,, ,, ,, ,,	when boiling	60	6·60 7·10	10·90	13·6
* ,, ,, ,, (2)	before heating	60	28·2 27·2	33·1 34·8	

* Old samples.

The figures in Table VI indicate that extremely low results are obtained when solid potassium iodide is added to a boiling solution of the oil and solvent. On the other hand, when a saturated soln. of potassium iodide is used the results are similar whether it is added to a cold or boiling soln. of the sample.

With highly oxidised oils and fats the weight of sample taken should be reduced so that the titration does not exceed 10 ml of 0·002 *N* sodium thiosulphate, otherwise low results are obtained. This was recommended by Lampitt and Sylvester.[6]

EFFECT OF TEMPERATURE ON THE DECOMPOSITION OF POTASSIUM IODIDE—By adjusting the conditions of experiment so that the potassium iodide was added at a higher temperature than the boiling point of the solvent mixture, the value obtained (1·85)* was much greater than by the ordinary method (0·60). Blank tests on the mixed solvents (0·45) and on the glacial acetic acid (3·30) indicate that the high temperature of boiling acetic acid (118° C.) is sufficient to liberate iodine from the potassium iodide acetic acid mixture.

EFFECT OF ALLOWING THE SAMPLE TO STAND IN THE SOLVENT BEFORE CARRYING OUT THE DETERMINATION—With fresh oils and fats no appreciable effect is noticed within one hour. However, with highly oxidised oils and fats (*e.g.*, peroxide value greater than 10) low results are obtained if the sample is allowed to stand in the solvent.

The foregoing investigation of the Lea methods shows that the results are influenced by

(*a*) intensity of the heating, *e.g.*, the time taken to bring the reaction mixture to the boil (see Table I);
(*b*) oxidation of the reaction mixture during the determination (see Table I);
(*c*) the weight of sample taken, if it is highly oxidised oil or fat (see Table VI).

* ml of 0·002 *N* sodium thiosulphate.

The above results demonstrate that the prevention of oxidation during the determination is only accomplished when inert gas is bubbled through the reaction mixture before and during the heating period.

G. K. Giles,[4] in his investigation of the Taffel and Revis[5] method, also found that atmospheric oxygen was eliminated more thoroughly when inert gas was bubbled through before and during the reaction.

Methods at Room Temperature

Where large numbers of tests have to be carried out, the passage of inert gas through the reaction mixture, together with heating, entails a considerable amount of manipulative work. This disadvantage can be eliminated by adopting a room temperature method. The authors therefore investigated this procedure.

Wheeler[2] described a room temperature method, in which no precaution against oxidation by air was adopted.

For details of the following room temperature experiments see recommended method at the end of this paper.

Table VII

Room Temperature Methods

Effects of Time of Reaction, Use of Inert Gas and Use of Solid Potassium Iodide or its Saturated Solution

Material	Total time of reaction mins.	Peroxide values: Solid KI No inert gas	Solid KI Inert gas bubbled through reaction mixture	Sat. KI soln. No inert gas	Sat. KI soln. Inert gas bubbled through reaction mixture
*Deodorised groundnut oil (1)	15	—	—	—	26·2
,, ,, ,, ,,	30	—	—	—	29·7
,, ,, ,, ,,	60	32·4	27·0 27·0	33·4	31·3 30·4
* ,, ,, ,, (2)	60	32·3 33·7	26·5 27·3	32·81 32·6	29·5
,, ,, ,, ,,	135	—	—	36·4 37·8	29·4
Edible palm oil	60	—	—	0·75 0·70	nil nil
,, ,, ,, ...	150	—	—	1·60 1·70	—
Hydrogenated linseed oil ..	60	—	0·55	—	0·50 0·55
Cooking fat (old sample) ..	60	—	243 284	—	272 280
Edible groundnut oil .. (Lea test 2·1, 2·2)	120	2·55	1·95	—	—
Edible groundnut oil ..	180	—	1·80	—	—
,, ,, ,, ..	7 hrs.	2·95	—	—	—
,, ,, ,, ..	24 ,,	7·80	—	—	—

* Old samples.

The results in Table VII show that:

(*a*) Unless inert gas is bubbled through the reaction mixture during the determination, the values increase with increase in time of reaction (*cf.* Table I).

(*b*) When inert gas is bubbled through the reaction mixture the following results are obtained:

(1) contant values in approximately one hour;

(2) low values with solid potassium iodide (*cf.* Table V);

(3) similar values in both the high and the room temperature methods when a saturated soln. of potassium iodide is used (*cf.* Table V).

With a saturated solution of potassium iodide the reaction is about 70% complete within a minute, whereas with solid potassium iodide no appreciable reaction takes place within five minutes. This difference may be attributed to the slow solution of the solid potassium iodide in the solvent at room temperature.

The fact that in (*b*) (3) values of the same order are obtained under such divergent conditions of experiment as the high and room temperature methods gives strong support to the view that a true measure of the peroxide-oxygen is obtained when gas is bubbled through the reaction mixture.

Other Experiments

Finally, experiments were carried out to investigate the possibility of loss of peroxide-oxygen or liberated iodine, caused by the bubbling of inert gas through the reaction mixture.

Samples of crude groundnut oil were divided into two portions. One portion was set aside for the direct determination of the peroxide value by the Lea simplified method. A large volume of inert gas was bubbled through the other portion for twenty minutes. The residual gas in the sample was expelled by attaching the flask to a vacuum pump. The peroxide value was then determined as in the untreated portion by the Lea simplified method, and also by bubbling inert gas through the reaction mixture.

The results in Table VIII show that similar values were obtained on the untreated and treated portions of the sample. Therefore there is no loss of peroxide-oxygen when inert gas is bubbled through the reaction mixture.

TABLE VIII

Material	Time taken to bring to the boil secs.	Peroxide values: Treated portion No inert gas used	Peroxide values: Untreated portion No inert gas used
Crude groundnut oil (1) ..	30	0·95 1·00	1·00 1·05
,, ,, ,, (2) ..	60	1·85 1·55	—
,, ,, ,, ..	30	0·50 0·45	0·50 0·40

In addition to the experiments above, the following procedure was adopted with a sample of crude soya bean oil having a high peroxide value (about 30). About 5 g of the oil were dissolved in 15 ml of the Lea solvent and inert gas was bubbled through the soln. The escaping gas was bubbled through a second tube containing 20 ml of the solvent, to which had been added 1 ml of a saturated soln. of potassium iodide. After an hour no iodine was liberated from the reaction tube.

Two experiments were carried out to investigate the possible loss of liberated iodine. The peroxide value was determined on the above sample of crude soya bean oil. During the first determination the escaping gas from the reaction-tube was bubbled through a second tube containing 20 ml of the Lea solvent. No free iodine was detected in the latter tube. The previous experiment was repeated with the second tube containing alcohol to absorb any escaping iodine. Again, no iodine was found to have escaped from the first tube.

These experiments indicate that there is no loss of peroxide-oxygen or liberated iodine when the inert gas is bubbled through the reaction-mixture.

RECOMMENDED METHOD

The following room temperature method is recommended for the determination of peroxide values.

REAGENTS—(1) Solvent mixture: 2 vols. of glacial acetic acid + 1 vol. of chloroform. (2) 0·002 *N* Sodium thiosulphate, made up each day from 0·10 *N* soln. (3) A saturated solution of potassium iodide, freshly prepared. (4) Starch soln. (5) Carbon dioxide or nitrogen. *All the reagents must be of analytical quality.*

The test tubes (6 in. by 1 in.) used should be thoroughly washed with soapy water and then rinsed with hot water and allowed to stand in chromic acid for a few hours. They should then be rinsed thoroughly in water and finally with distilled water, and should be dried in an oven before use.

The test must be carried out away from windows and preferably with the aid of artificial light.

PROCEDURE—Weigh 1 g* of the oil or fat in the test-tube, add 20 ml of solvent mixture and 1 ml of the saturated potassium iodide soln. The tubes must be protected from any daylight during the reaction with potassium iodide. Also, immediately after the addition of the potassium iodide bubbling of the inert gas through the reaction mixture must be commenced. Allow the reaction to proceed for one hour at room temperature. Transfer the contents of the tube to a 300-ml conical flask and wash out the test tube with distilled water. Titrate the soln. with the sodium thiosulphate soln., using starch as indicator. The starch should not be added until the end-point is almost reached.

The peroxide value is declared as millilitres of 0·002 *N* sodium thiosulphate required for 1 g of the oil or fat.

* Adjust the amount of oil or fat taken to bring the titration figure to not more than 10 ml. If the sample under test has a high melting point, the melted oil or fat should be dissolved in the solvent mixture while still liquid, otherwise solution may not take place.

SUMMARY—Factors affecting the accuracy of the determination of peroxide value of oils and fats have been critically examined. In both the Lea and room temperature methods oxidation is only effectively prevented when inert gas is bubbled through the reaction mixture. A room temperature method of procedure is recommended.

In concluding, the authors wish to thank the Directors of Messrs. J. Bibby & Sons, Ltd., for permission to publish the results of this investigation.

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Peroxide Values and the Chromatographic Separation of Oxidation Products as a Guide to the Ageing of a Turbine Oil

BY K. A. WILLIAMS

A MEDIUM turbine oil had been used continuously for the lubrication of a generating set in an electrical power station for about nine years, in the course of which the set had been damaged by enemy action and part of the oil charge of 4000 gallons had been on fire.

Throughout this long period the oil gave very satisfactory service, but nearly 5 years after the "blitz" damage it was found to have started to deposit a sludge. This differed from the usual powdery deposit, and had a softening point so little above the normal working temperature of the oil that it appeared as a compacted and adherent layer at various points, notably in the centrifuge used continuously for cleaning oil in the set and in the Bibby coupling. This gummy sludge rapidly built up in quantity and, before the seriousness of the situation had been realised, the centrifuge was stopped by its accumulation and the governor gear became inoperative.

Damage to the machinery was averted, but a considerable loss of output of electricity occurred while the set was being cleaned; and this would have been quite serious had the stoppage happened in winter and not in late spring. The urgent need for avoiding any similar failure in winter, when full production is vital, led to a review of the analytical methods used for following the ageing of the oil; and it proved possible to supplement the methods previously used, as samples of the oil were in existence which had been taken at intervals.

The ageing of turbine oils is normally logged by periodic determination of neutralisation value, saponification value, demulsification value and viscosity, the standard methods described in "*Standard Methods for Testing Petroleum and its Products*," published by the Institute of Petroleum, being used. An indication of the approaching end of the useful life of an oil is shown by an abnormal increase in the rate at which these figures rise; but variations between different oils and in the conditions of their use preclude the selection of any fixed limits to the figures as a sure guide. Moreover, the actual rise that occurs before the oil becomes unserviceable is not great. It was therefore desired to find additional methods whereby marked deterioration could be more easily recognised.

An analogy was drawn between the composition of major constituents of lubricating oils (long-chain hydrocarbons) and of fatty oils (long-chain fatty acids). In both cases ageing is promoted by oxidation at points in the chains and by heat; and in fatty oils the termination of serviceability is known to be marked by a very rapid rise in the peroxide oxygen measured by the peroxide value. The peroxide value of a fatty oil is low, usually below 5, during the long period for which the oil is sweet and palatable. Onset of rancidity appreciable to the

taste is preceded by a sudden rise, generally to 20 or more, and is quickly succeeded by a rise to 200 or more.

The long initial sweet period of fatty oils can be lengthened by incorporation of inhibitors, many of which contain a poly-hydroxyphenol group. It is believed that these are oxidised preferentially to the oil containing them, or divert oxygen from attacking the oil, and that they confer protection in some degree depending on the amount present. Rapid deterioration of the oil follows as soon as added inhibitors, and any natural ones present, have reacted with or diverted as much oxygen as they can. The ageing of lubricating oils also can be successfully inhibited by the use of anti-oxidants of similar chemical character to those effective for fatty oils; and these oils are known to break down when the inhibitors have been used up.

Peroxides are known to develop in volatile petroleum products as these age, and to be inhibited by phenols. Such development and inhibition is described by Risby and Nisbet,[1] whose work clearly shows the rapid accumulation during decomposition and infers a short period of induction for these products.

From the above analogy it was expected that turbine oils would have low peroxide values during the long period in which they give good performance, and that the values would rise sharply towards the end of their usefulness. This prediction proved to be justified in the case of the oil under review. Figures are given in Table I for the peroxide values at different points in the oil's history; it will be seen that in course of several years' use the value rose to 6, compared with a rise to 4 in oil that was not used, and that eventually there was a rapid rise in the used oil to 48½. The oil showed signs of turbidity for the first time at a peroxide value of 42, and then began to deposit the gummy sludge. If the peroxide values are plotted in a graph against time it will be found that the resulting curve has exactly the form regarded as typical of the degradation of fatty oils, and is similar in form to curves based, for example, on the rise in saponification value during the induced oxidation of mineral oils.

The simplicity of the test and the very marked rise in the figures it yields at the critical period would appear to recommend it as an indication of the degree of deterioration of a turbine oil such as that under review.

It is believed that the test has not previously been applied to the evaluation of these oils, and further confirmation of its value was desired. Search was made for a method which would separate early oxidation products. It was argued that these are likely to be relatively insoluble in light petroleum and thus similar to oxidised fatty acids; and it was proposed to use a chromatographic method for the separation of the oxidised products from this solvent, an actively adsorptive agent being expected to be most effective for ensuring the stripping of the solvent. It may here be mentioned that the importance of this principle was soon established; many separations of relatively insoluble matter from solutions of fatty matter are complicated by the solubilising effect of the oil present; for example, it is difficult or impossible by filtration to remove lecithin satisfactorily from acetone solution in presence of oils; gossypol from cottonseed oil; or resinous impurities from a light petroleum solution of sulphur olive oil. The use of an adsorbent for the more insoluble constituent of the solution, offering an active counter to the solvent action of the oil, will, it is believed, go far to the solution of these analytical problems. Details of work now in progress in this direction will be communicated later.

A chromatographic method for the examination of used lubricating oils has been described by Lahiri and Mardles[2] at a meeting of the Institute of Petroleum and the British Rheologists' Club (April, 1945), employing activated silica columns 2 in. in diameter and 6 feet in length; details of its application and results were not available during the present study. The method used in my laboratory was similar to that put forward by Bolton and Williams[3] for the detection of fuel oil in whale oil, differing only in that 5 g of oil were used, dissolved in 100 ml of light petroleum, and that only one adsorption on alumina proved to be necessary. The ether eluate from this adsorption was filtered, evaporated, dried and weighed, and its percentage of the original oil was calculated.

The product may be termed "dissolved gum," and is an extremely viscous material, resembling in appearance and physical state the coating of fly-papers; it seems to be the precursor of the gummy sludge that is (later) precipitated from the turbine oil. A similar product may be extracted from used oils by means of certain organic solvents, notably alcohol.

The proportions of "dissolved gum" separated from the turbine oil under review are included in Table I. It will be seen that here again there is a long slow period of induction in which the proportion rises gradually to about 4 per cent., and that in the last stages the

rise is rapid. The advantage offered by this method lies in the fact that degradation products are actually separated and recovered in a simple manner, so that one need not rely on the intangible factors of the usually determined values.

TABLE I

	Peroxide value	"Dissolved gum" %
New oil	0·3	0·28
Unused oil, supplied in 1936, analysed in 1945	4	1·20
Used oil, supplied in 1936, used until:		
January, 1945	6	5·35
March, 1945	25	6·59
May, 1945	42	7·70
June, 1945	48½	9·29

Peroxide values were determined by the simplified method of Lea.[4]

SUMMARY—An unusual instance of the failure of a turbine oil is described, resulting in the deposition of a gummy sludge. By application of tests for the peroxide value and for the chromatographic separation of oxidised products the course of deterioration of the oil is shown to be similar to that of the atmospheric oxidation of fatty oils, an induction period being followed by a period of rapid oxidation. These tests are proposed as being of value in the routine examination of turbine oils to indicate the state of deterioration.

My thanks are due to the Metropolitan Borough of Fulham, Electricity Department, and to W. C. Parker, M.I.E.E., Borough Electrical Engineer, for approving the publication of this paper.

