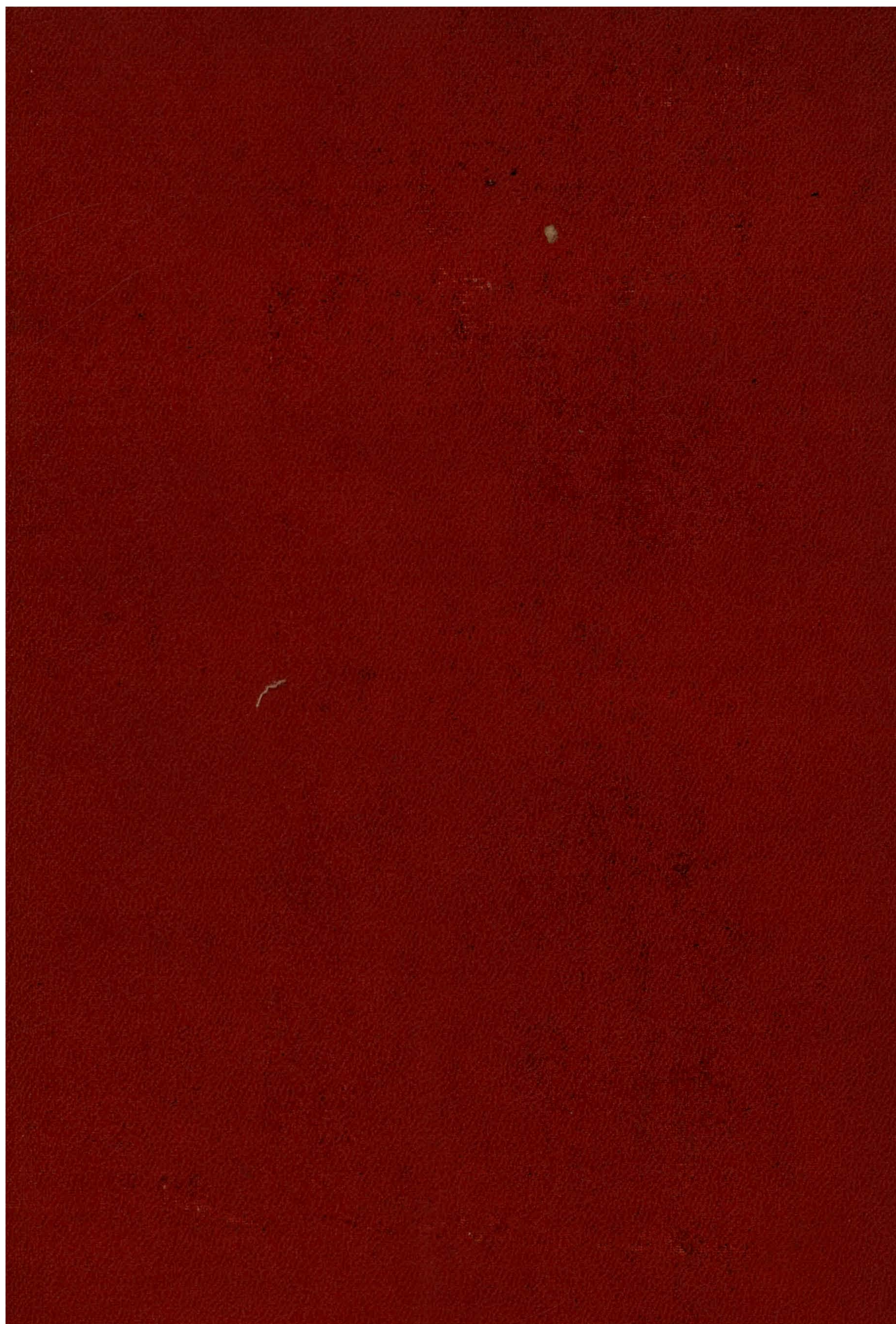

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The Journal of The Society of Public Analysts and Other Analytical Chemists

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Applications are invited for the position of Superintending Analyst in the above Department at a salary of £700 per annum rising by annual increments of £50 to £850 per annum, plus war bonus. Candidates should be Fellows of the Royal Institute of Chemistry in Branch E (The Chemistry, including Microscopy of Food and Drugs and of Water), should have had considerable experience in the analysis of foods and drugs, and should be conversant with the duties of an Official Gas Examiner. The successful candidate may be required to act as an assistant Gas Examiner without extra remuneration. The appointment will be subject to the Standing Orders of the City Council.

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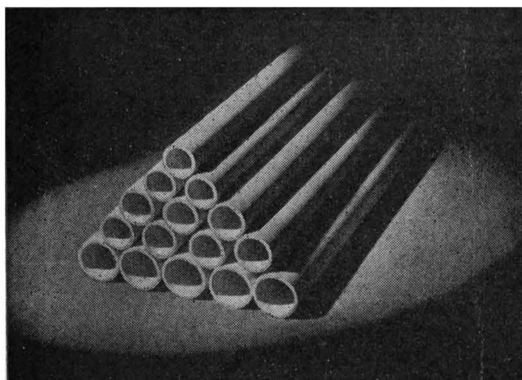

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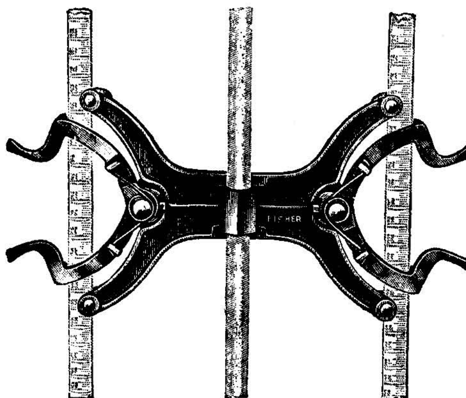

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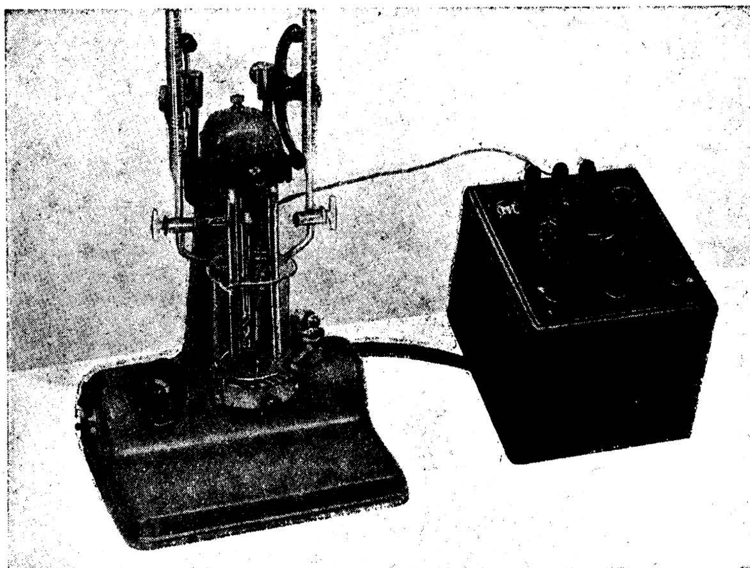

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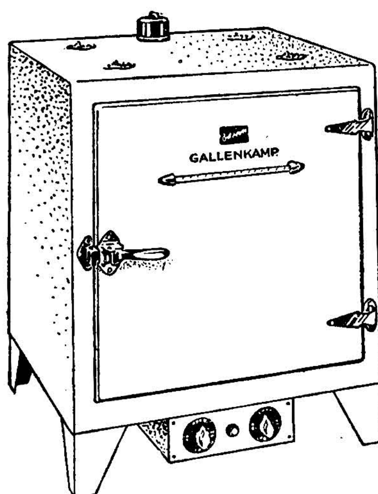

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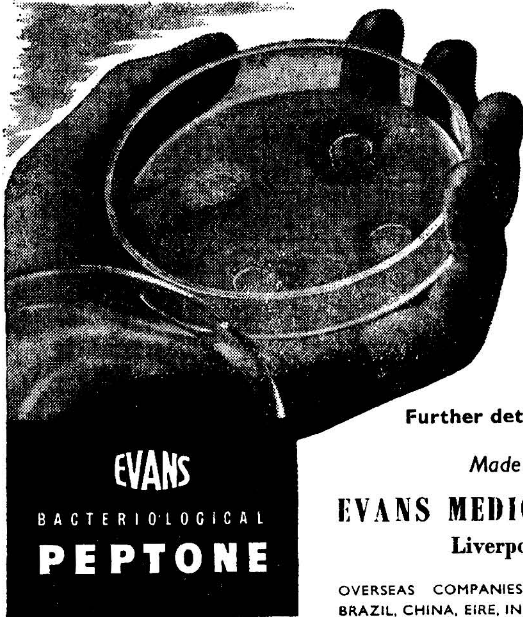

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PHYSICAL METHODS GROUP

THE following is the provisional programme for a meeting of the Group, on The Analysis of Radioactive and Stable Tracers, to be held in the English Theatre at Mason's College, Birmingham, at 6.30 p.m. on Friday, April 2nd, 1948:

"Measurement of β-Activity," by A. G. Maddock, B.Sc., Ph.D.
"Measurement of Radioactive Isotopes," by F. E. Whitmore, B.Sc., Ph.D., A.R.I.C.
"Determination of Abundance Ratios of Non-radioactive Isotopes," by E. R. Roberts, A.R.C.S., Ph.D., D.I.C., and E. R. S. Winter, A.R.C.S., Ph.D., D.I.C.
"Tracers in Biochemical Investigations," by W. J. Arrol, B.Sc., Ph.D.