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Electrometric Determination of Ascorbic Acid

BY H. LIEBMANN AND A. D. AYRES

(*Read at the Meeting of the Society on April* 4, 1945)

IN coloured extracts the direct visual titration of ascorbic acid with 2, 6-dichlorophenol-indophenol is impossible, and some other means has to be adopted to locate the end-point of the determination. Electrometric methods have been employed by a number of workers,[1,2,3] and excellent results may be obtained by a procedure suggested by Harris, Mapson and Wang. These authors use a mercury-coated platinum electrode and measure its potential against a calomel electrode as the titration proceeds. The e.m.f. remains steady during the titration and rises rapidly as soon as excess of the dye is present in the soln. under test. For routine analyses Harris's method has, however, a certain number of disadvantages. Potentials have to be determined after each addition of dye and a certain time has to be allowed for establishment of equilibrium at the electrode. Furthermore, the platinum electrodes must be freshly coated with mercury at frequent intervals.

It is well-known that equilibrium is rapidly attained with polarised electrode systems and it was thought that the "dead stop" method proposed by Foulk and Bawden[4] for iodimetric titration might be applicable to the titration of ascorbic acid with 2,6-dichlorophenol-indophenol. Apart from a possible increase in speed of titration a simplification of the experimental arrangement would be the main advantage, as neither special preparation of the electrodes nor the use of a standard reference cell and a potentiometer is necessary.

Two bright platinum wires are dipped into the acid extract under test and a small e.m.f., approximately 15 m.v., is applied. Polarisation occurs, setting up a small voltage of opposite sign, and the external e.m.f. can be adjusted so that no current flows in the system. A galvanometer in the circuit will show no deflection. These conditions remain unchanged during the course of the titration until an excess of the dye depolarises the electrodes, allowing a current to flow under the influence of the external voltage. The end-point of the titration

is, therefore, indicated by a sudden permanent excursion of the galvanometer from its zero position.

In our experiments, a 4-oz. wide-mouthed bottle served as a titration cell. This was closed with a cork stopper carrying the electrodes—bright platinum wires fused into glass tubes—and a leading tube for carbon dioxide. The tip of a 2-ml burette was inserted through a hole. A 2-volt accumulator with a 1,000 Ω fixed resistance and a 10 Ω potentiometer in series supplied the polarising voltage. A Cambridge "Spotlight" galvanometer of a sensitivity of 23 divisions/micro-amp. was used as current indicator.

The solution under test is pipetted into the cell and dye is added initially at the rate of 1 drop per second. No movement of the galvanometer occurs till the end-point is nearly reached, when the galvanometer is displaced, but returns to its zero position in a few seconds. The end-point is registered by a permanent deflection. During the titration the solution is vigorously stirred by a stream of carbon dioxide.

No difficulties are experienced in obtaining reproducible results if bright platinum wire electrodes are used. These are cleaned in chromic acid and washed with distilled water at the beginning of the experiments. Between the titrations they are stored immersed in water.

Some typical results are given in the following tables. The first set of data shows a comparison of visual and electrometric titration of solutions of ascorbic acid in trichloroacetic acid. The consistency of electrometric titrations can be judged by the first four figures in Table I, which represent single determinations on the same solution. All other results are the means of duplicate determinations.

TABLE I

TITRATION OF ASCORBIC ACID SOLUTIONS

Results are expressed as ml of dye solution

Electrometric ..	1·73	1·69	1·68	1·68	1·98	1·87	1·67	1·29	1·69	1·79*
Visual	—	—	—	—	1·99	1·92	1·80	1·30	1·63	1·78*

* Ascorbic acid in 8% acetic acid.

Extracts of brussels sprouts and broccoli leaves were prepared in the usual manner. Unless otherwise stated, a mixture of trichloroacetic acid and metaphosphoric acid was used as extracting medium. The results of electrometric and visual titrations are given in Table II. Here again, the figures represent the means of two determinations.

TABLE II

ASCORBIC ACID CONTENT OF VEGETABLES

Electrometric ..	Sprouts	31·4	53·6	58·9	92·5*	25·4*	36·1†	Broccoli	188·0 mg/100 g
Visual	Sprouts	35·0	53·5	60·5	89·6*	25·4*	36·9†	Broccoli	188·0 ,,

* Trichloroacetic acid as extracting medium.
† ,, ,, and citric acid as extracting medium.

In order to test whether naturally occurring pigments would interfere with the depolarisation of the electrodes, ascorbic acid determinations were carried out on samples of blackcurrant juice and extracts from beetroot. The colour of these solutions was very similar to that of unreduced dichlorophenolindophenol and no reliable results could be obtained by visual titration even when chloroform extraction, as suggested by McHenry and Graham,[5] was used. Known amounts of ascorbic acid were therefore added to the solutions and their recoveries were determined by the electrometric method. The results are given in Table III.

TABLE III

TITRATION OF ASCORBIC ACID IN COLOURED EXTRACTS

Product	Extract taken ml	Ascorbic acid: Added mg	Total found mg	From extract mg	Recovered mg	Recovery %
Blackcurrant ..	10	0·0	0·544	0·544	—	—
	5	0·231	0·509	0·272	0·237	102·5
	2	0·370	0·477	0·109	0·368	99·5
Beetroot	10	0·0	0·036	0·036	—	—
	10	0·231	0·264	0·036	0·228	98·7
	10	0·463	0·492	0·036	0·456	98·6

It appears that the "dead stop" method devised by Foulk and Bawden is well suited for the electrometric determination of ascorbic acid and that it has certain advantages over the procedures hitherto described.

The application of Foulk and Bawden's method to the determination of ascorbic acid was first suggested by Dr. C. G. Sumner, working in this Department, but wartime conditions made it impossible for him to test the practicability of this idea. We are grateful for his consent to the publication of our results. We are also indebted to the Metal Box Co., Ltd., and Mr. R. K. Sanders, Head of the Research Department, for permission to communicate this paper.

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Discussion

The President thanked the authors for their interesting paper and asked if in their method the condition of the surface of the electrodes was an important factor.

Dr. J. E. Page asked for particulars of the method used to stir the solution during titration.

Miss M. Olliver said that a modification of the McHenry and Graham visual method for estimating ascorbic acid in coloured solutions had been found exceedingly useful for routine control work; it was very rapid and, with experience, good agreement could be reached with results obtained by the Harris, Mapson and Wang electrometric method. Dr. Liebmann had stated that a pronounced change in E.M.F. took place when excess of dye was present; it appeared, therefore, that the described method would not have the advantage of the Harris, Mapson and Wang method, in which the absolute end-point could be determined. The results in Table I would be more conclusive if comparison had been made with the theoretical values instead of those obtained by visual titration. In Table II, one would have expected better agreement with sprouts extracts, being uncoloured, if both methods were applied to the same extracts. Finally, in view of Dr. Liebmann's remarks on the effect of iodide ions on the potential, Miss Olliver suggested that the possibility of interference of halide ions and other substances with the proposed method would need to be carefully investigated.

Dr. Liebmann, replying to the President, said that the magnitude of the polarisation E.M.F. set up at the electrodes, and hence the state of the surface of the electrodes, was not critical. By operation of the potentiometer the external voltage could be adjusted at will to balance the polarisation E.M.F. and to establish the condition of no current flowing at the beginning of the titration. In reply to Dr. Page, he said that the solutions were stirred by a vigorous stream of carbon dioxide, but mechanical stirring would be equally suitable. In reply to Miss Olliver, he said that neither the method of Harris, Mapson and Wang nor that now described could claim to yield "absolute" end-points. They both relied on the perception of an excess of the reagent by observation of a physico-chemical change. While Harris and his collaborators observed a rise in E.M.F. of an electrode in presence of an excess of dye, the end-point in the proposed method was indicated by the sudden flow of a current due to the depolarisation of an electrode by unreduced dye. Both were therefore indirect methods, and no advantage could be claimed for either on fundamental grounds. In both instances it was possible to determine the end-point by graphical interpolation. Iodide or other halide ions would not interfere with the titration. The decisive change at the end-point was the depolarisation of the cathode by excess dye. Throughout the titration the anode remained depolarised, probably first by the ascorbic acid and later by the reduced dye. Halide ions would also act as anode depolarisers and would therefore bring about no change at the already depolarised anode.

Notes on the Determination of Aniline in Mixtures of Aniline, Methylaniline and Dimethylaniline by the Picryl Chloride Method

By J. HASLAM and F. SWEENEY

For the speedy and accurate analytical control of industrial distillation experiments it became desirable to replace the ordinary methods for determining aniline in mixtures of aniline, methylaniline and dimethylaniline by other speedier tests. Two types of tests had been used for aniline determinations, both involving diazotisation and coupling. In one type coupling was effected with a measured excess of alkaline "R" salt solution, and in

the other with excess of *m*-phenylenediamine in buffered acetic acid medium, and in both instances the excess of coupling reagent was determined by titrating back with standard benzene diazonium chloride solution.

At this stage we became interested in an alternative method of determination of aniline known as the Picryl Chloride Method, described by Linke, Preissecker and Stadler.[1] The reaction on which the method is based is as follows:

$$C_6H_5NH_2+ClC_6H_2(NO_2)_3+NaHCO_3\rightarrow C_6H_5NHC_6H_2(NO_2)_3+NaCl+CO_2+H_2O$$

The authors named described alternative methods of test:

(*a*) *Gravimetric*—In this method the aniline is allowed to react with an ethyl acetate solution of picryl chloride in presence of sodium bicarbonate, and the reaction product is heated on the water-bath with a large excess of water; then, after standing, the solution is filtered and the chloride in the filtrate finally determined gravimetrically as silver chloride.

(*b*) *Volumetric*—In this method the procedure is as above, but, after digestion of the reaction product on the water-bath with a large volume of water the resulting solution is titrated with standard silver nitrate solution, with potassium chromate as indicator.

Linke, Preissecker and Stadler extended their observations to the examination of the reaction of picryl chloride with anthranilic acid and *o*-nitraniline; *o*-, *m*- and *p*-toluidine, *p*-aminophenol and *o*- and *p*-anisidine; secondary aromatic amines, monomethylaniline and diphenylamine; benzidine; naphthylamine; *m*- and *p*-phenylenediamine. Without giving any figures, they indicate that methylaniline reacts "hardly at all" with the reagent.

Nelyubina[2] used the picryl chloride method for the determination of aniline in presence of ethylaniline and diethylaniline and claimed to get more reliable results than by the older method of Reverdin,[3] who used the R salt coupling method.

Attention has been drawn to the piccryl chloride method in a private communication on alkyl-anilines submitted by W. L. Main. There is no indication in Main's paper as to whether he was working on the methyl, ethyl or other alkyl anilines, and the method which he used differed essentially from that put forward by Linke, Preissecker and Stadler. Main uses a benzene solution of picryl chloride and heats this under reflux with the sample for ½ hr. Where large proportions of aniline are involved pure dimethylaniline is used as an acid acceptor. The reaction product is washed with water and the aqueous washings are made alkaline with sodium carbonate, and then extracted with benzene. The chloride in the washed aqueous solution is finally determined by the Volhard method.

Main's final observations are as follows:

> "Status. Samples containing less than 5% aniline give results that are accurate to ±0·10 absolute. On samples containing 5% to 50% primary aromatic amine, the results are accurate to ±2% error. On samples containing more than 50% primary aromatic amine, the results are accurate to ±5% error. The results are usually on the low side."

For our purpose we had in mind rapid aniline determinations on two types of sample:

(1) A feed mixture containing approx.—aniline 50·0%, monomethylaniline 40·0% and dimethylaniline 10·0%;

(2) "Fractionation column bottoms" containing approx.—aniline 0·2%, monomethylaniline 96·0% and dimethylaniline 0·2%;

and we wished the analytical operations to be as simple as possible.

Such tests have been worked out. They are based on addition of the sample under test to an ethyl acetate solution of picryl chloride, followed by addition of sodium bicarbonate. After a comparatively short reaction time (¼ hr.) the mixture is extracted with water and the aqueous extracts are filtered. The chloride in the filtrate is finally determined by potentiometric titration with silver nitrate solution. This method is rapid and preferable to methods which involve the use of chromate indicator, since the chloride-containing solutions are coloured distinctly yellow.

METHOD—As used for the determination of aniline in feed samples (see (1) above)—Add approx. 0·4 g (accurately weighed) of the sample, from a Lunge Rey pipette, to 25 ml of an 8% w/v solution of picryl chloride in ethyl acetate (B.D.H. Laboratory Reagent) contained in a separating funnel (150 ml). Mix gently by swirling and then add 0·4 g of sodium bicarbonate. Allow to stand for 15 min. with occasional swirling; then add 40 ml of water and dissolve the sodium chloride produced during the reaction by gentle swirling. Separate the aqueous layer, filter it through a No. 40 filter paper (11 cm.) and collect the filtrate in a squat beaker (300 ml). Carry out two further washings as above, using

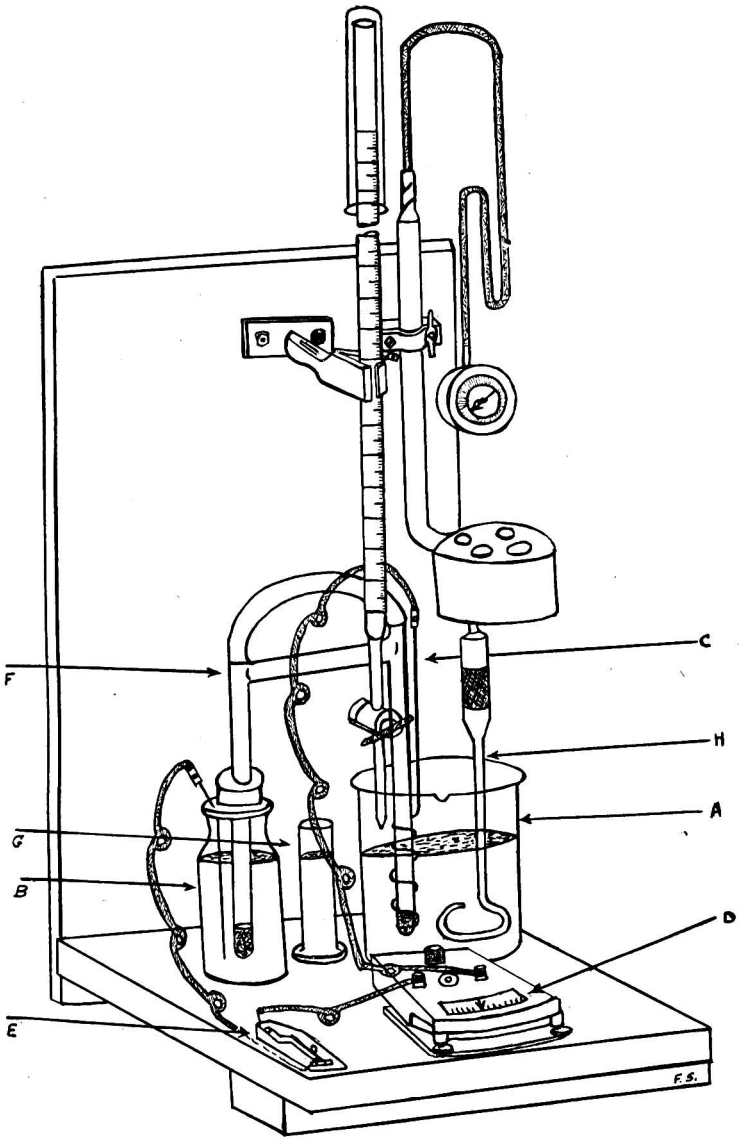

Fig. 1 ELECTROMETRIC DETERMINATION OF CHLORIDE

A. Titration vessel: a 300 ml squat beaker.
B. Reference half cell: a silver wire dipping into the following mixture:
   25·0 ml of $N/10$ sodium chloride
   25·05 ml of $N/10$ silver nitrate
   10·0 ml of N nitric acid
   40·0 ml of water
   contained in an amber glass bottle closed with a rubber bung carrying the silver wire and one limb of the salt bridge F.
C. Silver wire indicator electrode (wound round limb of bridge F to prevent fouling of stirrer).
D. High resistance galvanometer (Pye, dead beat pointer type; sensitivity approx. 12 divisions per micro-amp.).
E. Tapping key.
F. H type salt bridge fitted with a saturated potassium sulphate solution. The ends are drawn out to thick-walled capillaries and plugged with cotton wool. Test for air blocks, which must be completely absent.
G. Cylinder containing saturated potassium sulphate solution filled to the same level as the liquid in B; the free limb of the salt bridge F is dipped into it when not in use.
H. Variable speed stirrer.

successively 30 ml and 15 ml of water. Wash the filter paper two or three times with distilled water (using about 10 ml each time) and then add 10 ml of *N* nitric acid to the clear filtrate.