Arrangements have been made for a limited number of members and guests to take tea together at 5.45 p.m. in the Refectory at Mason's College. Price 1s. 3d. Members requiring tea must inform Mr. E. M. Joiner, B.Sc., F.R.I.C., at 15 Halton Road, Sutton Coldfield, Warwickshire, before Tuesday, March 30th.

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JANUARY, 1948 Vol. 73, No. 862

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

BIOLOGICAL METHODS GROUP

THE Third Annual General Meeting of the Biological Methods Group was held at 6 p.m. on Tuesday, December 16th, 1947, in the rooms of the Chemical Society, Burlington House, W.1. Mr. A. L. Bacharach was in the chair.

The following were elected as Officers and Members of the Committee for the forthcoming year:—*Chairman*—Dr. A. J. Amos. *Vice-Chairman*—Mr. N. T. Gridgeman. *Hon. Secretary*—Dr. E. C. Wood (Virol Ltd., Ealing, London, W.5). *Committee Members*—Mr. A. L. Bacharach, Dr. H. O. J. Collier, Dr. E. R. Dawson, Mr. H. E. Monk, Mr. S. A. Price, Dr. H. G. Rees; with, as *ex officio* Members, The President, Hon. Treasurer and Hon. Secretary of the Society and the Editor of THE ANALYST. Messrs. D. M. Freeland and J. M. Hamence were re-elected Hon. Auditors.

A vote of thanks was passed by the meeting to the retiring Officers and Committee Members for their services to the Group, with special reference to Mr. A. L. Bacharach, who had been Chairman of the Group since its inception and was, indeed, largely responsible for its formation.

Following the Annual General Meeting an ordinary meeting was held, at which the following papers were read and discussed:—"A Modified Method for the Microbiological Assay of Tryptophan, Methionine, Cystine and Tyrosine," by Dr. E. C. Barton-Wright and Mr. N. S. Curtis; "The Use of *Neurospora Crassa*, mutant 9185, for the Assay of Aneurine," by Mr. H. S. Harrison and Mr. E. J. Miller; "A Note on the Cup Method of Microbiological Assay and its Limitations," by Dr. W. F. J. Cuthbertson.

SCOTTISH SECTION

THE Thirteenth Annual General Meeting of the Section was held in Glasgow on January 14th, 1948, and the following Office Bearers were elected for the year:—

Chairman—Dr. H. Dryerre. *Vice-Chairman*—Dr. J. Sword. *Hon. Secretary and Treasurer*—Mr. R. S. Watson (City Analyst's Department, 20, Trongate, Glasgow, C.1). *Members of Committee*—Messrs. F. J. Elliot, A. N. Harrow, H. C. Moir, J. Sandilands, R. O. Scott and A. M. Smith. *Hon. Auditors*—Messrs. A. R. Campbell and W. M. Cameron.

DEATHS

WE deeply regret to report the deaths of

Charles Ainsworth Mitchell.
John William Pooley.
Walter Raymond Schoeller.
James Smith.
Sir Bernard H. Spilsbury, Honorary Member.

The Application of Statistical Methods to Food Problems

The following four papers were read at a Joint Meeting of the Society with the Food Group of the Society of Chemical Industry on December 4th, 1946.

The Inevitability of Statistics

BY D. J. FINNEY*

THE late Lord Hewart once aptly stated the attitude of the average man-in-the-street to statistics, and probably with equal truth he could have spoken for the average scientist-in-the-laboratory. "The world is wide," he said, "paper and ink are cheap, and there is room for all—poets, dramatists, and novelists, historians, philosophers, and biographers; yes, even chemists and economists and (for persons of ungoverned passions) the best of authorities on the repulsive subject of statistics." Possibly he was thinking primarily of the purely descriptive statistics published by the Ministry of Labour or by the Registrar-General, rather than of the methods of statistical science with which I am concerned in this paper; nevertheless, I doubt whether Fisher's "*Statistical Methods for Research Workers*" is any more popular with the general reader than are the Registrar-General's reports.

Yet the drawing of statistical inferences enters into daily life, both for the man-in-the-street and for the chemist-in-the-laboratory, in a multitude of ways; in their use of the process they may be as unaware of its true nature as was M. Jourdain of the nature of prose, but prose and statistics are alike inescapable. The man who says "To-morrow will be wet," unless an unvarying pessimist, is presumably basing his belief on past experience of weather similar to that of to-day; if conditions like to-day's, on a large proportion of occasions, have been followed by rain, the inference is drawn that rain is likely to-morrow, and the degree of confidence in the inference will depend upon the magnitude of this proportion. The chemist perhaps need not worry unduly because the laws of chemical reaction are found to be statistical rather than absolute truths: for many practical purposes, at least, he can continue to write his equations in the old style. But he is likely to be brought into daily contact with the fact that his weighings, his measurements, his analyses—all his quantitative determinations—are not perfectly reproducible, except in so far as the coarseness of scales of measurement may conceal the disagreement between separate determinations. If five people measure the length of a table to the nearest inch, they may show perfect agreement; if they use a scale graduated in 1/100 inches and express their results to the nearest 1/100 inch, perfect agreement is unlikely. The biologist encounters the problem of variability in measurable quantities in a more acute form than does the chemist. The weight increases of animals over a specified period will not be exactly equal, nor will the yields of wheat on adjacent acres of land, nor the numbers of eggs laid by individual insects, however carefully the experimenter controls all environmental factors. He is forced to take account of this variation in any statement he makes about mean values of his measurements. For example, in discussing a difference in mean weight between two groups of animals which have received different rations, he will have to allow for naturally occurring individual variations in weight, and may need to examine the possibility that the observed difference is attributable entirely to chance, the effect of rations being negligible. Such questions are essentially statistical in nature, and, in the last half century, close co-operation between biologists and statisticians has led to the development of a logical system for answering them.

I recently ventured to classify biologists who lack experience of statistics into three groups: those who know that all statistical concepts are too difficult for their comprehension, those who know that statisticians deliberately make simple questions appear difficult in order that they may practise the mysteries of their craft, and those who know that statistical analysis of their data is unnecessary. If I dared to make an equally cynical classification of analytical chemists, I should add that in preparing this paper I was thinking particularly of the third (and perhaps the largest) group, though some of what I shall say might be more applicable to

* Lecturer in the Design and Analysis of Scientific Experiment, University of Oxford.

the others. In spite of this starting point, and in spite of the title I have chosen, I am not contending that every scientist should keep a tame statistician in his laboratory, or that every scientific paper should have a statistical appendix. On the other hand, recent developments in statistical science have made it an integral part of so many investigations that both biologists and chemists are likely to benefit from having some knowledge of its potentialities and of the circumstances in which co-operation with a statistician is desirable. The physical scientist not infrequently argues that the quantities with which he is concerned are much less subject to variation than those which interest the biologist, and that in consequence he has little need to trouble himself with statistical arguments. To some extent this claim is justified, and I certainly have no wish to persuade chemists that they should indulge in an orgy of standard-error calculations in order to give an air of statistical respectability to their work. Far more important than the indiscriminate statistical analysis of every batch of figures is the cultivation of a statistical habit of mind. With this to guide him, the chemist (or the biologist) can judge whether the interpretation of his data requires a detailed statistical analysis and, if so, whether that analysis is of a standard pattern such as he feels competent to perform himself or whether he needs the assistance of a professional statistician.

Let me illustrate the function of statistical analysis by detailed discussion of a simple example. When a scientist calculates a mean of several measurements, he usually does so because he believes the mean to be a more precise value than any one of the individuals contributing to it. I here use the word "precise" not as descriptive of arithmetical accuracy but as referring to the closeness of approximation to an unknown ideal value. The botanist who records the weights of six plants is probably not interested in those six for themselves alone but rather as representative of a much larger class or population of plants that have been grown (or might be grown) under the same conditions; he expects the mean of his six measurements to be closer to the undetermined, and possibly undeterminable, mean of the whole population than would be the weight of a single plant. The calculation of a mean with this end in view is a statistical operation, just as truly as the most elaborate set of computations in a text-book of statistical methods. Now the chemist also is accustomed to taking an average of several measurements, and he does so for the same reason. For example, in analysing the constitution of a substance by means of observations and measurements on a small sample he obtains a figure that is not perfectly reproducible; one component of variation arises from the fact of his sample not being perfectly representative of the whole, another from the accumulated effects of small imperfections in the measurements made. He expects the mean of duplicate or triplicate determinations to be nearer to the true mean than would be a single determination. But what is meant by saying that he *expects* the mean to be nearer the truth? Clearly he cannot be certain of this, for his first determination might by chance (though he would not know it) be almost exactly correct, and averaging this with other values would give a result further from the truth. His expectation is only in the statistical sense that, if he habitually uses a mean of triplicate determinations rather than a single one, he will in the majority of instances be nearer the truth.

One important function of the statistician is to investigate the relative merits of alternative estimation procedures and to give numerical expression to the precision obtained. Though he may employ complex techniques and adopt unfamiliar terminology, he should obtain results according with a common sense view of the problem and the data, but having the great advantage of objectivity. Suppose that a certain process of measurement is repeated six times—the weighing of comparable batches of material, the analysis of comparable small samples from a large bulk or any other procedure giving results subject to some variation. Suppose, further, that the mean of the six values is 20 units. For many purposes, that mean needs to be supplemented by information on its precision, or reliability: with what assurance could we expect a second series of six values also to give a mean near to 20? Consider three sets of figures, each of which has the mean 20:

I	II	III
21	14	3
20	24	32
21	25	29
19	18	7
18	23	20
21	16	29

Common sense indicates that series I represents a fairly satisfactory determination of a mean value of 20, likely to be within 1 or 2 units of the truth; series II is much less satisfactory, since the individual values are so much more discrepant, though a true mean value between 15 and 25 seems reasonably sure; series III would usually be considered completely unsatisfactory, for though the mean is again estimated to be 20, the spread of the separate values about this figure is too great for much confidence to be placed in it.

These statements are vague and subjective. Perhaps the most obvious objective method of expressing the precision of the estimate is by means of the range of the observed values, 18 to 21 for series I, 14 to 25 for series II, and 3 to 32 for series III. Further consideration shows this method to be wasteful of information, particularly in long series, since it ignores completely all the remaining values, which obviously have something to tell about the dispersion. Also the range is unstable in the sense that it attaches undue importance to the chance occurrence of a single extreme value. An additional difficulty arises in the comparison of series of different lengths, for the longer the series the greater the opportunity for the occurrence of extremes and consequently, in general, the greater the range.

For these and other reasons, the statistician prefers to use the *standard error* calculated from the individual values. The term is perhaps unfortunate, and for the benefit of any to whom it is new I may explain that the word *error* is not used in the sense of a careless or faulty experimental technique. No stigma attaches to the possession of a standard error, though considerable effort may be expended on keeping it within reasonable limits! I shall not enter into details of its calculation, but shall be content to say that all the individual values are used. In our three series, the standard errors of the means are estimated to be 0·52, 1·88 and 5·05 units respectively. If the series arose from three different methods of estimating the same quantity, the standard errors would indicate the relative reliabilities of the methods. Other things being equal, we would prefer the method giving the smallest standard error, since this would give the least probability of any specified difference between the estimate and the true value of the quantity estimated. More exactly, we may say that the further the true value differs from 20, the less likely would the experimenter be to obtain the results quoted; if an arbitrary standard of unlikeliness, such as a probability of 5 per cent. or less, is agreed, this can be used in the specification of comparable limits for the three estimates. In fact, with this degree of uncertainty, the true mean for the first series may be asserted to lie between 18·7 and 21·3, that for the second between 15·2 and 24·8, and that for the third between 7·0 and 33·0. The deviations from 20·0 are proportional to the standard errors, the factor of proportionality being determined by the number of observations in a series and by the selected level of probability. There can be no complete certainty in these statements, but the three inferences are of equal reliability and that reliability can be made greater by choice of a more stringent criterion: the probable limits of error, or *fiducial limits*,* would then be more widely spaced. Furthermore, fiducial limits of the same reliability can be calculated whatever the length of the series, so providing a standard of comparison for series of different lengths. An increase in the number of observations will decrease the standard error of the mean in proportion to the square root of that number.

This simple example is typical of an important class of problem in which statistical concepts are valuable. The procedure in any one instance is dependent upon the character of the data and the way in which they have been obtained. The point I am anxious to make is not that the chemist should be able to use the various statistical methods himself—though with practice he may attain facility with many of them, and the chief danger is less that he will find them too difficult than that he will misapply them—nor indeed that he should always submit his results to a statistician. What is vital is that he should appreciate the main features of the statistical argument and should know when a statistical analysis is essential to the proper interpretation of his work. Often I have heard the remark "I haven't enough figures to make a statistical analysis worth while," yet the scantiness of data may be a strong argument for the necessity of expert statistical advice. From abundant data clear-cut decisions and valid conclusions may possibly be reached without great finesse, but when

* Strictly speaking, the figures I have quoted for the fiducial limits depend upon an assumption that the original frequency distribution is of a particular type known as normal, but departures from this law are often unimportant. The standard errors of the means are each based on six observations only and are therefore themselves subject to errors of estimation. Consequently the t-distribution, here with five degrees of freedom, must be used for calculation of the fiducial limits instead of the normal. The deviate for 5 per cent. probability is 2·57, not the familiar 1·96, and the multiple of each standard error gives the required range on either side of the mean.

information is restricted to very few results the closest possible co-operation between chemist and statistician may be required if nothing is to be wasted and if unjustifiable deductions are to be avoided. The choice seldom lies between using and not using statistics, often between using invalid or inefficient methods and using the specialised techniques of the expert who, though he may lack knowledge of the chemical aspects of the data, has a claim to be heard on matters connected with his own study.

The help of the statistician is not confined to the analysis and interpretation of experimental or sampling results. His contribution to the planning of an experiment or of a sampling programme for estimating a certain quantity is often more valuable than his analysis of the results. Indeed, good experimental design may make the main features of the results so clear that lengthy computations are unnecessary. Realisation of the intimate connection between the method of collecting data and the appropriate statistical analysis is one of the most important developments of modern statistical science. Often the results of past investigations may be used not only for their immediate purpose but also for predicting the precision that might be obtained in future similar work. For example, in sampling foodstuffs for the estimation of some particular constituent, the variability amongst duplicate estimates may be of the order to which biologists, rather than chemists, are accustomed, and careful planning is essential if the required precision is to be obtained at minimal cost. The procedure may involve several stages: within a large consignment, several units such as crates, boxes, or barrels are selected as the sources of the samples, from each of these one or more samples are withdrawn, each sample is sub-sampled for chemical analysis, and finally duplicate or triplicate analyses are made on each sub-sample. The mean value eventually obtained for the consignment will have components of error variation attributable to each stage of the sampling: duplicate chemical analyses will not be in perfect agreement; sub-samples from the same sample will differ to a greater extent than can be explained as due to analytical variations; samples from different crates will differ more widely than samples from the same crate. From data already in existence, or from a preliminary examination, the statistician can estimate the relative magnitudes of these components of variance, and thence can predict how the precision of the mean would be affected by changes in the intensity of sampling at any stage, as for example, by sampling more crates but taking fewer samples from each. His advice will enable future sampling to attain a specified precision at less cost in time and labour. The process of improvement in the design of the sampling process may be continued, as the accumulation of further data will increase the knowledge on which it is based.