Titrate the solution potentiometrically with *N*/20 silver nitrate as shown in Fig. 1, using a bottled end-point. The bridge between the bottled end-point and the titration beaker is filled with a saturated solution of potassium sulphate.

We are indebted to L. A. Haddock of I.C.I. General Chemicals for the details of this electrometric method of titrating chloride, which is based on earlier work of Callan and Horrobin.[5]

At the beginning of the titration it is important that the level of the liquor in the bottled end-point jar shall correspond with a height of approximately 50 ml above the level of the liquid in the titration beaker, since it is desirable that liquid shall not "seep" from the titration beaker to the bottled end-point jar in the course of the titration. The end-point is sensitive to 0·05 ml of *N*/20 silver nitrate.

The *N*/20 silver nitrate used for titration is standardised against 50 ml *N*/20 pure sodium chloride solution (prepared from dried AnalaR sodium chloride) by the potentiometric method as above, 10 ml of *N* nitric acid being added. One ml of *N*/20 silver nitrate ≡ 4·653 mg of aniline.

Where the proportion of aniline in the feed mixture is excessively high, *e.g.*, 60%, it is desirable to repeat the test on a smaller amount of sample so that a volume of approximately 40 ml of *N*/20 silver nitrate is used up in the final titration.

The blank on the reagents was never more than the equivalent of 0·15 ml of *N*/20 silver nitrate and was neglected.

The following results were obtained on application of the test to re-distilled aniline and known mixtures.

Composition of sample	Wt. of sample taken for test g	Titration *N*/20 $AgNO_3$ ml	Aniline found %
Re-distilled aniline (total base equiv. to 99·8% aniline)	0·1818	38·27	97·97
	0·1810	38·07	97·86
Mixture 1			
Aniline 35·95%	0·4189	32·04	35·59
M.M.A. 50·40% D.M.A. 13·65%	0·5085	39·14	35·81
Mixture 2			
Aniline 50·37%	0·3962	42·81	50·28
M.M.A. 39·42% D.M.A. 10·21%	0·4055	43·7	50·14
Mixture 3			
Aniline 64·76%	0·3005	41·65	64·49
M.M.A. 27·85% D.M.A. 7·39%	0·2970	41·15	64·47

M.M.A. = monomethylaniline D.M.A. = dimethylaniline

For the determination of aniline in fractionating column bottoms (see p. 414) the same procedure as is outlined above is followed except that approximately 6 gm (accurately weighed) of the sample are used in the test.

We have shown that the picryl chloride reagent has negligible effect on D.M.A. M.M.A. free from primary amines, prepared for us by L. Seed by treatment of M.M.A. of low primary amine content with phthalic anhydride, exerts a slight influence on the test and this has to be allowed for in the determination of small amounts of aniline in mixtures, the composition of which corresponds with fractionating column bottoms. When the test was applied to D.M.A. and M.M.A. (free from primary amines) the following results were obtained.

Sample	Wt. of sample taken g	Titration *N*/20 $AgNO_3$ ml	Equivalent aniline found %
D.M.A.	5·683	0·35	0·029
	6·572	0·36	0·025
M.M.A. free from primary amines	6·281	2·38	0·18
	6·248	2·38	0·18

In view of these figures we decided to deduct 0·18% from the aniline figure in tests on small aniline—large M.M.A. mixtures.

We were in possession of a well-fractionated sample of M.M.A. which had previously been shown by the R salt method to contain 1·23, 1·21% of aniline. When the picryl chloride method (using the correction of 0·18%) was applied to this sample and to mixtures of aniline with it the following results were obtained.

Sample	Wt. of sample taken g	Titration $N/20$ $AgNO_3$ ml	Aniline found %
Fractionated M.M.A.	6·332	19·48	1·25
	6·143	18·64	1·23
Mixture of above fractionated M.M.A. and aniline, prepared so as to contain 2·21% of total aniline	6·377	32·4	2·18
	6·203	32·26	2·24
Mixture of above fractionated M.M.A. and aniline, prepared so as to contain 3·15% of total aniline	6·082	43·68	3·16
	6·175	44·11	3·14

Our experience of the picryl chloride test, both for feed mixtures and for fractionating column bottoms of high M.M.A. content shows the following advantages and disadvantages of the test.

Advantages—(1) The test is simple and rapid. (2) The results obtained are of a high degree of reproducibility. (3) Large numbers of tests may be carried out in parallel. (4) Only one simple standardisation is involved in the test.

Disadvantages—Care has to be taken in handling the reagent which, it is understood, is liable to produce dermatitis on sensitive skins.

In our work, rubber gloves have been worn, and the method has been used successfully for a period of approximately 18 months without any evidence of dermatitis amongst the chemists engaged in the work.

SUMMARY—Simple and rapid methods have been developed for the determination of aniline in two types of mixture: (1) A feed mixture containing approx. 50% of aniline, 40% of M.M.A. and 10% of D.M.A., and (2) Fractionating Column Bottoms containing approx. 0·2% of aniline, 96% of M.M.A. and 0·2% of D.M.A. The method is based on the reaction of the aniline in the sample with a solution of picryl chloride in ethyl acetate in presence of sodium bicarbonate as acceptor of hydrochloric acid liberated in the reaction. The sodium chloride produced is extracted with water and the aqueous solution titrated potentiometrically with standard silver nitrate solution.

Aniline reacts almost quantitatively in the test, monomethylaniline only very slightly, whilst the reagent has a negligible effect on dimethylaniline. The advantages and disadvantages of the method are discussed.

Since this work was carried out, our attention has been drawn to a recent paper by Spencer and Brimley,[6] who describe an alternative method of carrying out the picryl chloride test. The authors use the principle of the picryl chloride method, but make use of either diethylaniline or dimethylaniline as acid acceptor; the hydrochloric acid liberated in the test is subsequently titrated with standard alkali solution.

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IMPERIAL CHEMICAL INDUSTRIES LTD., RESEARCH DEPT.
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Estimation of Tetryl, alone or in Mixtures with T.N.T., by Ferrous Ammonium Sulphate

By B. N. MITRA and M. SRINIVASAN

Cope and Barab[1] have shown that the nitroamino group in tetryl and certain other nitroamines, such as nitro-urea and nitro-guanidine, is quantitatively converted into nitric oxide in the nitrometer; the volume of nitric oxide is thus a possible measure of tetryl. Another method,[2] less specific and confined to its estimation in mixtures with T.N.T., makes use of the relative insolubility of tetryl in carbon tetrachloride. In this method, due to Taylor and Rinkenbach,[2] rigorous conditions have been prescribed; but even following these conditions the authors admit an error of 2% for the method, the error increasing with the percentage of T.N.T. present in the mixture.

The nitrometer method of estimating tetryl has been developed by Lehmstedt.[3] According to him, the method is both rapid and accurate; applied to a 1 : 1 mixture of tetryl and T.N.T., it gave values of 49·8, 49·5 and 49·7%, and it does not affect tetranitroaniline.

Desvergnes[4] finds that the estimation of tetryl in the nitrometer has limitations; *e.g.*, tetryl is not easily soluble in the restricted volume of conc. sulphuric acid used, nor is it easy to get the mixture into the nitrometer quantitatively. Desvergnes raises, however, another objection which is difficult to understand, namely, that it takes a long time (about 4 hr.) to complete the liberation of NO. He has no doubt that the dissolution of tetryl in sulphuric acid could be hastened by warming, but not without decomposition and consequent low values.

There would thus appear to be no established method for the accurate estimation of tetryl. We therefore began with an examination of the nitrometer method. Although we could not substantiate in their entirety the criticisms of this method by Desvergnes, our own experience of the method is that it is cumbersome; the dissolution of tetryl in the small quantity of sulphuric acid fixed by the working conditions of the nitrometer is not only time-consuming but unsatisfactory. Working with pure tetryl, it was difficult to get a value more than 97% by the nitrometer.

It thus became clear that there is a real need to estimate the nitric acid released from tetryl on dissolution in sulphuric acid, by a method less strenuous than the nitrometer method. The estimation of nitric acid by titration against ferrous ammonium sulphate suggested itself. Preliminary trials showed that this titration has the very great advantage over gas measurement, that there is no limit to the volume of sulphuric acid to be used, obviating the main difficulty of dissolving tetryl and insuring the quantitative release of nitric acid.

Method—About 0·75 g of finely ground tetryl (100 B.S. mesh) is accurately weighed into a 350-ml conical flask, 100–120 ml of ice-cold conc. sulphuric acid are added and the flask is stoppered. The mixture is kept at ice temperature until dissolution is complete and then titrated against ferrous ammonium sulphate as described by Mitra and Srinivasan[5] in the estimation of guncotton. The standardised ferrous solution (note iii) is run in very slowly, with continuous mixing, through a fine jet attached to the burette, the end being well below the surface of the acid. Mixing must be very thorough in order to avoid local concentrations of $Fe^{\cdot\cdot}$ which may cause a precipitate to form, vitiating the results. Near completion, the standard should be added, drop by drop. The end-point is indicated by a permanent pink colour. ($FeSO_4$: NO complex.)

Notes: (i) The tetryl should be finely subdivided to help dissolution.

(ii) Temperature of dissolution and titration should not exceed 0° C. Strict adherence to this low temperature is very necessary, not so much to prevent loss of nitric acid (the normal precaution in these titrations) as to avoid a browning of colour which occurs in varying degrees at laboratory temperatures and masks the end-point. If solution is completed at 0° C. the colour is pale yellow.

(iii) The ferrous ammonium sulphate solution used in this titration (120 g/litre in 60% sulphuric acid wt/vol.) is rather dilute, the concentration fixed being the optimum for the working conditions of the experiment (about 20 ml titre for 0·75 g of tetryl). This dilute solution, in contradistinction to the strong

solution used in guncotton estimation, shows a tendency to change on keeping. To avoid frequent standardisation of the solution, therefore, it is best preserved in a bottle closed with an automatic burette, the air space being filled with hydrogen. The solution is standardised against potassium nitrate (0·25 g).

Accurate results for pure tetryl are obtained by following the above details (Table I). As nuclear nitro groups are unaffected by sulphuric acid, the method is applicable with equal accuracy to estimation of tetryl in mixtures with T.N.T. (Table II).

The mechanism of the reaction between tetryl and sulphuric acid, about which there is little definite information in the literature, is under investigation.

RESULTS—

TABLE I

Nitroamine nitrogen % in tetryl (theoretical = 4·88)
Found: 4·89; 4·87; 4·83; 4·85; 4·85; 4·91; 4·91; 4·90; 4·85; 4·89; 4·88; 4·91; 4·92
Average 4·88%: Standard deviation 0·091; coeff. of variability 1·9%.

TABLE II

ESTIMATION OF TETRYL IN MIXTURES WITH T.N.T.

Tetryl present:	50·03%	30·03%	80·04%
,, found:	49·84%	29·73%	80·04%
	49·84%	29·70%	80·05%

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INSPECTORATE OF MILITARY EXPLOSIVES
KIRKEE, INDIA

The Determination of Mercury as Mercuric Iodate

BY C. H. R. GENTRY AND L. G. SHERRINGTON

THE usual method for the gravimetric determination of mercury is by precipitation and weighing of mercuric sulphide.[1] It is not applicable in presence of zinc, cadmium or copper, and the necessity of removing any sulphur carried down with the ppt. makes the method somewhat tedious. The gravimetric determination of mercury as mercurous chloride, after reduction of the mercuric salt with phosphorous acid, is lengthy and gives decidedly low results.[2] The use of anthranilic acid[3] for the pptn. and estimation of mercury has little to recommend it, whilst extensive use of thionalid[4] is limited by the cost of the reagent.

There would thus appear to be a need for a rapid and precise method for the gravimetric determination of mercury. In the course of some experiments on the determination of lead, as the iodate, interference from mercuric salts suggested a method for the determination of mercury, and both gravimetric and volumetric procedures have been investigated.

THEORETICAL—From the data of Smith and Joshi, the solubility of mercuric iodate in 1% nitric acid solution at 25° C. is known to be 8·0 mg per 100 ml. It is apparent that a considerable excess of iodate ions is required for quantitative pptn., and the use of such an excess is permissible, as mercuric iodate shows no tendency to formation of bi-iodate or complex iodate.

If the ionisation constant of iodic acid at 25° C. is taken as 0·19, the solubility of mercuric iodate in a 1% nitric acid soln. containing 2 g of iodic acid per 100 ml can be approximately calculated. Under these conditions the solubility is about 3×10^{-9} g-mol. per litre, equivalent to $1{\cdot}6 \times 10^{-7}$ g of mercuric iodate per 100 ml.

This result suggests that a quantitative estimation of mercury as mercuric iodate should be possible in a soln. containing 1–2 ml of nitric acid and 1–2 g of iodic acid per 100 ml.

EXPERIMENTAL—*Standard Mercury Solution*—A standard soln. of mercury was prepared by heating under reflux a known weight of spectrographically pure mercury with conc. nitric acid in an all-glass apparatus and diluting the cooled solution to a known volume in a standard flask. The final soln. containing about 0·1% of nitric acid was tested and found to be free from mercurous ions.

Precipitation of Mercuric Iodate—A measured volume of the standard soln., containing about 0·3 g or 0·1 g of mercury, according as the subsequent estimation was to be gravimetric or volumetric, was pipetted into a 250-ml beaker, diluted to 100 ml and treated with 1 ml of conc. nitric acid. The liquid was brought to the boiling point and a warm saturated soln. of 2 g of iodic acid was added slowly with vigorous stirring. The beaker was left on the hot plate for five min. with occasional stirring and then rapidly cooled to room temperature. The mercuric iodate settled out as a heavy white precipitate. The following paragraphs, (*a*) and (*b*), describe the treatment of the ppt. for gravimetric and volumetric determinations respectively.

(*a*) *Gravimetric Determination*—The precipitate was filtered through a grade 4 sintered glass crucible, complete transference being achieved with a jet of wash liquid containing 1% of nitric acid and 2% of iodic acid, together with careful use of a "policeman." The ppt. was washed five times with the wash liquid and then three times with small portions of cold water. The ppt. and crucible were dried at 140° C. to constant weight. Drying for one hour was found to be sufficient, and subsequent drying for one day did not affect the weight of the ppt. Weight of dried ppt. × 0·3644 = weight of mercury.

(*b*) *Volumetric Determination*—The precipitate was filtered through a paper pulp pad and washed as in the gravimetric procedure. The pad and ppt. were transferred to a stoppered flask. The funnel was washed with a 10% solution of potassium iodide, the washings being added to the contents of the flask. A further 3 g of potassium iodide were added and the soln. was diluted to 150 ml. Finally, 5 ml of conc. hydrochloric acid were added and the liberated iodine was immediately titrated with $N/10$ sodium thiosulphate, with addition of starch indicator just before the end-point. The sodium thiosulphate was standardised against potassium permanganate, with oxalic acid as the primary standard. One ml of $N/10$ thiosulphate ≡ 1·672 mg of mercury.

Results—The following typical results indicate the precision of the method. The results are expressed in terms of the total weight of mercury in the standard solution.

Expt.	Method	Result expected g	Result obtained g	Error %
1	Gravimetric	9·6983	9·697	−0·01
2	,,	,,	9·691	−0·07
3	,,	,,	9·696	−0·02
4	Volumetric	,,	9·66	−0·4
5	,,	,,	9·66	−0·4
6	,,	,,	9·68	−0·2
7	,,	8·408	8·39	−0·2
8	,,	,,	8·39	−0·2

The percentage error in a number of gravimetric determinations has been found to be always less than 0·1%. The volumetric determination using sodium thiosulphate has shown an average percentage error of −0·3%. However, by standardising the sodium thiosulphate against pure mercury by the method described the average error would be only ±0·1%.

INTERFERENCES—Either nitrate or sulphate solutions should be used for the precipitation, as the presence of halide ions prevents the precipitation of mercuric iodate.

The principal interfering cations are silver, lead, bismuth, titanium, zirconium and ferric iron. Under the conditions described, complete precipitation of silver, lead and small amounts of ferric iron is obtained provided that a digestion time of thirty minutes is given. If required, the silver and lead in the precipitate could be estimated and suitable corrections applied. The method can be applied in presence of cadmium, aluminium, manganese, chromium, nickel, cobalt, zinc, calcium, the alkali metals and moderate amounts of barium, strontium and copper. Antimony and tin are separated in the preparation of the nitric acid solution.