This example, like my earlier one, is over-simplified, but it illustrates the benefit to be gained from attention to the statistical analysis. From the close relationship between how the figures are obtained and the form of their statistical analysis, I would like to draw two morals. The more obvious is that the statistician must be given the full facts if his conclusions are to be valid; too often points thought to be irrelevant are withheld from him, until a chance enquiry during the analysis discloses that what he understood to be figures for single samples are really means of pairs, or that samples he believed to have been taken at random were in fact taken subject to some systematic or balancing conditions. The second moral, which, if consistently followed, would destroy the need for the first, is to discuss your investigation with the statistician while you are planning it, instead of merely bringing him the results for analysis.

I cannot attempt to summarise all the ways in which statistical techniques may be useful in food problems, for few aspects of applied statistics can be dismissed as irrelevant. I have mentioned sampling estimation, and as a particular use of sampling I may refer you to the technique known as *quality control.* Though developed to assist in the control of industrial manufacture, the methods are of general utility in providing a systematic and objective routine for the inspection of any product whose qualities may vary with time and must be kept within reasonable limits of tolerance in order to satisfy producer and consumer; they may prove to be as useful for butter as for guns. Experimental design, an art as well as a science, has been developed mostly to meet the needs of field trials on agricultural crops, but its importance is by no means limited to problems of primary food production. In the laboratory also the statistician can render great service in helping to design experiments which will provide efficient tests of questions under examination. Organoleptic experiments, for example, are in certain formal aspects analogous to field-plot trials, and their planning so as to eliminate personal factors, sensory fatigue, and variation in materials may be on similar lines; moreover, the analysis of their results, whether expressed in rankings or in marks, may involve both standard and specialised statistical techniques. A subject in which analytical chemists

and biologists work in close collaboration is that of biological assay, and to this statisticians have made many contributions; the validity of the assay method, the planning of constituent tests so as to obtain an estimate of satisfactory precision, and the eventual calculation of that estimate all involve interesting problems of statistical theory and practice.

I have spoken of the chemist and the statistician as separate persons, not because they are necessarily distinct but in order that I might emphasise my belief that the analytical chemist can and must have some understanding of statistical *principles*, even though he may not unreasonably decline to devote his time to routine statistical *calculations*. Some may wish, in addition, to perform their own statistical analyses rather than to depend upon a professional statistician, and, for those who can spare the time, this is perhaps the best way of becoming familiar with the underlying principles. The extent to which the chemist is his own statistician must vary with individual inclination and circumstances. Few will master the subject so completely as to dispense with all expert advice; none, I venture to think, are incapable of the elementary stages. Providing that he has some grasp of basic principles, the chemist has no more need to follow the intricacies of mathematical theory than he has to know how to construct a table of logarithms before he can use a slide rule (an analogy that I owe to Mr. Bacharach). Recognition of his own limitations, however, may be as important as confidence in his own abilities, if he is to avoid misapplication of methods comparable with attempts to use a slide rule for subtraction! His chief danger is not the difficulty of the calculations: these may be tedious, but they seldom require more than an elementary knowledge of arithmetic. On the other hand, the unwary are frequently entrapped by forgetting that the *possibility* of performing certain arithmetical operations provides no guarantee that the corresponding statistical technique is appropriate to the data.

I have called my paper "The Inevitability of Statistics," not "The Infallibility of Statisticians." I ask you to remember that, as an exact science, statistics has not the long history of chemistry. Both theory and practice are developing rapidly, and their development can be stimulated and assisted by well-informed criticism from other sciences. In inviting criticism, however, I am not abrogating the claim of statistics to be considered a specialised branch of knowledge, for I am convinced that the man who is trained and experienced in the intrinsic properties of numerical data is to-day essential to pure and applied science. There is a strange temptation for the same man who professes complete inability to understand the mathematical basis of the subject, and even abhorrence for any attempt to do so, to make confident assertions that certain methods are obviously correct or obviously incorrect! The statistician is entitled to demand of his critics that they examine the evidence with due care before they pass judgment on statistical theory. He is aware that his methods are sometimes incomplete and involve approximations of uncertain validity, and he welcomes help in their improvement; less welcome is destructive criticism of standard techniques having no more basis than use of the word "obviously."

I have tried to show you that modern statistical science can help the chemist, and that he will understand his own science the better if he knows the general principles on which statistical analysis is based. Much of the importance of the contribution of the statistician to research problems derives from his concept of "information," a word I have already used several times; in closing, I would like to draw your attention to the special sense in which we speak of information in statistics. In the last quarter-century we have realised the possibility of assigning a numerical measure to the total quantity of information, relevant to a specific question, contained in a given body of data. Alternative methods of analysis can then be compared in terms of the degree to which they recover the available information; though extraction of the whole may not always be practicable, we have an absolute criterion for the efficiency of any method proposed. This concept is invaluable in the planning of an experiment or of a sampling investigation, for it enables the statistician to aim at maximising the yield of information on the points at issue. I cannot do better than end by quoting a few sentences of R. A. Fisher, himself the pioneer in this branch of the theory of statistical estimation. Fisher says:

> "The statistician is no longer an alchemist expected to produce gold from any worthless material offered him. He is more like a chemist, capable of assaying how much of value it contains, and capable also of extracting this amount and no more. In these circumstances, it would be foolish to commend a statistician because his results are precise, or to reprove him because they are not. If he is competent in his craft, the value of the result follows solely from the value of the material given him. It contains so much information and no more. His job is only to produce what it contains."

The Use of Statistical Methods in Research on Food Canning

By W. B. ADAM

STATISTICAL analysis has long been regarded by biologists, economists and workers in many other fields of scientific research as an essential instrument for the proper design and interpretation of experiments. The fact that chemists have been slower in recognising its value may be due to a natural tendency to distrust results that are expressed in terms of probability, but nevertheless these methods are being increasingly used by the analyst no less than the research chemist in studying problems connected with the food industries. The object of the chemist engaged in food research may be to show the effect of introducing a new factor into a process to be tested, or to separate the several effects of a number of factors operating together, and the choice of a suitable statistical treatment enables this to be done in such a way that the maximum information is extracted from the analytical results.

The object of the present paper is to give some illustrations of the application of statistical methods to research on food canning, and the examples chosen indicate the use of significance of means and correlation coefficients. Reference is also made to the use of analysis of variance. The data quoted are restricted to published figures based on chemical tests and field trials, and no examples are given to show how statistical tables may be used in calculating the probability of survival of bacterial spores under various conditions of heat treatment, as this subject is considered to be outside the scope of the present discussion.

THE NORMAL DISTRIBUTION CURVE—

The fundamental curve where variation is continuous is usually the Normal Distribution Curve. If a variate is normally distributed and a limited number of observations are made on the same "population," the mean of the results will be the best estimate of the true mean, and the degree of dispersion—and hence the shape of the distribution curve—will be specified when the standard deviation has been estimated. The probability of occurrence of any particular measurement can be calculated from the estimated mean and standard deviation, but the frequency curve obtained, though often of interest, is seldom of value in itself. The commonest use of the mean and standard deviation is in calculating the significance of the differences between means—*i.e.*, whether two means, obtained from different sets of experiments, are separated by an amount greater than can be attributed to errors of random sampling.

SIGNIFICANCE OF MEANS—

In a large class of experiments the aim is to test the effect of introducing one new factor, while holding other factors constant. The whole of the samples included in the experiment are thus drawn from a single population of which one portion contains the test factor, and the object of the statistical analysis is to determine whether an observed difference between the means of the test samples and the controls is due merely to sampling errors or must also be attributed to the effect of the new factor introduced. This may be done by calculating the standard deviation for either series. From this figure the standard error of each mean may be calculated, and also the standard error of the difference of the means. The figure obtained by dividing the difference between the means by the standard error of this difference is known as t, and, from Fisher's tables of t values, it is possible to estimate the degree of probability that the observed difference between the means is due to sampling errors. A probability of less than one in twenty ($P = 0{\cdot}05$) is conventionally regarded as significant.

Example 1—The following example, taken from a paper by Dickinson,[1] shows the use of significance of means. The problem was to examine the effect of different methods of treating the surface of de-tinned steel strips on the rate of corrosion under standard conditions. He first determined the mean losses in weight of ten abraded strips and ten unabraded strips. The results, with the principal steps in the calculation, are shown in Table I where the means are seen to be significantly different ($P = 0{\cdot}04$). The standard error of the difference between the means is the square root of the sum of the squares of the standard errors of the individual means. As the standard errors of the two means in Table I were the same this accounts for

the factor 2 beneath the square root sign. The estimate of P was obtained from the tables of t with t = 2·23 and 18 degrees of freedom.