CONCLUSIONS—The gravimetric determination of mercury as mercuric iodate compares very favourable in speed and precision with other methods for the gravimetric determination of mercury.

The separation and subsequent volumetric determination of mercury as mercuric iodate, whilst of more limited application, is a simpler procedure than the usual method involving separation of the sulphide and final estimation by the thiocyanate procedure.

SUMMARY—The precipitation of mercury as mercuric iodate and its subsequent gravimetric and volumetric determination are described. Possible interferences from some other elements are discussed.

In conclusion, we wish to thank Mr. J. A. M. van Moll and the directors of Philips Lamps, Ltd., for permission to publish this work.

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The Determination of Small Quantities of Silica, Alumina and Lime in Tungsten Carbide Powder

BY C. E. A. SHANAHAN*

TUNGSTEN carbide powder used in the production of cemented carbides must be as free as possible from such impurities as silica, alumina and lime, as these tend to form non-metallic inclusions and voids in the sintered pieces. It is therefore important to have a simple and reliable method for their determination, suitable for routine application. The author has experimented with the well-known method of determining silica, thoria, etc., in tungsten metal, involving the separation of the tungsten by volatilisation as chloride and oxychloride. The results show that the method can be used to determine small quantities of silica, alumina and lime in tungsten carbide powder.

Friedheim, Henderson and Pinagel[1] have determined silica in tungsten by a method involving volatilisation of the tungsten with gaseous hydrochloric acid at "cherry-red" heat. Jannasch and Leiste,[2] employing a similar procedure at lower temperatures, used carbon tetrachloride vapour and carbon dioxide as the volatilising atmosphere. Later, Brophy and Van Brunt[3] recommended an atmosphere of gaseous hydrochloric acid and oxygen. Smithells[4] suggests using a chloroform-air mixture.

The author has worked with tungsten carbide powder, employing two furnace atmospheres, *viz.*, gaseous hydrochloric acid—air, and chloroform vapour—air mixtures, varying the temperatures of volatilisation in both cases.

APPARATUS—A Sillit rod horizontal tubular furnace (heating zone about 5 in. in length) taking a Morgan triangle H tube (26 in. long by $1\frac{1}{16}$ in. int. diameter) and capable of maintaining temperatures up to about 1100° C. is used as the heating unit. The refractory tube is closed at both ends with rubber bungs, one having an inlet and the other an outlet tube ($\frac{1}{4}$ in. bore). Passing through one of the bungs is a quartz sheathed Pt-Pt.Rh. thermocouple, the "hot" junction of which is positioned at the centre of the heating zone very close to the silica boat containing the tungsten carbide sample. Fumes leaving the furnace are passed over the surface of dilute hydrochloric acid to decompose most of the volatile tungsten compounds, and thence to the fume chamber. The gaseous hydrochloric acid—air atmosphere is simultaneously produced and dried by passing hydrochloric acid gas (made by dropping concentrated sulphuric acid on to ammonium chloride) and air through two delivery tubes into conc. sulphuric acid, and allowing the mixed gases to flow to the furnace tube. Dry air which has been bubbled through chloroform in a Drechsel bottle is used as the chloroform vapour—air atmosphere.

PROCEDURE—Weigh 2·5 g of the tungsten carbide into a silica boat (3 in. long by $\frac{3}{4}$ in.

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wide by $\frac{7}{16}$ in. deep) and introduce it into the hot furnace having the required atmosphere. Adjust the flow of gas to about 2 bubbles per sec. and after 6 hr. withdraw the boat and transfer the white residue to a small platinum crucible and determine the silica by volatilisation as silicon tetrafluoride. Fuse the residue with 1 g of A.R. potassium bisulphate and dissolve the cooled melt in 10 ml of conc. hydrochloric acid and 50 ml of water. Precipitate the aluminium hydroxide by ammonia, using methyl red as indicator, and purify by repptn. Ignite and weigh as Al_2O_3. Precipitate the lime as oxalate in the standard manner, ignite and weigh as CaO.

If the silica percentage only is required, very small amounts may be estimated by the following procedure based on a method described by Snell.[5] Transfer the residue from the boat to a platinum crucible and fuse with 0·6 g of A.R. sodium carbonate. Dissolve the residue in water and transfer a convenient aliquot to an 8 in. × 1 in. boiling tube. To another tube add standard sodium silicate soln. from a microburette so that the amount of silica present is approx. the same as that in the assay. (Prepare the standard silicate soln. by fusing 1 g of pure freshly ignited silica with a small quantity of A.R. sodium carbonate, dissolving in water and diluting to 1 litre; 1 ml ≡ 0·001 g SiO_2.) Acidify the solns. in the tubes with 1 : 1 acetic acid and add 2 ml in excess. Heat the tubes in boiling water to drive off carbon dioxide, and then add 5 ml of 10% aqueous ammonium molybdate soln. followed by 2 ml of saturated aqueous sodium sulphite soln. Develop the molybdenum blue colour by further heating for about 30 min. Cool and compare the colours in Hehner tubes. When the silica content is very low, it is essential to run a blank determination.

RESULTS—The values obtained for the silica, alumina and lime contents of a well mixed sample of tungsten carbide powder, employing various volatilisation temperatures are given below, together with the results obtained by the longer methods of Lundell, Hoffman and Bright.[6] In all instances the analyses were performed on 2·5 g of sample.

HYDROCHLORIC ACID GAS—AIR MIXTURE

Volatilisation temp. °C.	Silica g	Alumina g	Lime g
650	0·0081	0·0121	0·0031
700	0·0078	0·0121	0·0033
750	0·0079	0·0120	0·0030
795	0·0078	0·0121	0·0036
850	0·0079	0·0119	0·0031

CHLOROFORM VAPOUR—AIR MIXTURE

Volatilisation temp. °C.	Silica g	Alumina g	Lime g
616	0·0081	0·0123	0·0030
650	0·0080	0·0123	0·0034
727	0·0083	0·0108	0·0029
750	0·0079	0·0092	0·0032
775	0·0052	0·0033	0·0035
823	0·0028	0·0022	0·0031
850	0·0019	0·0021	0·0035

METHODS OF LUNDELL, HOFFMAN AND BRIGHT

Silica (g)	Alumina (g)	Lime (g)
0·0080	0·0122	0·0032

With the gaseous hydrochloric acid—air mixture, variation of the temperature of volatilisation over the range investigated does not affect the accuracy of the results. The higher temperatures are preferable, as these increase the rate of volatilisation. With the chloroform vapour—air atmosphere the volatilising temperature should not exceed about 650° C., as higher temperatures cause partial volatilisation of the silica and alumina, the amount volatilising increasing with rise of temperature. Both types of atmosphere are therefore capable of yielding accurate results, and it has been found that the volatilisation rate at about 600° C. with the chloroform—air mixture is as great as that with the gaseous hydrochloric acid—air mixture at much higher temperatures.

As a test on the method for determining the silica colorimetrically, pure freshly ignited silica was added in varying amounts to a high purity tungsten carbide powder (SiO_2=0·02%).

After homogenising the mixtures by trundling, they were analysed, using an assay weight of 2·5 g. The results are as follows:

Silica added g	Silica found g
None	0·0005 C
0·0007	0·0013 C
0·0012	0·0019 C
0·0019	0·0022 C
0·0030	0·0032 G
0·0056	0·0057 G

C = Finished colorimetrically G = Finished gravimetrically

SUMMARY—A method of determining small quantities of silica, alumina and lime in tungsten carbide powder, involving the separation of the tungsten by volatilisation as chloride and oxychloride, is shown to be accurate.

Volatilisation of the tungsten in a mixture of gaseous hydrochloric acid and air does not result in any appreciable loss of the constituents being determined, even when temperatures in the neighbourhood of 850° C. are employed. With a chloroform vapour—air mixture, however, the volatilisation temperature must not exceed about 650° C., otherwise partial volatilisation of the silica and alumina will occur.

A colorimetric method based on the production of molybdenum blue from the reduction of ammonium silicomolybdate has been shown to be reliable where the silica content of the sample is low.

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July 2, 1945

The Determination of Traces of Sulphur Dioxide—with special reference to the Determination of Sulphur in Ferro-alloys

BY G. INGRAM

(*Read at a Joint Meeting of the Microchemistry Group with the North of England Section, at Manchester, on May* 25, 1945)

AMONG the commoner constituents of organic compounds, sulphur alone has proved a difficult element to determine in trace amounts unless large quantities of sample are used. This also applies to its estimation in steels, from which even with 1 g samples the amount of sulphuric acid produced by combustion is so small that a micro burette is necessary for its titration with *N*/200 alkali.

Few attempts have been made to apply micro technique to any of the three methods available for determining sulphur in steels. This is understandable, as titrimetric or gravimetric procedures are unsuitable for amounts of sulphur of the order of 10 μg. One method has been described by Klinger, Kock and Blaschezyk[1] employing the evolution procedure with 100 mg samples. Considerable controversy has, however, appeared from time to time in the literature regarding the accuracy of the evolution method, which, according to the authors mentioned above, is subject to an error of $\pm 10\%$. Moreover, the use of 10 mg samples would undoubtedly increase the errors already found by various authors.

The most reliable and simple method for the conversion of trace quantities of sulphur into an easily workable form consists of oxidation to sulphur oxides, which is rapidly accomplished by combustion treatment.

The difficulty in determining sulphur dioxide lies, not in its absorption, but in its

subsequent estimation. The normal amounts of sulphur oxides produced by combustion of 0·5–1 g samples may be absorbed in iodine, silver nitrate or hydrogen peroxide soln. Of these, the last is most suitable in that sulphuric acid is obtained free from interfering products of reaction.

Of methods that can be used for determining this sulphuric acid, besides the titrimetric or gravimetric procedure, the turbidity method of Obermer and Milton[2] can be used for a sulphur content of 80 μg or more; this amount corresponds to a drum reading of 0·16 on the Spekker absorptiometer. For smaller amounts a more sensitive method is desirable, especially if the weight of sample taken is to be reduced to micro quantities, *e.g.*, 10 mg. To obtain this sensitivity, together with the necessary accuracy (error 5% or less), a colorimetric procedure is described below, suitable for the determination of sulphuric acid obtained from 50–10 mg of material containing 0·01% or more of sulphur.

Method

The method is based on interaction of the sulphuric acid with cupric oxide, and photometric determination of the copper sulphate formed, with the Spekker absorptiometer. Its application to organic combustion methods is restricted to materials free from elements, *e.g.*, nitrogen or halogens, that will produce other acids than sulphuric acid. With steels this complication does not occur. Carbon dioxide, being driven off during the preliminary treatment, has no harmful effect.

Determination of Copper—The copper is determined after reaction with sodium diethyldithiocarbamate,[3] which produces a colour sufficiently intense to enable 2 μg of copper, equivalent to 1 μg of sulphur, in a volume of 5 ml to be readily determined.

The complex formed is only sparingly soluble in water, but at low concns. is completely dissolved by alcohol. During the actual determination the amount of water present must not exceed 30%.

The following dilutions (in terms of weights of sulphur) have been found suitable by experience: 1–15 μg should be made up to 5 ml, 15–20 μg to 7 ml and 20–40 μg to 10 ml. In the actual determinations carried out the 7 ml dilution was chosen because a micro cell was not available at the time. The alternative was a 1·0 cm oblong cell which had a capacity of just under that amount.

Reagents—*Cupric oxide*—Wire form M.A.R., in 5 mm lengths, purified by boiling 10 g in 1% sulphuric acid soln. to remove dust and soluble salts and then leaching with conductivity water until the washings give no colour reaction with the reagent.

Hydrogen peroxide soln.—To 20 ml of freshly boiled conductivity water add 0·2 ml of *N*/100 alkali and 1 drop of methyl red indicator. Then add from a burette a solution prepared from 10 drops of M.A.R. hydrogen peroxide (100 vol.) and 50 ml of conductivity water, until the neutral point is reached.

Alcohol—Purified by boiling under reflux with, and distilling from, caustic soda. A colourless solution should be obtained on treating 7 ml with the reagent.

Sodium diethyldithiocarbamate reagent—1% soln. in alcohol.

Procedure—Combustion of the sample, whether steel or organic material, is carried out in the normal way. The sulphur oxides are absorbed in the absorber shown in Figure 1, which is constructed of quartz glass. For combustions of organic material it is sealed permanently to the quartz combustion tube, and for steels it is connected by rubber tubing, or better still, by fixing with a heat-resisting cement, to the porcelain combustion tube as used for steels.

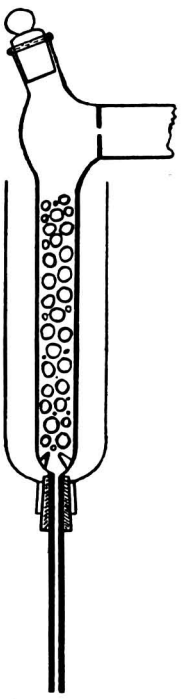

Fig. 1

The absorber is 7 cm. in length and 1 cm. in diam. and filled with quartz beads or chips. The exit terminates in a tip 6 cm in length which during the combustion dips into a platinum crucible 4 × 3 cm provided with a lip to facilitate removal of the contents. Washing out is effected by means of the stoppered inlet situated at the junction of the combustion tube and absorber. To prevent evaporation of the liquid absorbent by the rapid exit of gases from the hot combustion tube, the absorber is cooled by a water jacket.

Before starting a combustion run 1 ml of the neutral hydrogen peroxide solution through the absorber and allow the excess to collect in the platinum crucible. A similar quantity is required for the blank (see p. 426).

When the combustion is completed rinse the bead tube out by lowering the crucible and washing the beads with conductivity water *via* the stoppered inlet. Use 5–6 ml of water in all.

Evaporate the contents of the crucible, protected by an inverted funnel, down to 1 ml on a boiling water-bath. Add 0·5 g of the prepared copper oxide, and swirl the contents of the crucible to ensure intimate contact. Evaporate off the remaining liquid. Run a further ½ ml of water down the sides of the crucible so that the contents are intimately mixed. Evaporate to dryness again.

Dissolve the resulting copper sulphate in a few drops of water, and filter through a micro sintered funnel, washing out in succession with alcohol and 2 or 3 drops of water. Collect the filtrate in a 10-ml stoppered graduated cylinder contained in a side-armed test tube. Alternatively a filter stick may be employed for this transference.

Add 1 drop of *N* nitric acid, 1 ml of the reagent solution and further alcohol to make up to volume in the cylinder. Mix the contents thoroughly and, after 10 minutes to allow complete colour development, read off the resulting yellow colour on the "Spekker," using the appropriate cell and Chance No. 7 dark blue filter.

Ascertain the amount of copper present from the calibration curve and divide the result by 2 to obtain the amount of sulphur in μg.

EXPERIMENTAL—(1) *Calibration Curve*—A standard soln. of copper sulphate was prepared and dilutions were made to give different copper contents. One ml portions of these were treated as described above and made up to 7 or 10 ml (Table I).

TABLE I

Copper present	Spekker readings	
	Volume	Volume
μg	7 ml	10 ml
1	0·021	—
2·5	0·056	—
5	0·105	0·070
8	0·170	—
10	0·215	0·140
15	0·312	0·210
20	0·422	0·280
25	—	0·348
30	—	0·420
35	—	0·492
40	—	0·560

(2) *Determinations of sulphuric acid*—*N*/100 sulphuric acid was prepared, accurately standardised by titrimetric and gravimetric methods and stored in a wax-lined bottle. Ten ml of it were diluted to *N*/1000, of which different quantities were taken and submitted to the technique described above. The results given in Table II show that an accuracy of 100 ±2% was obtained.

TABLE II

Sulphur present	Final volume	Spekker reading	Sulphur found	Error
μg			μg	%
16·0	10 ml	0·444	15·9	−0·6
14·4	,,	0·402	14·5	+0·7
13·5	,,	0·375	13·4	−0·75
12·4	,,	0·340	12·2	−1·6
11·2	,,	0·305	11·0	−1·8
10·7	,,	0·300	10·7	nil
9·6	7 ml	0·405	9·6	nil
8·3	,,	0·355	8·4	+1·2
8·0	,,	0·342	8·0	nil
6·4	,,	0·275	6·5	+1·56
5·08	,,	0·218	5·1	+0·4
3·2	,,	0·140	3·25	+1·56
2·84	,,	0·125	2·9	+2·12
2·84	,,	0·120	2·8	−1·41
2·45	,,	0·100	2·4	−2·04
2·45	,,	0·102	2·4	−2·04
1·6	,,	0·070	1·6	nil
1·6	,,	0·068	1·6	nil

(3) *Sulphur determinations on steel samples*—Two samples of steel were submitted to combustion and gave the following results.

TABLE III

Steel sample No.	Taken mg	Sulphur found μg	Sulphur found %	Sulphur present %
E 6290	10·41	2·8	0·027	0·027
,,	11·05	2·9	0·026	,,
K 1627	11·93	5·8	0·048	0·049
,,	12·13	6·0	0·049	,,

Blank = 0·024 Spekker reading.

Samples of organic compounds containing sulphur were also submitted to combustion and the sulphuric acid obtained was diluted to 100 ml. Portions of this solution were taken, and results obtained which agreed well within the experimental error allowed for normal procedure.

(4) *Determination of blank*—Add 1 ml of the prepared hydrogen peroxide solution to the crucible containing the copper oxide and proceed as in the sulphuric acid determination.

ERROR OF THE METHOD—Errors affecting the accuracy of the method are few in respect of the actual estimation of sulphuric acid. As the method involves the handling of extremely minute quantities the utmost caution is necessary for complete success.

The chief sources of error likely to produce erratic results are impurities from the wash liquors used, and from the atmosphere. Both alcohol and conductivity water must be kept in well-stoppered quartz containers. Prior to the preparation of the absorbent the water must be boiled in a quartz flask. For the transference of these liquids a quartz dropping pipette is useful.

To illustrate the effect of ordinary glass apparatus, it may be mentioned that in the preliminary experiments low results were obtained with $N/1000$ acid that was stored in ordinary flasks for a few hours. In about 24 hours the normality, as deduced from the results found, had been reduced by half. This was due to alkali from the glass vessel. Also, considerable loss occurred when pyrex dishes were employed for the evaporation and reaction of the sulphuric acid solution. Extraordinarily high blanks were obtained when distilled water improperly purified was used.

Attempts were made to remove volatile acids, *e.g.*, nitric or halogen acids produced when organic compounds containing nitrogen or halogen were submitted to combustion. The same technique was employed as described in an earlier paper.[4] However, in this instance, low and negative results were obtained owing to loss of sulphuric acid during evaporation of the volatile acids.

SUMMARY—The method described for the determination of trace amounts of sulphur dioxide was developed to make possible the satisfactory estimation of sulphur in ferro-alloys and organic compounds containing trace amounts, using the minimum of material, *e.g.*, 10–50 mg. This method can be applied to the determination of sulphur dioxide in amounts above 2 μg.

Absorption is effected with neutralised hydrogen peroxide solution, the resulting sulphuric acid is allowed to react with copper oxide, and a colorimetric procedure is used to determine the amount of soluble copper salt formed. The amount of copper found, divided by 2, equals the sulphur content of the sample.

Results given show that an accuracy of 100 ±2% can be attained.

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DISCUSSION

Mr. N. STRAFFORD asked the author (*a*) if it could be assumed that the presence of carbon in the sample was without influence on the results, (*b*) if the presence of nitrogen caused interference, and (*c*) if it was easy to obtain a representative 10 mg sample of steel.

Mr. INGRAM replied that—(*a*) Carbon dioxide retained by the absorbent solution was removed from the latter during the preliminary heating on the water-bath before the addition of the copper oxide. (*b*) Nitrogen caused no interference except in the combustion of organic compounds; attempts to remove the acid then formed, by preliminary evaporation of the absorbent solution, had failed, so far. In the combustion of steels the nitrogen present, being in such small amounts and in absence of an active catalyst, was swept out of the combustion tube in the uncombined state. (*c*) The method was intended as a micro-estimation, when only limited weights were available, as, for example, of top surface layers of segregations. No doubt in ordinary circumstances it would be difficult to obtain a representative 10 mg sample from a large bulk of material, but then it would not be necessary to use the micro method.

Notes

THE RAPID EXTRACTION OF FAT FROM POWDERS

REFERRING to the note of Manley and Wood upon "The Rapid Determination of Fat in Powders" (ANALYST, 1945, **70**, 173), I also can testify to the usefulness of the modified Bolton and Revis apparatus, for it has been repeatedly employed in this Laboratory since 1924 in the following manner for the determination of fat in chocolate for manufacturing control and in other fatty powders.

A 9 cm Greens No. 804 (fat-extracted) filter paper is flute-folded and weighed on a watch glass. About 1 g of the shredded or molten chocolate is placed in the apex of the paper and weighed. For rapid control tests it is not necessary to oven-dry the paper, but obviously this precaution may be taken if a stoppered weighing bottle is employed. The paper is inserted in an extraction chamber (made by A. M. E. Sherwood, Ltd., 25, Dingwall Road, Croydon), measuring about 20 cm × 5 cm top diameter, ground for insertion of reflux condenser at the top and with standard ground joint for fat flask at the bottom, where the paper rests on three inward projecting points made just above the narrowing lower part of the chamber.

Light petroleum spirit (b.p. 40–60° C.) is used as solvent and an extraction time of 1 hour is given. The solvent is boiled in an air-bath, heated by a resistance element, for it has been found that the solvent action of steam arising from a boiling water-bath causes a small but progressive loss in the weight of the extraction flask.

The continuous drip of solvent on to filter papers that have absorbed fat or contain fatty materials, placed in the glass thimble of the Bolton and Revis apparatus with ground glass joints has also proved its worth. The packing of this glass thimble with wool and paper as directed by Bolton and Revis has only been employed when it has been desired to extract fat from a substance upon which other determinations were to be made. Even in this instance, the quantity of fat obtained is limited by the capacity of the glass thimble. When quantities of about 50 g of cake or biscuit crumb have to be extracted for gaining the fat in bulk, a simple extractor made by a 500 ml tall-form beaker, round bottom flask, filter paper and support have sufficed. An 18 cm Greens fat-free paper is formed into a cup upon a former, made of a piece of $1\frac{7}{8}$ in. diameter steel shafting with champfered edges, and with a slight depression at the axis to help centring whilst the paper is being flute-folded around it. A wire loop or cotton thread ties the paper folds in position. The material is placed in the cup, which is then stood upon a glass rod support in the beaker. The support is tilted by having larger feet at one end, so that the solvent drains away more freely at the bottom edge of the cup. Solvent is placed in the beaker so that the cup is just above its top level and the percolation is effected by condensation from the surface of a round bottomed flask rigged as a condenser. It is necessary to see that the flask rests on the rim of the beaker in a close fitting manner. The fat extract may sometimes be cloudy, but it can be clarified by filtration or decantation prior to recovery of the solvent.

THE LABORATORY, MACFARLANE, LANG & CO., LTD. D. M. FREELAND
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June 20, 1945

THE TANNIN CONTENT OF SOME POTAMOGETON SPECIES AND ITS EFFECT ON THE COLOUR OF IRON-CONTAINING INLAND WATERS*

AT the junction of the Rio Paragua and the Rio Caroni, a tributory of the Orinoco, are several lakes the waters of which are of a deep black colour. These lakes are known to contain iron,[1] and for centuries their waters have been medically valued by the Caroni Indians.

When I visited the lakes in July, 1937, with Dr. F. N. Azara, they were thickly covered with aquatic vegetation. Dr. Azara kindly identified the potamogeton species according to the Bennet system, but on my return home I used the simplifications proposed by Dandy[2] and accepted by Andrews.[3] According to Azara's notes the species found are the same as in Africa, namely, *P. nodosus* Poir, *P. Schweinfurthii* Benn., *P. crispus* L., *P. octandrus* Boir, *P. panormitanus* Biv., *P. pectinatus* L.; also *P. natans* L., generally associated with the European flora. Estimations of tannin by the micro-method recently described,[4] using potamogeton tannin as a standard, as recommended by Mitchell,[5] gave an average of 0·87% irrespective of species or age of the material analysed.

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* In 1923 Dr. C. A. Mitchell sent me a specimen of *Potamogeton natans* L., in which I detected tannin. This was, I believe, the first time that that tannin was proved to be present in potamogeton.

THE VOLUMETRIC DETERMINATION OF TIN AND ANTIMONY IN BRASSES AND BRONZES AFTER THEIR SEPARATION BY DISTILLATION

RECENTLY Harvey[1] has published a method for the determination of tin in ores, based on Scherrer's work[2] on the volatility of arsenic, antimony, and tin with hydrochloric and hydrobromic acids. Harvey, and also Law,[3] dealt with comparatively small amounts of tin, but the method seemed feasible also for the larger amounts to be dealt with in metallurgical analysis. Scherrer himself applied the distillation for this purpose, but only after an initial separation by the traditional methods. In the following, a method

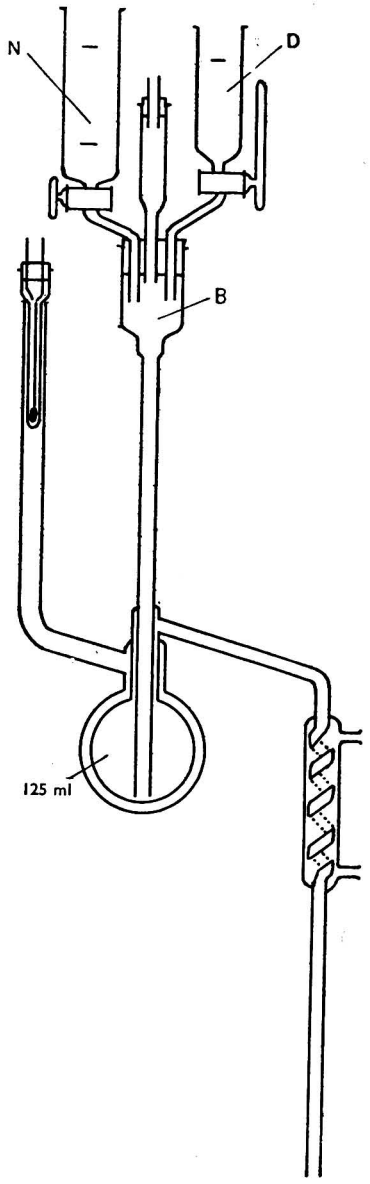

Fig. 1

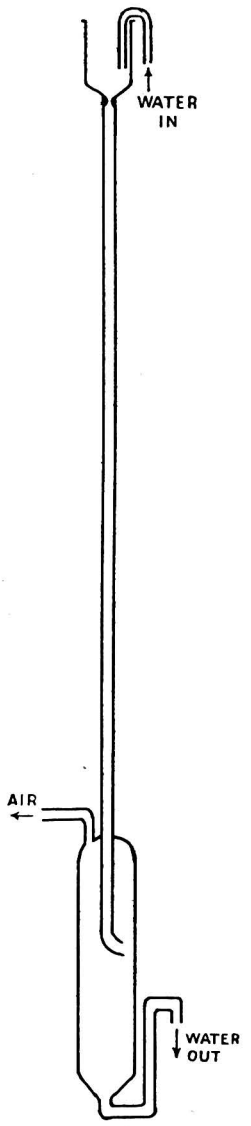

Fig. 2

similar to one described by Biltz,[4] based on the direct distillation of tin and antimony from the solution of the alloy is described, and an apparatus which reduces the necessary attention to a minimum. The time required for the determination of tin or antimony is approx. 3 hours. The samples were dissolved in hydrochloric acid and hydrogen peroxide (see for example Brückner[5]), introduced into the apparatus and distilled at 160° in a bath of boiling cyclohexanol. For the determination of tin the distillate was reduced by nickel and titrated with a solution of potassium iodate. Antimony was determined by titration with potassium bromate after reduction of the distillate by sodium sulphite.

Harvey's apparatus was modified as shown in Fig. 1 to replace the oilbath by a bath of cyclohexanol. No thermometer is required, as the boiling of the cyclohexanol (b.p. 160° C.) automatically controls the

temperature of the distillation. A length of glass tubing 12 mm in diam., and 200 mm long as a reflux condenser is sufficient to retain the cyclohexanol. A mercury temperature controller is introduced with its bulb 75 mm inside the condenser and connected with a bell and a lamp to give warning when the cyclohexanol is boiling. A short spiral condenser (3 turns) was provided to reduce the height of the apparatus and the cooling water was used in the apparatus shown in Fig. 2 to provide the air current required for the distillation. Funnel D was marked at 25 ml, and a further funnel holding 60 ml was provided for the diluent, bearing marks at 15 and 45 ml. The stem of funnel B was slightly widened on top to take a small stopper bearing a tube connected with a Bunsen pump to allow the emptying and cleaning of the apparatus without dismantling. The heating may be effected by a gas burner, or by an electric heater of 160 watts (8 ft. Nickel-Chromium Wire 42 Gauge at 240 volt.).

Procedure—Dissolve 2·5 g of the sample in the form of fine borings or sawings (free from oil) in a tall 400 ml conical beaker in 20–30 ml of conc. hydrochloric acid by careful addition of 10–15 ml of hydrogen peroxide (100 vol.) in two to three portions of 5 ml. Transfer the solution into the apparatus, rinse the beaker with 10 ml of water to which 10 ml of sulphuric acid are added before pouring it into the apparatus. Rinse the beaker down once more with 10 ml water which are used to wash down the funnel B. The beaker, in which 30 ml water are placed, is used as the receiving vessel. Place the stopper bearing the two funnels in position after filling the stem of funnel D with the acid mixture. Start the heating and turn on the cooling water, passing a flow of air (about 3–5 bubbles per second) through the apparatus; the air enters through the third tube in the stopper carrying funnels D and N. Fill funnel D with 25 ml of hydrobromic-hydrochloric acid mixture (1 HBr sp.gr. 1·48; 3 HCl sp.gr. 1·18) and funnel N with 15 ml of acid mixture and 30 ml of water. The temperature should reach the boiling point of the cyclohexanol in about 45 minutes, and when the boiling cyclohexanol sets the alarm ringing run in the acid from funnel D at a rate of 12 to 15 drops per minute, taking care that sufficient acid remains to keep the stem of the funnel filled. By the addition of the acid the temperature is lowered sufficiently to silence the alarm in a few minutes. When all the acid is run in the temperature rises again until the alarm sounds. Add the diluent from funnel N in a slow stream (taking about ½ minute) and continue the distillation until the alarm sounds. Add a further 25 ml of acid mixture at 12 to 15 drops per minute, and when the alarm sounds again discontinue heating, remove the beaker, and reduce the distillate by boiling with a nickel spiral and titrate with a solution containing 3 g of potassium iodate, 20 g of potassium iodide, and 2 g of sodium hydroxide per litre (1 ml ≡ about 5 mg of Sn), which has been standardised against an alloy of known tin content or a sample of pure tin taken through the described process. Fill the apparatus with water, which is withdrawn by inserting into the bottom of Funnel B a stopper carrying a tube connected to a Bunsen pump. Repeat this rinsing twice more. The distillation requires about two hours and the entire determination about three hours, but hardly any attention is required during the distillation so that a number of tests may be run simultaneously by one operator.

The determination of antimony was first tried by distillation with hydrochloric acid only, but the results were erratic. The same routine as for the distillation of tin was then tried, using hydrobromic-hydrochloric acid mixture, and was found to be satisfactory if the distillation was carried out carefully, reducing the flow of air to a minimum when the acid mixture is dropped in, as the antimony compounds are more volatile than the corresponding tin compounds. The yellow colour of the bromides makes it difficult to observe the end-point of the titration. The bromides are therefore replaced by chlorides by addition of sufficient potassium chlorate (about 5 g) to the distillate and boiling to expel the liberated bromine before the reduction with 2 g of sodium sulphite in the usual manner and titration with $N/10$ potassium bromate using methyl orange as indicator. After the antimony titration the distillate may be reduced by nickel and titrated with potassium iodate to determine the tin.

In Table I the results of a number of determinations carried out by the described method are shown. Tests 1 to 4 were carried out on pure tin, tests 7 to 13 on tin-copper mixtures and tests 15 to 21 on actual alloys. Test 24 shows that the described procedure is able to deal with amounts of tin equal to 20 per cent. in weighings of 2·5 g. Tests 31 to 39 were carried out with pure antimony and with mixtures of antimony, tin and copper.