TABLE I

EFFECT OF ABRASION OR PATTERN

De-tinned Strips

Test	Loss in weight, mg.	Mean
A Abraded	59, 63, 63, 68, 66 58, 85, 74, 72, 77	68·5
B Unabraded	44, 47, 83, 67, 53 55, 51, 70, 54, 58	58·2

Sum of squared deviations A = 654
B = 1266
Total = 1920

Degrees of freedom, n − 2 .. = 18

Standard deviation .. .. = $\sqrt{106 \cdot 6}$

S.E. of either mean .. .. = $\sqrt{\frac{106 \cdot 6}{10}}$

S.E. of difference between means = $\sqrt{\frac{106 \cdot 6}{10} \times 2} = 4 \cdot 62$

Difference between means .. = 10·3

t = 2·23 P = 0·04

There was thus evidently some real difference between the two sets of strips, but there was no indication whether the observed difference was chiefly due to the effect of abrasion of the one lot of strips or to the existence of a "pattern" on the surface of the unabraded strips. A similar test on strips cut from blackplate (the results of which need not be quoted) showed that the mean loss in weight of the unabraded strips (without pattern) was again significantly less (P = 0·01) than that of the abraded strips, and a third test showed that the

TABLE II

EFFECT OF SPRAYING TREES WITH BORIC ACID

Test	Percentage of gummed fruits	Mean
A Control ..	59·5, 88·3, 86·8, 79·1, 41·5, 75·1	71·7
B Boric acid ..	66·8, 35·5, 46·6, 54·4, 22·4, 56·8	47·1
	Results after angular transformation	
A Control ..	50·5, 70·0, 68·7, 62·8, 40·1, 60·1	58·70
B Boric acid ..	54·8, 36·6, 43·0, 47·5, 28·3, 48·9	43·18

Sum of squared deviations A = 659·7
B = 451·0
Total = 1110·7

Degrees of freedom, n − 2 .. = 10

Standard deviation .. .. = $\sqrt{111 \cdot 07}$

S.E. of either mean .. .. = $\sqrt{\frac{111 \cdot 07}{6}}$

S.E. of difference between means = $\sqrt{\frac{111 \cdot 07}{6} \times 2} = 6 \cdot 09$

Difference between means .. = 15·52

t = 2·55 P <0·05

initial rate of loss of de-tinned strips was significantly greater (P < 0·01) than that of unabraded plain steel strips.

From these figures it was possible to show that abrasion had caused a marked increase in the rate of corrosion and that the effect of "pattern" had probably been slight. The important point to note, however, is that in all three tests there was considerable overlap

in the individual results of the two sets of readings, and it was only by statistical analysis that the significance of the means could be ascertained.

Example 2—The second example, which is taken from a paper by Adam and Gillespy,[2] is concerned with the effect of spraying plum trees with boric acid to check the formation of gum-spots in the fruit. The results are shown in Table II, where the mean percentages of gummed fruits in the six untreated and six treated trees were 71·7 and 47·1, but, although the extent of gumming of the treated trees fluctuated widely, the means for the two sets of trees were significantly different ($P < 0{\cdot}05$). The treatment could thus be regarded as having resulted in a reduction in gumming.

In dealing with percentages, where the limits of variation are 0 to 100, each percentage p may be transformed to angular degrees using the formula $p/100 = \sin^2\phi$, the values obtained for ϕ being used in the test for significance. This angular transformation is of value where the percentages vary considerably, and was used on the data shown in Tables II and III.

A second experiment was made to show the effect of injecting boric acid into the branches of plum trees and here a higher degree of control was obtained by making injections of boric acid into one arm of a forked branch and distilled water into the other arm. By this means each test was paired with its own control. The results are shown in Table III where the two means—69·4 and 44·7 per cent.—are again significantly different ($P \simeq 0{\cdot}05$).

TABLE III

EFFECT OF BORIC ACID INJECTIONS

Tree	Percentage gummed fruits		Transformed percentages		Differences
	Control	With boric acid	Control	With boric acid	
I	56·5	19·5	48·7	26·2	+22·5
II	56·8	47·4	48·9	43·5	+ 5·4
III	66·4	31·3	54·5	34·0	+20·5
IV	87·4	37·1	69·2	37·6	+31·6
V	80·7	50·0	64·0	45·0	+19·0
VI	68·7	83·0	56·0	65·6	− 9·6
Mean	69·42	44·72	56·88	41·98	+14·90 (n = 6)

Sum of squared deviations of differences .. .. .. = 1075·3

Degrees of freedom, n − 1 .. = 5

Standard deviation of differences = $\sqrt{\dfrac{1075{\cdot}3}{5}}$

S.E. of mean of differences .. = $\sqrt{\dfrac{1075{\cdot}3}{5 \times 6}} = 5{\cdot}99$

Difference between means .. = 14·90

$t = 2{\cdot}49$ $P \simeq 0{\cdot}05$

Many other examples could be given of the use of this type of statistical analysis in problems connected with field trials, analytical technique and processing tests in research on food canning.

CORRELATION COEFFICIENTS—

In scientific investigations it is often important to ascertain whether two or more factors influence one another, and in such circumstances the degree of association can be estimated by means of the *correlation coefficient*, which takes into account not only the dispersion of each variate separately but also the extent to which the deviation of one variate from its mean affects (or is affected by) the deviation of the other variate from its mean. Where the effect of several factors is being studied it is sometimes possible to disclose a significant correlation of a pair of factors if the influence of one or more of the other variates is eliminated. This can be done by calculating the appropriate *partial correlation coefficient*. The use of correlation coefficients has been of great value in studying problems associated with food canning and has been preferred in most cases to the calculation of regression coefficients, in

spite of the fact that the utilisation of the data is less efficient where the former method is adopted.

Example 3—In fruit canning one of the heaviest sources of loss to the trade is due to the formation of "hydrogen swells." In lacquered cans hydrogen is formed chiefly by corrosion of the steel baseplate, and the effect of the minor constituents of the steel on the rate of formation of hydrogen in cans was studied statistically by Hoar, Morris and Adam.[3] Cans made from a representative "population" of 28 lots of hot-rolled tinplate were packed with each of the common fruits under standard controlled conditions and stored at a constant temperature until they became hydrogen swells. The steel base from each can was subsequently analysed and various laboratory corrosion tests were conducted on strips cut from the plate. Two of the various correlations worked out are given in Tables IV and V as examples of the information disclosed by the use of correlation coefficients—information which could not be obtained by experiments based on the deliberate control of each factor separately. Partial correlation coefficients were also worked out.

In Tables IV and V the letters used in the subscripts of the correlation coefficients are as follows: T = time required for cans to form hydrogen swells; S = sulphur content of steel; P = phosphorus content of steel; Cu = copper content of steel.

The results of the statistical analysis showed that the sulphur content of the steel base was not correlated with the rate of formation of hydrogen swells but that the phosphorus content showed a negative correlation and the copper content a positive correlation. From the metallurgical standpoint it was also interesting to note that the sulphur and phosphorus concentrations in the steel were positively correlated and the phosphorus and copper concentrations negatively correlated.

TABLE IV

CORRELATION OF TIME TO FORM HYDROGEN SWELLS WITH STEEL BASE COMPOSITION

Fruit	r_{TS}	r_{TP}	$r_{T\,Cu}$
Blackcurrants ..	− 0·33	− 0·54†	+ 0·55†
White cherries ..	− 0·02	− 0·23	+ 0·32
Gooseberries ..	− 0·25	− 0·47†	+ 0·50†
Loganberries ..	− 0·13	− 0·35*	+ 0·65†
Yellow plums ..	− 0·08	− 0·39*	+ 0·29
Raspberries ..	− 0·00	− 0·36*	+ 0·57†
Strawberries	− 0·23	− 0·47†	+ 0·37*

Levels of significance: r = 0·335 for P = 0·05 (marked *)
r = 0·430 for P = 0·01 (marked †)

TABLE V

CORRELATION BETWEEN CONSTITUENTS OF STEEL BASE

Fruit	r_{SP}	$r_{S\,Cu}$	$r_{P\,Cu}$
Blackcurrants ..	+ 0·79†	− 0·12	− 0·41*
White cherries ..	+ 0·71†	− 0·16	− 0·59†
Gooseberries ..	+ 0·77†	− 0·07	− 0·37*
Loganberries ..	+ 0·77†	− 0·07	− 0·36*
Yellow plums ..	+ 0·66†	− 0·13	− 0·53†
Raspberries ..	+ 0·69†	+ 0·10	− 0·50†
Strawberries ..	+ 0·78†	− 0·07	− 0·39*

Levels of significance: r = 0·355 for P = 0·05 (marked *)
r = 0·430 for P = 0·01 (marked †)

Correlation coefficients have also been used to demonstrate a relationship between the gumming of plums and the rainfall during the ripening period, and also to indicate which of several objective chemical or physical tests for the degree of maturity of canned peas agrees most closely with subjective tests based on visual and organoleptic impressions.