TABLE I

No.	Weighed in	Found
1	0·0432 g Sn	0·0423 g Sn
4	0·1145 g Sn	0·1149 g Sn
7	0·0490 g Sn + 2·450 g Cu	0·0494 g Sn
12	0·1900 g Sn + 2·300 g Cu	0·1920 g Sn
13	0·0440 g Sn + 1·450 g Cu + 1·000 g Zn	0·0440 g Sn
15	2·500 g gunmetal (9·62% Sn)	9·66% Sn
20	2·500 g phosphorbronze (9·90% Sn)	9·96% Sn
17	2·500 g brass (1·33% Sn)	1·26% Sn
21	2·500 g bronze (7·40% Sn)	7·43% Sn
24	0·4980 g Sn + 2·000 g Cu	0·4990 g Sn
31	0·1000 g Sb	0·0994 g Sb
34	0·0500 g Sb	0·0491 g Sb
38	0·0500 g Sb + 0·2060 g Sn + 2·250 g Cu	0·0486 g Sb, 0·2070 g Sn
39	0·1000 g Sb + 0·1980 g Sn + 2·200 g Cu	0·0980 g Sb, 0·1960 g Sn

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ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

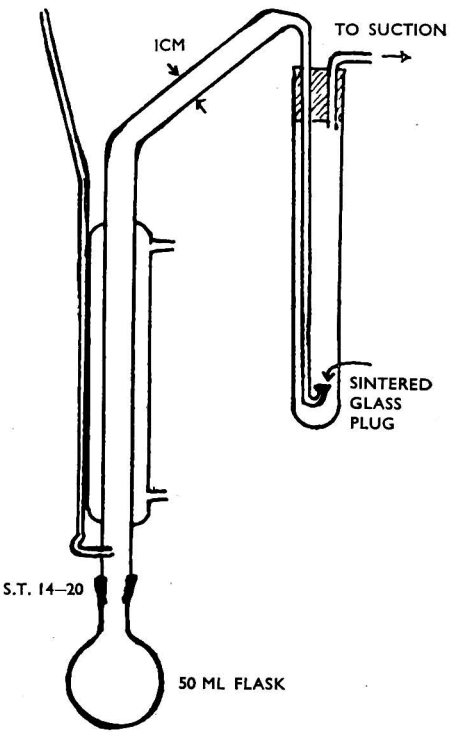
Chemical Determination of Penicillin. J. J. Murtaugh and G. B. Levy (*J. Amer. Chem. Soc.*, 1945, **67**, 1042)—The method is based on detmn. of the acid formed when penicillin is inactivated by penicillinase. Adjust separately to *p*H 8·0 an aliquot of penicillin of 5000–20,000 units, in about 10 ml, and 1–2 ml of enzyme soln. containing 1000–2000 penicillinase units (McQuarrie, Liebmann, Kluener and Venosa, *Arch. Biochem.*, 1944, **5**, 307). Use one set of electrodes, with a *p*H meter, to adjust the penicillin soln., and a second set to adjust the enzyme soln. Add the enzyme soln. to the penicillin soln. and maintain the mixture at about *p*H 6·8 by gradually adding 0·02 *N* sodium hydroxide, using the second set of electrodes. After a few mins. the penicillin is inactivated and the *p*H becomes constant. Complete the titration by rapid adjustment to *p*H 8·0. With pure crystalline penicillin (G) recoveries were over 98%, and apparently good accuracy is also obtained with less pure commercial prepns. With salts of four manufacturers, using different processes, the results corresponded with those of the turbidimetric microbiological assay. In precision tests replicate analyses showed a dispersion in results of the order of 1%. The error is significantly less than that of present microbiological assay methods. The method is applicable to all aqueous penicillin solns. of potencies over 200–500 units/ml which do not contain excessive amounts of buffer.

E. M. P.

Biochemical

Colorimetric Method for the Micro-estimation of α-Alanine in Blood. B. Alexander and A. M. Seligman (*J. Biol. Chem.*, 1945, **159**, 9–19)—Ninhydrin reacts with amino acids to give carbon dioxide and the corresponding aldehydes, and the reaction has been used for the estimation of total amino acids by measuring the carbon dioxide evolved. A method of estimating α-alanine has now been devised, in which the amount of acetaldehyde formed in this reaction is measured. The acetaldehyde is separated from the reaction mixture by aeration and the colour produced by reaction with *p*-hydroxydiphenyl is measured. α-Alanine was recovered quantitatively from blood and from hydrolysates of polypeptides.

Prepare a protein-free blood filtrate by adding 1·0 ml of blood to 7·0 ml of water, and then 1·0 ml of 10% sodium tungstate soln. and 1·0 ml of 0·66 *N* sulphuric acid, and centrifuge. Put 5·0 ml of the centrifugate into the flask of the specially constructed aeration still shown in the figure, and add 1·0 ml of 1% ninhydrin soln., 2 ml of phosphate buffer *p*H 5·5 (3·5 g of K_3PO_4 added to 100 ml of 20% KH_2PO_4 soln.) and a glass bead. Connect the flask to the aeration still and put 8·0 ml of 1% sodium bisulphite soln. into the receiver, which is immersed in an ice-bath. Apply gentle suction to the apparatus and gently boil the soln. in the flask for 75 min. with a steady stream of air passing through the apparatus. Remove the receiver and transfer the bisulphite soln. quantitatively to a graduated centrifuge tube; make up the soln. and washings to a vol. of 10 ml. To 1 ml add one drop of 4% copper sulphate soln., followed by 6·0 ml of conc. sulphuric acid from a burette. Cool the test tube to 37° C. and add two drops (0·1 ml) of a 1·5% soln. of *p*-hydroxydiphenyl in 0·5% sodium



hydroxide soln. Shake the mixture vigorously and put the test-tube in an incubator at 37·5° C. for 30 min. and then in a boiling water-bath for 1·5 min. Cool to room temperature and compare the violet colour formed with that given by a standard α-alanine soln. in a Klett photoelectric colorimeter with filter 540. Alternatively, prepare a calibration curve using pure α-alanine solns. The relationship between concn. and colour is linear between 20 and 100 μg. Accurate results cannot be obtained with amounts less than 20 μg of α-alanine in 5 ml of blood filtrate, but by making a more concentrated blood filtrate, by using a larger vol. or by aerating the acetaldehyde into a smaller vol. of bisulphite soln. or redistilling the

bisulphite-acetaldehyde soln., as little as 0·5 mg per 100 ml of blood can be estimated.

Previous statements that α-alanine reacts incompletely with ninhydrin are due to the slowness of the reaction, and theoretical yields are only obtained if the reaction mixture is boiled for at least one hour. Although aspartic acid can yield acetaldehyde on reaction with ninhydrin, the yield is greatly influenced by the *p*H and, when the reaction is carried out at *p*H 5·5–5·6, interference from this source is negligible. Interference due to aldehydes formed from other amino acids is largely avoided by the aeration process and, of the volatile aldehydes that accompany acetaldehyde, only the following react with *p*-hydroxydiphenyl: formaldehyde (from glycine), *iso*valeraldehyde (from leucine), *n*-valeraldehyde (from norleucine) and *n*-butyraldehyde (from norvaline). The last is the only one to give a violet colour. The intensity of the colour developed during the reaction of *p*-hydroxydiphenyl with leucine was found to vary with the temperature of the sulphuric acid mixture when the reagent was added, the colour decreasing with rise of temperature. The colour produced from α-alanine, on the other hand, did not vary with temperature, so that interference from leucine was considerably reduced by adding the reagent at a higher temperature. Under these conditions, 22 parts of leucine gave the same colour intensity as 1 part of α-alanine. Interference from norleucine and norvaline proved to be more difficult to circumvent, but the effect of both was appreciably reduced by raising the temperature to 37° C. Since these amino acids rarely occur in proteins, their effect on the estimation of alanine is not regarded as serious. F. A. R.

Specific Micro-method for the Estimation of Acyl Phosphates. F. Lipmann and L. C. Tuttle (*J. Biol. Chem.*, 1945, **159**, 21–28)—Anhydrides of carboxylic and phosphoric acids appear to be intermediates in carbohydrate metabolism, and a method of estimating one of these, acetyl phosphate, has been described previously (Lipmann and Tuttle, *J. Biol. Chem.*, 1944, **153**, 571). This was based on the differential precipitation of calcium acetyl and inorganic phosphate, with subsequent estimation of phosphate in the separate fractions. A more specific method is now described, in which the reaction of acyl phosphates with hydroxylamine is utilised, the acyl part of the anhydride being converted into hydroxamic acid:

$$R.COOPO_3'' + H_2NOH \rightarrow R.CONHOH + HOPO_3''$$

The hydroxamic acid forms, with ferric iron, a bright purplish complex which can readily be estimated. Since acyl phosphates are unstable even in moderately acid or alkaline solns., deproteinisation is best carried out after the formation of the hydroxamic acids, which are very stable compounds.

Add 0·5–1 ml of the test soln. (*p*H 5·5–7·5) to a mixture of 1 ml of hydroxylamine soln. (nearly neutralise a 28% soln. of hydroxylamine hydrochloride (4 *M*) by addition of an equal vol. of 14% sodium hydroxide (3·5 *M*); the mixture has a *p*H of 6·4 and as it is not stable it is best prepared daily from stock solns.) and 1 ml of acetate buffer, *p*H 5·4 (a 1 : 4 mixture of acetic acid and sodium acetate soln., both 0·1 *M*). Adjust the vol. to 3 ml and leave for 10 mins. at room temperature. Add, in the following order, 1 ml each of diluted hydrochloric acid (1+3), 12% trichloroacetic acid and a 5% soln. of $FeCl_3.6H_2O$ in 0·1 *N* hydrochloric acid, remove the protein precipitate by centrifuging and measure the colour in either a Klett-Summerson colorimeter or an Evelyn colorimeter, using filters 54 and 540 respectively. If an Evelyn colorimeter, is used, the vols. should be doubled. Readings should be made within 5–30 min., after which the colour fades slowly. The reaction is specific for carboxylic acid anhydrides but, in conc. soln. and on heating, acid amides and, to a lesser degree, esters will react with hydroxylamine. The colour is much less intense, however, than that formed with acyl phosphates or acid anhydrides. A satisfactory standard soln. can be prepared with succinic anhydride in place of acetyl phosphate, which is not readily available. For this purpose dissolve 1·0 g of succinic anhydride in 40 ml of the 2 *M* hydroxylamine soln., leave for 10 min. and dilute to 100 ml. One ml of this soln. diluted 40-fold gives a colour equivalent to 2·0 micromoles of acetyl phosphate. F. A. R.

Colorimetric Estimation of Arginine in Protein Hydrolysates and Human Urine. A. A. Albanese and J. E. Frankston (*J. Biol. Chem.*, 1945, **159**, 185–194)—The estimation of arginine by the method of Sakaguchi (*J. Biochem. Japan*, 1925, **5**, 133) and its modifications suffer from certain difficulties which the present workers attribute to a fault in the concn. of sodium hypochlorite originally recommended by Sakaguchi. The use of 0·06 *N* sodium hypochlorite in conjunction with urea has been found to give a more stable colour than that obtained with either 0·3 *N* or 0·06 *N* sodium hypobromite. The use of this concn. of hypochlorite also permits the test to be carried out at room temperature (20–25° C.) without detectable loss of colour, stability or intensity, it obviates the need for adjusting the quantity of the reagent to the nitrogen content of the sample and it gives relatively low reagent blanks. When the procedure was applied to urine, interference due to glycocyamine and methylguanidine was encountered. Separation of arginine from the sample by means of Permutit was unsuccessful as it was impossible to elute the arginine quantitatively, but satisfactory results were obtained by subtracting the colour obtained with an aliquot treated with Permutit from that obtained without treatment. This overcame all difficulties except that due to the presence of methylguanidine.

Prepare hydrolysates by heating 5-g samples of the protein with 25 ml of 6 *N* hydrochloric acid under reflux for 24 hr., estimate the nitrogen content of the hydrolysate by the micro-Kjeldahl method and remove excess of acid by concn. under reduced pressure; filter to remove humin. Alternatively, heat 5-g quantities of protein with 25 ml of 6 *N* sulphuric acid under reflux for 24 hr. and remove the excess acid as calcium sulphate. Put aliquots of the hydrolysates (not more than 5 ml and containing 100–400 μg of arginine) into 10-ml graduated Klett-Summerson photoelectric colorimeter tubes. Dilute to 5 ml if necessary and add 1 ml of 10% sodium hydroxide soln. and 1 ml of a soln. of α-naphthol (100 mg in 100 ml of 95% ethanol). After 5 min. add 1 ml of 0·06 *N* sodium hypochlorite soln. (checked by iodimetric titration) followed exactly 1 min. later by 2 ml of 20% urea soln. Evaluate the colour, which is stable for 15 min., in a Klett-Summerson colorimeter using filter S 54. Calculate the results from the value obtained with a standard soln. of *l*(+)-arginine hydrochloride (12·05 mg in 100 ml of water). To estimate arginine in urine, collect a 24-hr. sample

in brown bottles containing 50 ml of 15% (*v/v*) hydrochloric acid and 1 ml of 10% alcoholic thymol soln. and dilute to 2 litres. Run 20–25 ml of the urine (*p*H 5–6) at the rate of 1 drop per sec. through a column of 10 g of activated Permutit in a 150× 15 mm tube. Put 5 ml of the filtrate and 5 ml of the original urine sample into colorimeter tubes and treat as described above. The difference between these readings (corrected for the reading obtained with a reagent blank) is proportional to the arginine content. The recovery of arginine added to protein hydrolysates and urine ranged from 98 to 101% of the theoretical. F. A. R.

Separation of Purine Nucleosides from Free Purines and Estimation of the Purines and Ribose in these Fractions. S. E. Kerr and K. Seraidarian (*J. Biol. Chem.*, 1945, **159**, 211–225)—The free purines, adenine, guanine and hypoxanthine, are quantitatively precipitated by silver nitrate in presence of sodium trichloroacetate and 0·02–0·05 *N* sulphuric acid, whereas in dilute solns. the nucleosides, adenosine, guanosine and inosine, remain in soln. All these purines and nucleosides are quantitatively precipitated by silver nitrate when the soln. is made slightly alkaline with sodium hydroxide. A method of estimating nucleosides and free purines in trichloroacetic acid extracts of tissues based, on these observations, is described.

Make the neutralised, protein-free filtrate slightly acid with 10% acetic acid and add the minimum amount of 8% uranyl acetate soln. to give a slight excess in the supernatant liquid. Centrifuge to remove precipitated nucleotides and, if necessary, filter the supernatant liquid and washings. Measure the vol. of the filtrate, acidify with sulphuric acid until the soln. is 0·05 *N* and then add 0·02 vol. of *M* silver nitrate to precipitate the free purines. Centrifuge within an hour, wash the precipitate and again centrifuge. Wash the precipitate a second time to remove a product which interferes with the subsequent determination of ribose and discard the wash liquor. The precipitate is the "acid silver precipitate." To the combined supernatant liquid and first washings add *N* sodium hydroxide until the mixture is alkaline to phenol red, centrifuge and wash the "alkaline silver precipitate" in the same way as the "acid precipitate." Heat each silver precipitate on a boiling water-bath for 30 mins. with 15 ml of 0·5 *N* hydrochloric acid, filter hot through asbestos (not paper) and collect the filtrate in a centrifuge tube with a conical tip graduated at 35 ml. Dilute to 35 ml with 0·5 *N* hydrochloric acid and estimate the ribose in each soln. by the method described below. The estimation is carried out before removal of the uranium as much of the ribose is otherwise lost in the uranium precipitate formed on neutralisation; uranium does not interfere unless present in excessive quantities. For the ribose estimation take aliquots containing 0·02–0·05 mg of ribose corresponding to 1 ml of the "acid silver extract" and 0·2 ml of the "alkaline silver extract." After completion of the ribose estimation but before precipitation of the purines, precipitate the uranium from the remainder of the extract by neutralising to phenolphthalein with 20% sodium hydroxide soln. and then discharging the indicator colour with 5% acetic acid. Centrifuge and transfer the supernatant liquid to a 50-ml centrifuge tube with a conical tip. Dissolve the precipitate in a few ml of *N* sulphuric acid and re-precipitate. Heat the combined supernatant liquids in a boiling water-bath and precipitate the purines by addition of copper sulphate and 40% sodium bisulphite soln.; determine the content of individual purines by published methods.