ANALYSIS OF VARIANCE—

It is sometimes convenient to introduce two or more test factors into a single experiment and yet to be able to determine the effect of each factor separately. This may be done, in a suitably designed experiment, by an *analysis of variance*. For this the variance of the whole of the measurements contained in the available data is first calculated. In its simplest

form the degree of this dispersion is due to two causes (*a*) the variation within each series tested (*i.e.*, errors due to random sampling) and (*b*) the differences between the means of the various series into which the data are grouped. In general, the variance of each factor, and that of any operative interaction between the factors, are compared with the residual error of the experiment. By using tables of the distribution of this variance ratio it is possible to find the degree of probability that the observed ratio could have occurred by chance, the usual levels of significance being accepted.

Analysis of variance appears to have been used very seldom in problems concerned with canning processes, and no suitable example can be quoted. On the other hand, this method of statistical analysis is commonly employed in connection with field trials on fruit and vegetables and has been used in a recent experiment on the effect of pre-cropping sprays on the reduction of blackcurrant leaf spot disease which was linked closely with a canning test. The results are described in a paper by Adam, Dickinson, and March.[4] There appears to be scope for the use of analysis of variance in connection with problems more directly concerned with the effects of the various canning operations and there is little doubt that experiments along these lines will be undertaken in the near future.

It is hoped that the examples given in this paper have shown that statistical methods can be of value in researches connected with food canning, provided attention is given to the proper design of the experiment. Statistical methods are of value to the analyst as well as to the research chemist, because they may disclose relationships not otherwise apparent between factors, and also because they encourage a healthy critical attitude towards the figures obtained from analytical determinations. Not only is the possibility of error thus recognised, but the probable extent of the error is estimated. It should be realised, however, that no more information can be extracted from the results than was permitted by the design and conduct of the experiment, and so the use of statistical methods cannot be regarded as a substitute for precise and accurate methods of chemical analysis. It is not suggested that statistical analysis is helpful or necessary in every class of experiment with which the food chemist is associated, but in certain types of chemical analyses, and in planning research work, the judicious use of these methods is now a well-established and commendable practice.

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The Evaluation of the Nutritive Value of Animal Feeding-stuffs

BY K. L. BLAXTER*

THE first stage of any investigation in any field is generally one of qualitative description, which is soon displaced by quantitative measurements. This is very evident in the nutrition of farm animals. Thus, Wolff's[1] first feeding standard for cattle displaced the much older qualitative observations, such as those made by Young[2] in his famous tours of England and Wales. A more recent example is the careful work of Guilbert and Hart[3] in determining the carotene requirements of ruminants, this work following the much older work[4,5] which indicated that a dietary deficiency of vitamin A or carotene would result in the death of cattle.

In the nutrition of large animals these quantitative measurements have as their final objective a practical problem: to formulate, from the available feeding stuffs, rations that are adequate to meet the requirements of the various classes of farm stock. I would like, to-day, to factorise this problem and to show several of the ways in which statistical method is of value in dealing with the problem.

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Perhaps the best method of approach is to describe the problem more fully, and to show the difficulties that arise. In feeding farm animals we have to consider the feeding stuffs themselves, the animal's ability to utilise them, and the requirement of the animal for the nutrients contained in the feeding stuffs.

In dealing with feeding stuffs it is often not realised how great is the variation in chemical composition between samples. Variations in the proportion of a constituent of over 100 per cent. as judged by coefficients of variation, are of common occurrence, especially in the vitamin contents of particular foods and in the protein content of hays. There is less variability with grains and processed feeding stuffs, but the fact remains that ruminants, our most important domesticated animals from an economic point of view, have a food supply for which no exact analytical figures can be predicted. Any mean estimate is subject to a large error, and although it is possible, by a system of sub-classification on the basis of a quality rating, to reduce this variation, the residual variability is still of the order of 25 to 35 per cent.

The first stage in the utilisation of any nutrient is its absorption from the digestive tract. Here again there is a very considerable variability, though little is known of its extent, largely owing to the expense of conducting experiments with large farm animals. In experiments at Weybridge[6] we have shown differences amounting to 10 per cent. between sheep from the same flock and of the same age, fed the same amount of food, in their ability to digest their ration. Similar differences have been recorded with dairy cows,[7] and these differences between individuals have been shown to persist for several years and to be true individuality factors. Even more disturbing in estimating the nutritive value of a particular food is the fact that the absorption of a nutrient from that food is affected by the ingredients forming the rest of the dietary. This interaction of food in digestion is particularly noticeable in the ruminant, in which associative digestibility effects amounting to nearly 40 per cent. have been recorded[8].

When we come to the requirement of the animal for particular nutrients an even more complex situation is evident. Farm animals are not uniform in relation to their food requirements, and even within a single breed individuals can vary very markedly. A more important feature which must be considered in estimating requirement, however, is the amazing ability of farm animals to adapt themselves to their nutritional regimen. In this respect Mitchell[9] has re-worded the theorem of Le Chatelier to read—"If an animal in equilibrium with its food supply is subjected to nutritional stress, such as an inadequate or excessive supply of one or more of the essential nutrients, the animal will react in such a way as to minimise or to undo entirely the effects of the nutritional stress." An example of this is the ability of the dairy cow to adapt herself to a low calcium dietary over many years[10].

This then is the background of variability with which one is dealing in research with large animals: a variable food supply, and a highly variable animal population, both from the point of view of their utilisation of feeding stuffs and their particular nutrient requirements. It follows that considerable care must be taken both in planning experiments to determine the nutritive value of food and in interpreting the results of such experiments if they are to be of general applicability. This variability has been recognised in the past, but has been regarded as very much of a nuisance and attempts have been made to minimise it. Such methods have reached perfection in studies on small laboratory animals, where animals have been bred to almost complete homozygosity, the environment is rigorously controlled throughout life, and highly purified diets are fed. Owing to the size of farm animals and their slow reproduction rate, such a method is difficult to apply to them, although the use of monozygotic twins and purified dietaries have recently become very useful techniques[11,12,13]. These methods, however, have one limitation. Although they tend to reduce variation and the general background of variability, they tend to make it more difficult to use the results inductively and to apply them direct to animals on the farm. In other words some of the information that has been lost in minimising variation would be of value in using the results to make estimates applicable to farm conditions. The method minimises rather than measures variation. Largely for this reason statistical method is of value in dealing with these problems of large animal nutrition, for by properly planning an experiment one is provided with the basis for induction, that is, for application of the results obtained in the experiment to the population as a whole.

Despite such a distinction, methods of controlling variation in animal experimentation, (*a*) by minimising it and (*b*) by measuring it, are not mutually antagonistic and are in many

ways complementary. Thus, sound experimental design is never an excuse for shoddy experimentation. The only distinction which we wish to draw is that our problem is such that, as we wish our results to be of wide application, then we should not feel at a disadvantage because it is difficult to minimise variation due to factors such as genotype, breed, nutritive status, etc., providing that by proper planning and design of our experiment we can measure the magnitude of the variation due to these factors. In any experiment it is logical to minimise some factors—those which are of no value in increasing the precision of our mean estimate of the effect. We can conclude therefore that in studies with large animals, where our results are to be applied to the population as a whole, variability must be measured and taken into account in any estimation of nutrient requirement or dietary adequacy.

This attitude, however, may in certain respects be regarded as of the nature of a stop-gap. It states that we should endeavour to measure by statistical method the variation due to factors over which we have no very adequate laboratory control, and take them into account in the estimation of food values and requirements. The superior method is to find the cause-and-effect mechanism for each particular variation and to correct the mean estimates for each particular set of conditions. This is the ultimate aim, but until it is possible to measure each cause and its effect the wider view of the variance of a requirement is perhaps the better one.

As an example of the use of this method of approach we can take a specific one relating to a dietary requirement. Thus, an estimate of the population variance of the calcium requirement of adult wether sheep would enable us to fix a value for calcium requirement such that only one animal out of every hundred would not receive sufficient. If we then found that we could separate from this total variation an age factor such that the older animals required more calcium to maintain calcium equilibrium, then an estimate of requirement based on such an age distinction would be a much better estimate of requirement. Even so, within each age group there would still remain a variation due to other factors which had not been separated—those hereditary, environmental and dietary factors which vary in the whole population. The initial estimate of variability would include all factors affecting the calcium requirement, and it is the factorisation of this variability that increases the precision with which the individual's requirement is estimated.

In large animal nutrition we still know but few of the factors that cause variation, and of those factors we have quantitative data for only a small proportion. In order to make sure that an individual receives the correct amount of food we can at least measure this variation and take it into account, even though we do not know exactly what it is, other than a rather vague "between animal" or "between flock" variation.

While statistical technique is of value in helping the interpretation of experimental results in this way, when we come to the problem of estimating experimental effects themselves statistical technique is of even greater value. We have to measure these effects in the nutrition of farm animals in terms of body growth, milk yield, egg production, nutritional balance or blood levels. These measurements are subject not only to errors of observation but also to variation due to factors other than the specific nutritional one under observation. In the case of a cow's milk yield, for instance, the season of the year, her age, how long since she has calved, whether she is pregnant, her hereditary constitution, the presence of disease organisms and other factors all affect her milk yield. By analogous reasoning we can in part minimise this variation and in part measure it in order to control it effectively. We can minimise variation due to disease incidence by using only healthy cows as our experimental animals, and by making sure that the process of milking the cows is standardised we can minimise a further portion of the total variation, for this too is a potent cause of variation in milk yield. Owing to the difficulty of selecting cows that are exactly the same in respect of the other non-nutritional causes of variation, the methods of analysis of variance and co-variance are those with which we have to deal.

To take a specific example. If we are concerned with measuring a nutritional effect on milk yield we can compare directly one group of cows with another, one group receiving a control diet and the other the experimental diet under consideration. The difference in the milk yields of the cows in this case will be subject to innumerable causes of variation besides the one under review; in fact, if there were six cows in each group, the difference which one could detect as due to the treatment difference would have to be of the order of 50 per cent. before we were safely outside the range of variation due to non-nutritional factors. Our first step is to place a restraint in the design, so that we can measure and remove

part of this variation. This can be done by pairing the cows initially on the basis of their age or productivity. By use of the analysis of variance we can obtain an estimate of this variation and reduce the residual variability, thus increasing the accuracy of our mean estimate of the effect of the dietary. This single restraint will reduce the difference we can measure to 25 per cent.[14] Further introduction of obvious causes of variation into the pairs, by pairing on the basis of both age and stage of lactation, results in further increases in precision. The second step is to use co-variance analysis to measure that part of the variation in milk yield which is due to the variation in their milk production before the experiment was started. This results in further increases in precision, enabling us now to estimate differences of the order of 12 per cent.[14] The next stage can be the introduction of restraints on a factorial plan, which results in further slight reductions in the size of our error variance, but an even greater advance is to measure that particular term with which we are to be concerned, the variation between cows, similar in all respects, that is due to the effect of the ration under consideration. This is done by a change-over in the type of design so that each cow receives each treatment in turn, and the experiment is so designed as to include the restraint introduced by the pairing technique, and to make possible the measurement of any general change in milk yield from one period to another. Such designs have a high precision and result in an estimation also of the variation with which we have been concerned previously—the variation between individuals in the effect of a particular ration, as measured in this case by milk yield changes. With more than two treatments under consideration the gain by using these designs is much greater, and it has been found possible to measure effects of only 5 per cent.[14,15] by using developments in experimental design of the type we have just considered.

Such an increase in the precision of our experimental result, its freedom from variation due to extraneous sources of variation with which we are not concerned, together with a valid estimate of the variation between animals due to the experimental treatment, is a great advance on the older methods of experimentation, in which attempts were always made to reduce all variability to the minimum. These methods are of general applicability to most nutritional problems and their use has an added advantage in that experimental effects do not now need to be so drastic nor differences in dietary so large as formerly. Without the measurement of variability our conclusions would have had but little significance.

In the interpretation and application of the results of a single experiment such as this one, several factors have to be considered before arguing from the sample to the population. An important one is due to the fact that in practice the herd, rather than the individual, tends to be the unit of rationing. The variability of the requirement is thus not necessarily the standard deviation of the individual requirement, but rather the standard error of a herd mean or total requirement. For this and other reasons there has been an attitude that for practical application one must have practical conditions. This has been the attitude taken by the workers dealing with group experimentation,[16] in which groups of animals are compared, without replication of the groups, because such replication is considered unnecessary if the groups are sufficiently large. In such circumstances it is impossible to calculate the particular term needed—the variance of the difference between the groups which is due to the dietary effect.

This position is much complicated also by the fact that the herd or flock tends to behave as a unit so far as variability is concerned. Thus, differences in nutritive status, breed, genetic efficiency and food composition, are largely herd differences and not differences to be found between animals of the same herd. We are thus led to the conclusion that the herd or flock by treatment interaction is the best measure of the variability of a requirement or the nutritive value of a food, so far as the use of such figures for application to the whole population is concerned. This in turn suggests that the planning of experiments to determine the nutritive value of foods, or the requirement of an animal, should include a replication on the unit of a herd or flock. The range of variability of the requirement will then be given by two components of the total variance, one dependent on the number of animals making up the herd and the other derived from the interaction term. It is possible that, in the future, the nutrient requirement of an animal will be expressed in this form, and the need for an arbitrary correction to allow for the possible inefficient animal or for the wide differences in conditions will be replaced by an exact measure of this variability.

These are but two of the ways in which statistical method helps in large animal research. Its application to the problems of the individual experiment in assessing the significance of,

and making allowance for, the errors caused by disturbing influences and in reducing the mass of experimental data is the more obvious one, and the one which is often most accentuated in any discussion of the value of statistical method. The milk yield example is an example of this type.

The importance of the other aspect of statistical method, however, is often overlooked, and it is often forgotten that statistics are the mathematics of inductive reasoning. I have tried to show that in large animal nutrition where one has to think in terms of application, the role of statistical method in planning an investigation that is to be used as a basis for nutritional standards is a primary one, especially in view of the large background of variability which we have discussed. We can conclude, therefore, by saying that in nutritional research with farm animals statistical technique provides a method of controlling and measuring variation which is complementary to the refined techniques of animal experimentation that can be used to minimise unwanted variation. As such, statistical method must be an integral part of every worker's equipment in attacking the complex relationships that are apparent in the study of the nutritive value of foods and the nutrient requirements of farm animals.

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Application of Statistical Methods in Calculating Proportions of Ingredients in certain Food Products

BY E. H. STEINER

A MAJOR problem with which food chemists and analysts are frequently confronted is the estimation of the proportion of certain raw ingredients in processed or otherwise treated foods. By the term "raw ingredient" is meant such materials as meat, fruit, eggs or milk, which form the basis of all the more important food products. The determination of the proportion of any of these ingredients involves the estimation of the amount present of one or more proximate constituents, such as protein, soluble solids, sugar or ash, which are characteristic of the particular ingredient. The percentage of the constituent determined in the food product is compared with the percentage in the ingredient itself, and by dividing one by the other (and multiplying by 100) the percentage of ingredient present is found.

In practice, however, it is found that the amounts of any constituents present in the raw ingredients themselves exhibit considerable natural variation. It is thus impossible to assign, say, a definite insoluble-solids content to strawberries or an invariable percentage of protein

to pork. It is generally realised that this variation exists in so far as it is customary to collect data for the values of the constituent in the ingredient, and to use the arithmetic mean of all the values as divisor in calculating the percentage of ingredient in the processed material. The percentage so calculated is then regarded as the most likely one for the sample of material in question. This percentage may not be the true one, as the particular specimen (unknown) of the raw ingredient incorporated might have a value for the constituent determined greater or less than the mean value used in the calculation. Although this is recognised, there is not generally any indication of the reliability of the "most likely" percentage or of what may be considered to be the range within which the true percentage of the ingredient probably lies. It is in the attempt to assign a measure to this range that the value of the statistical approach becomes evident.