The estimation of *d*-ribose is carried out as follows: Put the soln., containing 0·02–0·05 mg of ribose, into a test tube graduated at 15 ml, add 0·3 ml of *N* hydrochloric acid and dilute to 5 ml. Into a similar tube, put 3 ml of a standard soln. of ribose in 0·1 *N* hydrochloric acid (1 mg per 100 ml) and dilute to 5 ml. Add to both tubes 5·0 ml of conc. hydrochloric acid containing 0·02% of $FeCl_3$ and 0·3 ml of a 10% alcoholic orcinol soln. Immerse the tubes in a boiling water-bath for 20 min., cool, dilute to 15 ml and compare the colours in a colorimeter, using a Wratten filter E 22. Arabinose gives the same colour intensity as ribose, and may be used as a standard. Recoveries of ribose added to pure solns. and to solns. of adenosine and adenylic acid ranged from 96 to 102%. Recoveries of adenine and adenosine added to trichloroacetic acid extracts of brain ranged from 86 to 96% of the theoretical. F. A. R.

Microbiological Estimation of Nicotinic Acid, Nicotinamide and Nicotinuric Acid. B. C. Johnson (*J. Biol. Chem.*, 1945, **159**, 227–230)—*Leuconostoc mesenteroides* 9135 responds to nicotinic acid, but not to nicotinamide at low concns., nor to nicotinuric acid. In conjunction with *Lactobacillus arabinosus*, which responds to all three, it can be used to determine the amount of each compound in a mixture. Cultures of *L. mesenteroides* are carried on the following medium: yeast extract 1%, Difco peptone 1%, glucose 1% and agar 1·5%; they are sub-cultured every two weeks. Prepare the inoculum by transfer from the stock-culture to 10 ml of the diluted basal medium containing 1 μg of nicotinic acid and, after 24 hr. incubation, centrifuge and re-suspend in 10 ml of sterile saline. Use one drop of the suspension per tube for inoculation. The basal medium has the following composition: acid-hydrolysed vitamin-free casein, 10 g; glucose, 60 g; sodium acetate, anhydrous, 40 g; cystine, 20 mg; *l*-tryptophan, 10 mg; salt soln. A,* 10 ml; salt soln. B,* 10 ml; riboflavin, 500 μg; aneurine, 500 μg; calcium pantothenate, 500 μg; biotin, 10 μg; pyridoxal, 100 μg; adenine, 20 mg; guanine, 20 mg; xanthine, 20 mg; asparagine, 50 mg; *p*-aminobenzoic acid, 100 μg; distilled water to 1 litre. This medium is double-strength, 5 ml being used per tube. It gives a maximum titration of 12–15 ml of 0·1 *N* sodium hydroxide per tube. As *L. mesenteroides* utilises *pseudo*-pyridoxine rather than pyridoxine it is desirable to use either pyridoxine treated with hydrogen peroxide (Carpenter and Strong, *Arch. Biochem.*, 1944, **3**, 375) or pyridoxal itself. After inoculation, incubate the tubes at 30° C. for 72 hr. and titrate the acid formed with 0·1 *N* sodium hydroxide. In some instances a considerable amount of carbon dioxide may be formed and interfere with the end-point of the titration. This can be eliminated by autoclaving the tubes and cooling before titration. The organism only responds to nicotinamide in relatively high concns., *e.g.*, 1 mg per tube produces acid equivalent to 8 ml of 0·1 *N* sodium hydroxide. To determine nicotinamide, autoclave for 1 hr. at 15 lb. pressure with 0·6 *N* sulphuric acid, neutralise with saturated baryta soln., dilute to 0·2 μg per ml, filter and assay as before. The difference between this and the

* *Cf.* ANALYST, 1945, **70**, 139, footnote.

previous assay represents the amount of nicotinamide that has been converted into free nicotinic acid. To determine nicotinuric acid, assay with *L. arabinosus* by the method of Krehl, Strong and Elvehjem (*Ind. Eng. Chem. Anal. Ed.*, 1943, **15**, 471) and from the result subtract the value obtained with *L. mesenteroides* after hydrolysis with 0·6 *N* sulphuric acid. F. A. R.

Growth Stimulants in the *Lactobacillus arabinosus* Biotin Assay. V. R. Williams (*J. Biol. Chem.*, 1945, **159**, 237–238)—It has been previously observed that in the microbiological assay of biotin, pantothenic acid and riboflavin, certain lipoids act as growth stimulants for *Lactobacillus helveticus*. It has now been found that the assay of biotin with *L. arabinosus* is also sensitive to lipoids present in rice polishings and, unless these are removed, high results are obtained. F. A. R.

Determination of Cobalt in Biological Material. G. H. Ellis and J. F. Thompson (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 254–257)—The method, which is suitable for amounts of cobalt between 0·02 to 25 μg, depends on the formation of the coloured complex of cobalt with *o*-nitrosocresol. The complex is extracted from the aqueous soln. with ligroin (b.p. 70–90° C.) and measured in a photoelectric colorimeter fitted with 10-cm absorption cells of 1·8 ml capacity or in a spectrophotometer with a 5-cm cell of 2·8 ml capacity. Unless large quantities of cobalt are present, the ligroin appears colourless, as the maximum absorption occurs in the near ultraviolet. Samples for analysis are either wet- or dry-ashed. They are treated with hydrofluoric and perchloric acids and evaporated to dryness to remove silica, if necessary. The residue is dissolved in hydrochloric acid and iron is removed by extraction with dithizone or diethyldithiocarbamate dissolved in carbon tetrachloride. Sodium nitrosocresol soln. is added to the residue after removal of the carbon tetrachloride by distillation, the cobalt complex is extracted with ligroin and the absorption is measured. Special solutions, requiring purification either by extraction with carbon tetrachloride or distillation, are needed, and for these the original paper should be consulted. C. F. P.

Microchemical

The Oxidimetric Determination of Ammonia. I. The Determination of Small Amounts of Ammonia by Titration with Permanganate. W. Hurka and I. Rudžić (*Mikrochem. ver. Mikrochim. Acta*, 1943, **31**, 9–14)—Ammonia is pptd. as insol. cobaltinitrite, along with a small known amount of added potassium, which acts as a "carrier." The ppt. is dissolved and titrated with potassium permanganate soln., either directly or after addition of oxalic acid soln. Ammonia plus added potassium is thus determined, and the former obtained by difference. Amounts of ammonia as small as 3 μg may be determined by this method. Double-distilled ammonia-free water is used for all solns. *Cobaltinitrite Reagent*—Dissolve 120 g of sodium nitrite in 180 ml of water. Cool 210 ml of the soln. in ice and add an ice-cold soln. of 25 g of cobalt nitrate and 12·5 g of glacial acetic acid in 50 ml of water. Keeping the soln. ice-cold, draw through it a current of air until free from nitrous fumes. The reagent may be kept in the ice-chest for a few days only. *Procedure*—Mix 1 ml of 0·005 *N* potassium chloride and 1 ml of the test soln. To 1 ml of the mixed soln. add 6 ml of water and 1 ml of silver nitrate soln. Leave for 1 hr. and centrifuge. Cool 4 ml of the centrifugate in ice and add 1 ml of 96% ethanol and 2 ml of cobaltinitrite reagent. Leave for 3 hr. (or overnight) in the ice-chest and then centrifuge and suck off the supernatant soln. with a capillary hooked at the lower end. Wash the ppt. twice with ice-cold 50% ethanol and remove all traces of the latter by drying at a max. temp. of 40° C. Alternatively, use a little ice-cold water for the second washing, when the ppt. may be left damp. To the ppt. add 6 ml of 0·01 *N* potassium permanganate and 2 ml of approx. 2 *N* sulphuric acid. Disperse the ppt. in the soln., warm in water at 50° C. and stir continuously until the ppt. dissolves. (Evolution of gas must not occur.) Add 4 ml of 0·01 *N* oxalic acid and titrate at 70° C. with 0·01 *N* permanganate to the first pink colour lasting for 30 sec. with continuous stirring. At the end-point, the soln. is somewhat turbid. Alternatively, direct titration may be used, but extreme care is required. Disperse the ppt. in 1 ml of 0·01 *N* permanganate and 2 ml of approx. 2 *N* sulphuric acid. Immerse in water at exactly 50° C. and stir continuously; evolution of gas must not occur. As the colour fades, titrate slowly with 0·01 *N* permanganate until the ppt. disappears. The end-point is as before. The empirical relation: 1 ml of 0·01 *N* $KMnO_4 \equiv 30{\cdot}7$ μg of ammonia was used, but determination of this factor and of that for the added potassium is recommended. J. T. S.

Qualitative Inorganic Microanalysis without Hydrogen Sulphide. R. Belcher and F. Burton (*Metallurgia*, 1945, **31**, 317–319; **32**, 37–39)—In adapting the procedure of Mee ("*A New Scheme of Elementary Qualitative Analysis*," London, 1942) to the microscale the authors have retained the essentials, but certain separations and confirmatory tests are different. The group-numbering is altered to include the alkali metals. Precautions against the spreading of certain of the metals into several groups are also retained, although on the micro-scale this spreading is much reduced. More care is required than in the conventional scheme.

GROUP I

(Tests applied to portions of the original substance)

1. Apply Nessler Test for NH_4 in "hanging drop" cell.
2. Boil with ammonium phosphate soln., cool, centrifuge, and discard the residue. Divide the soln. into 2 portions.
   (*a*) Add excess $ZnCO_3$, heat in water-bath for 2 to 3 min., stir well and centrifuge. Discard the residue. Test the soln. for **Na** with zinc uranyl acetate.
   (*b*) Boil with excess 2 *N* NaOH to expel all NH_3. Acidify with dil. CH_3COOH and test for **K** with sodium cobaltinitrite.

GROUP II

Treat 2 to 3 drops of the soln. of the substance with 11 *N* HCl. Centrifuge. *Solution*—See Group III.

Residue—Wash, suspend in water and transfer to a slide. Remove excess liquid and scrape into a compact mass. Dry and sublime **Hg** (Mercurous). Confirm by ammonia test. Extract the residue with hot 2 *N* HCl.

Residue—Is **Ag**; confirm by *p*-dimethylaminobenzalrhodanine test.	*Solution*—Apply iodide or chromate test for **Pb.**

GROUP III

Solution from Group II—Evaporate if necessary to original bulk. Add an equal vol. of satd Na_2SO_4 soln. and twice the vol. of ethanol. Rub the sides of the cone with a glass thread, leave for 2 to 3 min. and centrifuge. *Solution*—See Group IV.

Residue—Wash with hot water, extract with 10% CH_3COONH_4, and centrifuge.

Residue—Wash once with hot water. Digest for 5 min. with 10% Na_2CO_3, cool and centrifuge. Discard the soln., wash the residue and examine by ethanol extraction (Belcher and Burton, *Metallurgia*, 1944, **31**, 42; ANALYST, 1945, **70**, 389). Alternatively, dissolve in CH_3COOH, warm, add excess $K_2Cr_2O_7$ and centrifuge.		*Solution*—Apply $(NH_4)_2S$ test for **Pb.**
Residue—Is **Ba.**	*Solution*—Add 1 to 2 drops of NH_4OH and $(NH_4)_2CO_3$, centrifuge and discard the soln. Wash the residue, dissolve in CH_3COOH, neutralise with NH_4OH, saturate with solid NH_4Cl and add fresh satd. $K_4Fe(CN)_6$. Centrifuge.	
	Residue—Is **Ca.**	*Solution*—Add excess of $(NH_4)_2CO_3$. A white ppt. shows **Sr.** Confirm by rhodizonic acid test.

GROUP IV

Solution from Group III—Expel ethanol, cool, add NH_4OH carefully until ammoniacal. Warm, add 2 *N* HCl until slightly acid or until any ppt. formed just redissolves. Add 10% $Na_2S_2O_3$ soln., warm for 2 to 3 min., and centrifuge. (If only a white ppt. of sulphur forms, Group IV is absent.) Test soln. for complete pptn.; if more ppt. forms, add to main residue. *Solution*—See Group V.

Residue—Add a few drops of water and a fragment of $(NH_4)_2CO_3$. Digest and centrifuge.

Residue—Wash, digest with Na_2CO_3 soln., and centrifuge.			*Solution*—Acidify with 2 *N* HCl. A yellow ppt. shows **As.** Confirm by ammonium molybdate test.
Residue—Wash, digest with 8 *N* HNO_3 and centrifuge.		*Solution*—Add 2 *N* HCl until ppt. forms. Centrifuge, discard the soln., and dissolve the residue in 11 *N* HCl. Divide into 2 portions (*a*) Test for **Sn** by cacotheline. (*b*) Test for **Sb** by Rhodamine B.	
Residue—Is **Hg** (mercuric). Confirm by *p*-dimethylaminobenzalrhodanine test.	*Solution*—Add NH_4OH and centrifuge.		
	Residue—Is **Bi.** Confirm by thiourea test.	*Solution*—Confirm **Cu** by α-benzoinoxime test.	

GROUP V

Solution from Group IV—Evaporate to original bulk, add 2 drops of *aqua regia*, digest and centrifuge to remove sulphur. Add excess NH_4OH and 3–4 drops $(NH_4)_2CO_3$. (If phosphates are present, first render *ca.* 5 *N* to HCl and add 6 drops of zirconium nitrate soln. Stir, warm, centrifuge, and add another drop of reagent to ensure complete pptn. Add a few crystals of NH_4Cl. Discard the residue.) Warm and centrifuge. *Solution*—See Group VI.

Residue—Wash, digest with excess 2 *N* NaOH and centrifuge.

Residue—Extract with 2 *N* HNO_3 and divide into 3 portions. (*a*) Test for **Fe** with $K_4Fe(CN)_6$. (*b*) Test for **Bi** by bismuthate test. (*c*) Test for **Cr**; convert to chromate and apply diphenylcarbazide test.	*Solution*—Divide into 4 portions. (*a*) Test for **Sn** by cacotheline. (*b*) Test for **Al** by aluminon. (*c*) Acidify with CH_3COOH and add $K_2Cr_2O_7$. A yellow ppt. shows **Pb.** Centrifuge and test soln. with ammonium oxalate soln. A white ppt. shows **Ca.** (*d*) If **Pb** is absent, test for **Ca** as above.

GROUP VI

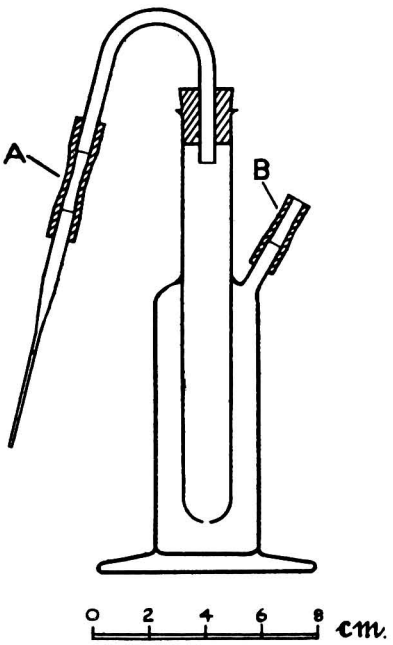
Solution from Group V—Add 2 *N* NaOH. Boil until all NH_3 is expelled. Centrifuge.

Residue—Wash, dissolve in 2 *N* CH_3COOH, and test portions as follows.
(*a*) Test for **Ni** by dimethylglyoxime.
(*b*) Apply KCNS test in acetone for **Co.**
(*c*) In presence of **Ni** or **Co** add KCN; if absent omit.
(i) Add $(NH_4)_2S$. A yellow ppt. shows **Cd.** Dissolve in HCl and confirm by diphenylcarbazide.
(ii) Test for **Mg** with Titan Yellow.

Solution—Add $(NH_4)_2S$. A white ppt. shows **Zn.** Confirm by (*a*) diphenylamine test, (*b*) Rinnmann's Green test (Benedetti-Pichler, *Ind. Eng. Chem., Anal. Ed.*, 1934, **4**, 336).

J. T. S.

Semimicro Gas Generator. (Miss) M. D. Barnes (*J. Chem. Education*, 1945, **22**, 322–323)—The unit is compact and delivers gas at a convenient rate and pressure. The hole at the bottom of the inner vessel should not exceed 2 mm. A charge of 15 ml of 6 *N* hydrochloric acid and a 1 c.c. piece of



ferrous sulphide is used to generate hydrogen sulphide. To operate, remove the pinch-clamp from A and close B either with a clip or with the thumb. If a clip is used, the pressure exceeds 1 atmosphere and is adequate for pressure pptns. The generator may also be used for carbon dioxide, nitric oxide and hydrogen. J. T. S.

Micro-determination of Copper with the Polarograph. C. Carruthers (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 398–399)—The method has been devised primarily for the determination of copper in animal tissues. The copper is precipitated with salicylaldoxime and the amount of the reagent used in the process is determined polarographically. The diffusion current of salicylaldoxime in a phosphate buffer mixture of *p*H 5·4 is proportional to its concentration. The wave height decreases from *p*H 3·7 to 7·8 and disappears at *p*H 9·1. The half-wave potential of salicylaldoxime at *p*H 5·4 is approx. −0·98 volts with respect to the saturated calomel electrode. Four to 15 μg of copper can be determined with an error of about 3%, whilst 0·15 to 0·6 mg can be determined with an error of between 1 and 3%. Ferric iron, sodium, potassium, calcium and magnesium do not interfere; zinc interferes strongly. B. S. C.

Amperometric Determination of Sulphates. W. C. Davies and C. Key (*Ind. Chemist*, 1943, **19**, 167–169)—The amperometric titration of sulphate with lead nitrate solution, using a dropping mercury cathode, is rapid and accurate (*cf.* Kolthoff and Pan, *J. Amer. Chem. Soc.*, 1940, **62**, 3332). In presence of aluminium difficulty is encountered owing to the solubility of lead sulphate in aluminium nitrate solns. By adjusting the *p*H, keeping the concn. of aluminium below a certain max., and adding alcohol, residual sulphate in pptd. alumina may be determined. *Procedure*—Heat 1 g with 25 ml of 30% nitric acid until a syrup and a small amount of white residue remains. Add 25 ml of water, warm, and dilute to 50 ml, rendering just acid (red end-point) to thymol blue (use instead methyl orange if the aliquot to be taken is such that after the addition of alcohol, the titration soln. contains less than 0·125 g of Al_2O_3 per 30 ml). Filtration is unnecessary. Transfer to a titration cell having a mercury pool anode an aliquot containing less than 0·4 g of Al_2O_3. Add 2 vols. of alcohol (industrial spirit), and adjust, if necessary, to just acid to thymol blue (or to methyl orange, as above). Bubble hydrogen through the soln. for 10 min. to remove dissolved oxygen. Apply an e.m.f. of about 1·2 volt and titrate with 0·05 *M* lead nitrate soln., passing the gas stream and noting the current after each addition. (A shunted Cambridge mirror galvanometer of max. sensitivity 950 mm per microamp., of period 5·6 sec., and of resistance 120 ohm was used to measure the current). When the concn. of sulphate is low, the gas stream should be passed for 3 min. after each addition to allow complete pptn. Not until the end-point has been passed does the current increase appreciably. Correct the current for the diluting effect of the reagent (Kolthoff and Pan, *loc. cit.*), and plot the corrected current against the vol. of reagent added. Extrapolate the steeply-rising portion of the curve to cut the vol. axis and read off the titre. (1 ml of 0·05 *M* $Pb(NO_3)_2$ = 0·004 g of SO_3.) Good results were obtained with samples containing from 0·5 to 4% of SO_3. To determine sulphate in boiler-water, etc., add an equal vol. of alcohol, render just acid to methyl orange, and titrate. Evaporate, dilute to 25 ml, and titrate an aliquot if the concn. of

sulphate is low. In presence of ammonium salts add 20% sodium hydroxide and boil before proceeding. J. T. S.

Photometric Determination of Fluosilicic Acid in Hydrofluoric Acid. G. N. Cade (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 372–373)—This method for the determination of fluosilicic acid depends upon the reaction of this acid with ammonium molybdate to form silicomolybdic acid, which is determined photometrically, using 425 $m\mu$ filters. Most of the hydrofluoric acid is separated from the fluosilicic acid by evaporation in the presence of sodium chloride (Kolthoff and Furman, "*Volumetric Analysis*," Vol. 2, p. 124. Wiley, New York, 1929). The small amount remaining as sodium hydrogen fluoride is converted into fluoboric acid, and the fluosilicic acid is converted into silicomolybdic acid, which is then determined photometrically. After the blank and the calibration curve have been established, a determination requires about an hour. B. S. C.

Spectrophotometric Method for Determining Formaldehyde. C. E. Bricker and H. R. Johnson (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 400–402)—Chromotropic acid (1, 8-dihydroxynaphthalene-3, 6-disulphonic acid) reacts with formaldehyde to give a purple colour (Eegriwe, *Z. anal. Chem.*, 1937, **110**, 22). Procedure: Prepare the chromotropic acid soln. by dissolving 2·5 g of the pure dry powder in 25 ml of water and filter. This soln. gradually changes colour on standing, so a reagent blank must be carried out at the same time as the formaldehyde determination. The acid soln. can be used at any time up to 2 weeks after preparation. The sample to be analysed should contain less than 100 μg of formaldehyde and be between 0·4 and 0·9 ml. If a dry sample is to be analysed, place a suitable weight in a glass stoppered test tube and add 0·5 ml of water. Then add 0·5 ml of chromotropic acid soln. and slowly pour 5 ml of conc. sulphuric acid into the tube, shaking continuously. Stopper the test tube and place in a beaker of boiling water for 30 min. Cool, dilute, cool again and then dilute to approx 50 ml in a volumetric flask. When the soln. has reached room temperature adjust the volume accurately to 50 ml and measure the transmission of a suitable thickness of the soln. at wavelength 570 $m\mu$ with a spectrophotometer. Correct for the reagent blank and determine the formaldehyde present from a previously prepared calibration graph. This procedure may be used equally well for determining combined formaldehyde which is liberated by acid hydrolysis. When determining free formaldehyde, however, the chance of obtaining incorrect values as a result of such hydrolysis should be borne in mind. Other aldehydes do not give the purple colour with chromotropic acid, but some give other colours which may necessitate a modification of the method. Benzaldehyde, methyl alcohol and ethyl alcohol do not interfere, but higher aliphatic alcohols hinder the formation of the colour. Using 0·5 g samples it is possible to determine as little as 2 p.p.m. of formaldehyde in methyl and ethyl alcohols. The accuracy of the method is well within 5%. B. S. C.

Spectrographic Analysis of Magnesium Alloys. B. L. Averbach (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 341–348)—In connection with the development of a routine method of spectrographic analysis of a magnesium-base alloy, various types of sample have been investigated. A horizontal sand-cast disc, with a blind riser, attached to the test bar mould provides the most consistent sample. The areas next to the gate and in the centre should be avoided. The sample is prepared by taking a 0·16 cm cut from one surface of the disc on a lathe and finishing with a No. 120 Aloxite belt. This prepared surface is then used as one electrode and a spectrographically pure carbon rod as the counter electrode with normal spark excitation (capacity 0·014 μF; inductance 0·045 mH). To enable as many elements as possible to be determined with one exposure, the spectrum lines are split horizontally with a rotating sector at the secondary focus. The top half is exposed to four times as much light as the bottom half. With this arrangement aluminium, zinc, magnesium and manganese lines are read on the bottom half, whilst iron, silicon and copper are read on the upper half. The following analysis lines and magnesium reference lines are used for the construction of curves and for the determinations:

Element	Analysis line	Magnesium reference line
Aluminium . .	3587A	3330A
Zinc . .	3302	3330
Manganese . .	2610	3074
Silicon . .	2516	3074
Iron . .	2382	3074
Copper . .	3274	3074

For nickel (analysis line 3415A, magnesium reference line 3074A) about double the exposure with an inductance of 0·365 mH is most suitable. For an alloy containing approx. 6% of aluminium, 3% of zinc and 0·2% of manganese this method gives results for these three constituents with an accuracy of at least ±5% of the amount present. B. S. C.

Effect of Extraneous Elements on Spectral Line Intensity in the Cathode-layer Arc. R. O. Scott (*J. Soc. Chem. Ind.*, 1945, **64**, 189–194)—A survey has been made of the intensities and intensity ratios of the lines of a number of trace elements, with iron as the internal standard, when these trace elements are incorporated in various pure compounds which either occur in plant ashes, or may be used in chemical concentration methods (Scott and Mitchell, *J. Soc. Chem. Ind.*, 1943, **62**, 4; ANALYST, 1943, **68**, 313). The various base materials used were alumina, silica, calcium carbonate, calcium phosphate, sodium pyrophosphate, sodium chloride and sodium carbonate. Working curves were prepared for sixteen common trace elements in each of the seven base materials. An assessment of the magnitude of the effect of the base material was made from a study of the errors produced by determining the amounts apparently present in the other bases using the curve for the alumina base as the working curve. Studies were also made of the actual intensity changes in the various impurity lines produced by the various bases. Most of the trace constituents gave their highest line intensities in the alumina base. The more volatile trace constituents, *e.g.*, copper, silver, zinc, bismuth and cadmium, tended to be greatly depressed by sodium chloride. Chromium, vanadium and molybdenum were enhanced by calcium salts and depressed by silica. Constant intensity ratios for cobalt and nickel to iron were obtained in all bases except silica. The observed changes in the intensities and intensity ratios do not appear to be caused by the addition of easily ionised atoms

nor by variation in source temperature. In general, trace elements in the same periodic group are affected in the same manner. B. S. C.

Ink Coverage Determination and Relation to Printability. R. Buchdahl and M. F. Polglase (*Paper Trade J.*, 1945, **121**, July 19, *T.A.P.P.I. Sect.*, 25–27)—Three methods for the evaluation of the coverage of printing ink on paper are described. (1) A very thin copper plate (which can be fixed firmly to a heavy base plate during the printing operation) is used as printing surface. It is weighed after inking (*i.e.*, before printing) and after printing, and the decrease in wt. gives the coverage for the printed area. (2) A very small, known quantity of a suitable dyestuff (*e.g.*, methyl violet) is added to the ink and then extracted quantitatively from the print in ethyl alcohol. The dyestuff content of the resulting soln. (*e.g.*, 30×10^{-5} g per 100 ml) is determined colorimetrically, or by absorption spectroscopy. Interfering substances which are sol. in alcohol must be absent. (3) A very small known quantity of radioactive phosphorus pentachloride is added to the ink, and the amount present in the print is determined by means of a Geiger-Müller counter. The high-energy electrons emitted produce a momentary discharge in the counter-tube, which is amplified and transmitted to a mechanical recorder. The method must be calibrated against a print, the ink coverage of which has been determined gravimetrically; a calibration curve showing the wt. of ink plotted against the no. of counts per min. is used. This method is more rapid than the other two, but it is not absolute. The calibration against the standard print must be carried out whenever a test is made, because of the decay of radio-activity with time. The methods are used to evaluate the printability of paper. They yield satisfactory results if contrast and (to a lesser extent) scale of tones are the important print qualities in question. J. G.

Reviews

INDUSTRIAL OIL AND FAT PRODUCTS. By ALTON E. BAILEY. Pp. x + 735. New York: Interscience Publishers, Inc. 1945. Price $10.

This is undoubtedly one of the most important works on the subject that has appeared in recent years. Written from the industrial point of view, it clearly and concisely reviews in a critical manner the recent rapid advances in oil and fat technology besides dealing with the basic processes of the industry and their theoretical aspects.

Two preliminary sections review the chemical and physical nature of oils and fats and the composition, characteristics and availability of the raw materials from which they are prepared. The greater part of the book is concerned with a description and discussion of the commercially important oil and fat products and of the processes used in their manufacture. Here the author deals with cooking and salad oils, shortenings, butter and margarine, bakery products, soap, paints and varnishes and miscellaneous products; and he describes the various unit processes involved including extraction, refining and bleaching, deodorisation, hydrogenation, soap manufacture, fractionation of fats and fatty acids, splitting, esterification and inter-esterification, polymerisation and isomerisation, solidification, homogenisation and emulsifying.

A large amount of important material is included that is not available in most of the older books, some appearing for the first time, and the author has been critically assisted in the preparation of his manuscript by most of the outstanding oil chemists in his country. It is therefore not surprising to find oneself distinctly and very favourably impressed by the work. Minor adverse criticism of certain statements and conclusions is possible, but generally speaking the presentation of the discussion is admirable and accurate, and the oil trade will certainly be the richer for the author's painstaking effort.

It should perhaps be mentioned that analytical methods are not described in the book, though results obtained by the more practically valuable ones are recorded.

K. A. WILLIAMS

MODERN PLASTICS. By H. BARRON, Ph.D., F.R.I.C., A.I.R.I. Pp. xv + 680. London: Chapman & Hall, Ltd. 1945. Price 42s.

In the present work Dr. Barron completes his account of the closely related rubber and plastics industries, the forerunners being "*Modern Rubber Chemistry*" (1937) and "*Modern Synthetic Rubbers*" (1942, 1944). In the preface to "*Modern Plastics*" the author says that he is not writing for the experts but that he hopes to provide an over-all view of the industry for those with modest scientific and engineering knowledge; the book is written in a similar style to the earlier publications and should fulfil this intention. Certain materials have been omitted as being unavailable in this country and still of purely academic interest, a conclusion with which the non-specialist will agree. However, recent developments in America show that the outstanding heat and electrical properties of the silicone resins warrant

their industrial production and that in this country we can no longer afford to regard them only from a theoretical standpoint; they should certainly be included in a review of the plastics, even if this is of general character such as that now under consideration.

The book opens with three introductory chapters: the two types of resins, thermoplastic and thermosetting, and their historical development; raw materials and factors involved in their application to the manufacture of plastics; and condensation and polymerisation resulting in the formation of macromolecules. There follows a more detailed account of individual plastics which constitutes the major part of the book. Nine chapters deal with the thermosetting resins based on formaldehyde and phenol, urea or melamine. These include the production of moulding powders, varnishes and cast resins, together with the technology of their applications and the physical properties of the finished products; the last two of these chapters are devoted to laminated materials, plywood and improved (resin impregnated) wood. Nitro-cellulose, cellulose acetate and acetobutyrate, and ethyl cellulose are similarly described in four chapters, one of which is on injection moulding. Eight chapters set out the technology, applications and properties of vinyl derivatives: polythene, polystyrene, polyvinyl chloride, polyvinylidene chloride, methyl methacrylate, polyvinyl acetate and butyral. In this section the more detailed features comprise co-polymerisation and emulsion polymerisation, extrusion, plasticisers and laminated safety glass. This part of the books ends with three chapters on nylon, alkyd (or glyptal) resins and casein plastics. The concluding three chapters deal with the application of high frequency heating and with chemical analysis and physical testing of plastics.

The analytical chapter is treated somewhat unevenly, some of the processes being described at length (in spite of the intended reader with "modest scientific . . knowledge"!), while others are given in outline only. Some of the detail is both unnecessary for the specialist and meaningless to the non-chemist. Emphasis is laid upon the various published schemes for the qualitative examination of a plastic of unknown composition, while some of the raw materials are tested for quality by familiar methods. The customary physical tests are adequately described and summaries of typical results are given in tabular form. Reference is made to relevant British Standard Specifications, but it may be noted that No. 547—1934, quoted on p. 661, has now been superseded by No. 1137—1943 (*Synthetic-Resin Bonded-Paper Sheets for Use as Electrical Insulation at Power Frequencies*).

The book contains some needless repetition of material, a little careless writing and many typographical errors. These are generally readily detectable but might sometimes mislead the less well-informed reader. Some examples follow. P. 63: American production figures given in "gallons" may be U.S. or Imperial gallons. P. 110: A solution of phenol is used, yet "in order to keep it fluid the phenol is maintained at ·45° C." P. 153: "Asbestos is only melted with difficulty by phenolic resin. . . ." P. 242: Tensile strengths are given in "kilometers." P. 358: The table contains abbreviations the meanings of which are not stated and cannot be guessed. P. 383: "The use of temperatures up to 300° C. permits of the production of higher polymers with molecular weight up to 200° C." P. 565: An increase in iodine value is said to indicate that "the unsaturation has become less." P. 607: The sodium fusion test is given without warning that sulphur and nitrogen, if present, will interfere with the halide reaction; moreover, it appears that silver nitrate solution is added to the alkaline extract of the melt. Much useful information may be derived from "*Modern Plastics*" and it is a pity that it is marred in this manner.

G. H. WYATT

PHYSICAL METHODS GROUP

THE Annual General Meeting of the Group will be held at the Chemical Society's Rooms, Burlington House, London, W.1, at 6 p.m. on Wednesday, November 28th, 1945.

The business meeting will be followed at 6.30 p.m. by a meeting open to non-members, when the following papers will be read:

"The Barker Index: a Means of Identifying Crystals from their Shape," by Mr. R. C. Spiller, Reader in Mineralogy, University of Oxford.

"Examples of the Utility of the Barker Index in Analytical Chemistry," by Dr. M. W. Porter (The University Museum, Oxford) and Dr. A. E. J. Vickers (I.C.I., Billingham).

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