For a statistical treatment of the data it is convenient to regard any specimen or sample of a particular raw ingredient as a member of a population consisting of all possible specimens. The total of all possible members will be termed the *ingredient* population, and each specimen an individual. For example, any sample of strawberries analysed is considered as an individual selected from a strawberry population consisting of all possible samples. The ingredient population is associated with a *constituent* population comprising the actual values of the constituent determined in the individuals of the ingredient population. Thus we may consider the population of percentages of insoluble solids or of free acid in strawberry samples.

The varying values encountered for the constituent determined in different samples of an ingredient are, therefore, to be regarded simply as individuals, which belong to a population that is obviously so large that it may be considered infinite. The usual statistical measure of variation in any population is the standard deviation, which is a measure of the degree of scatter of the individual values in the constituent population about the mean value.

In all the cases examined the distribution of constituent values form symmetrical bell-shaped curves with the most frequently occurring values at or near the mean, and the frequency diminishing regularly the further the values are from the mean, in a manner that closely approximates to the theoretical normal distribution.

It is the property of such distributions that the frequency of occurrence of values greater or less than any given deviation from the mean depends solely on the standard deviation of the population. For example, 32 values out of 100 will deviate from the mean by an amount greater than the standard deviation itself; 5 values out of 100 will deviate by an amount greater than twice the standard deviation. Stated in terms of probability there is a probability of 0·68 that a value obtained will lie within a range of plus or minus the standard deviation from the mean, and a probability of 0·95 that it will lie within twice the standard deviation.

Owing to the impossibility of examining all individuals from the constituent population, neither the true mean nor the true standard deviation can be found exactly. Provided, however, that these values have been estimated from about 50 or more individuals selected at random from the population, the error involved will not be appreciable for present purposes. This point will be taken up again later.

Calculation of the probable limits to the proportion of ingredients

In applying these considerations to the analysis of food products each food is regarded as comprising one ingredient to be estimated. The remainder of the material will be treated as a diluent of that particular ingredient, whether this diluent consists only of a single material, *e.g.*, water, as in fruit pulps, or of several materials, as in jams, meat pastes or sausages. It will be assumed, first, that the exact value of the constituent determined in the material is known—that is, that no experimental error is involved in the analytical determination—and that the whole of the constituent as determined is contributed by the ingredient itself. (This is not so with some foodstuffs: with sausages, for example, allowance must be made for protein contributed by the cereal.) With these assumptions, the proportion of ingredient in the foodstuff is calculated as a ratio of two amounts of the constituent, one of which (the numerator) is known exactly, being found by analysis of the foodstuff itself, whilst the other (the proportion in the ingredient used) can have any one of a set of values which are distributed normally about a known mean with known standard deviation.

As mentioned previously it is possible to state with any desired degree of probability a range within which the value of the constituent in the ingredient lies. The question that then arises is what is the degree of probability desirable in fixing the limits to the ingredient

content? Obviously, the more certain we want to be, the wider apart must the limits to the probable value lie. Conversely, by making the limits closer together, the greater becomes the probability that the true proportion of ingredient may in fact lie outside the given limits. The degree of probability to be decided on is quite arbitrary, and it is proposed to adopt as probable limits those values of the constituent which are not exceeded by more than five values out of every hundred, *i.e.*, the limiting ordinates to the distribution curve will cut off 95 per cent. of the total area under the curve. This is usually referred to as a probability level of 0·05.

The figure that is normally of most importance to the analyst is the probable upper limit to the proportion of ingredient, as it is usually required to determine whether the food product complies with some specified standard. In adopting a probability level of 0·05 we stand to make a wrong decision once in forty times. This is because 2·5 out of every 100 of the constituent values for the ingredient may in fact lie below the lower limit (which fixes the upper limit to the proportion of ingredient, owing to the reciprocal relationship). That is, once in forty times we may reject a food product as being below standard, when in fact it is not so. Any attempt to reduce this chance of a wrong decision by widening the probable limits increases the chance of accepting a product as up to standard when in fact it is deficient in ingredient; nothing can be said as to the probability of making an error of this kind except that the narrower the limits chosen the less will be the chance of this error. The probability level of 0·05 is, therefore, adopted in the attempt to strike a balance between the risks of these two kinds of error (see Appendix).

In the "normal distribution" curve the ordinates which cut off 95 per cent. of the total area under the curve are situated at distances from the mean of $\pm 1{\cdot}96$ times the standard deviation. Hence, if

z = percentage of the constituent determined in a food product.
$\bar{x}$ = mean of all possible percentages x of the constituent in the ingredient itself.
σ = standard deviation of the constituent population.
I = percentage of ingredient in the food product.
x_1 = probable lower limit of value of determined constituent in the ingredient itself.
x_2 = probable upper limit of value of determined constituent in the ingredient itself.
I_L = probable lower limit of percentage of ingredient in food product.
I_U = probable upper limit of percentage of ingredient in food product.

we have (at a probability level of 0·05)

$$x_1 = \bar{x} - 1{\cdot}96\,\sigma \qquad x_2 = \bar{x} + 1{\cdot}96\,\sigma \quad \ldots \quad (1)$$

and

$$I_L = \frac{100\,z}{\bar{x} + 1{\cdot}96\,\sigma}\ \text{per cent.} \qquad I_U = \frac{100\,z}{\bar{x} - 1{\cdot}96\,\sigma}\ \text{per cent.} \quad \ldots \quad (2)$$

It is convenient to use the coefficient of variation as a measure of variation rather than the standard deviation itself, as it affords a better basis for the comparison of variation between different populations. If the coefficient of variation is denoted by V, then $V = 100\,\sigma/\bar{x}$. Also, the apparent, or most likely, proportion of ingredient is given by $I_A = 100\,z/\bar{x}$. Substituting these values in (2) gives the following equations for the lower and upper limits to the probable percentage of ingredient

$$I_L = I_A/(1 + 0{\cdot}0196\,V)\ \text{per cent.} \quad \ldots \quad (3a)$$

$$I_U = I_A/(1 - 0{\cdot}0196\,V)\ \text{per cent.} \quad \ldots \quad (3b)$$

It is seen from these relationships that the range of probable values of the proportion of ingredient above the most likely value is greater than the range below it. This is a consequence of the fact that the distribution of the proportion of ingredient in food products containing a *particular* constituent value is not a normal one.

It should be emphasised that the underlying assumption in equations (3*a*) and (3*b*) is that the values in the constituent population are "normally distributed." In so far as the constituent values in the ingredient are the result of a large number of complex natural processes operating together this is likely to be so, to a fairly close approximation. The values obviously cannot form a complete normal distribution, as this would imply the existence of proportions of the constituent less than zero and greater than 100 per cent. For any bell-shaped distribution, whether normal or not, the minimum proportion of values lying within the range $\pm 1{\cdot}96$ times the standard deviation is about 88·5 per cent.[1] Thus, in the absence of any knowledge about the nature of the distribution of constituent values, other

than that it gives a bell-shaped frequency curve, the use of equation (3*b*) will certainly not lead to the rejection of a food product wrongly more than 5·75 times in 100. That is, in the worst case we might expect to condemn a product wrongly about 1 in 17 times. As already remarked, however, the distribution of constituent values is unlikely in practice to depart far from normality.

APPLICATION TO FRUIT PULPS

To illustrate equations (3*a*) and (3*b*) some figures are given for the percentage of fruit in fruit pulps. The pulps are regarded simply as fruits diluted with water, so that the equations are strictly applicable. The percentage of soluble solids is taken as the chemical constituent determined by analysis, and the distribution of soluble solids percentages in various fruits has been worked out from data collected over a large number of years.[2] These distributions are approximately normal. Table I shows the probable range of fruit contents for various apparent percentages of fruit, for four different varieties.

TABLE I

RANGES OF PROBABLE FRUIT CONTENTS IN PULPS CONTAINING DIFFERENT APPARENT PERCENTAGES OF FRUIT

Fruit	Coefficient of variation, V	Apparent percentage of fruit, I_A			
		60 per cent.	70 per cent.	80 per cent.	90 per cent.
Strawberry ..	10·7	50 to 76	58 to 89	66 to 101	74 to 114
Gooseberry ..	11·0	49 to 76	58 to 89	66 to 102	74 to 115
Blackcurrant ..	16·6	45 to 89	53 to 104	60 to 119	68 to 133
Plum	18·0	44 to 93	52 to 108	59 to 124	67 to 139

It is clear from Table I that the range increases very rapidly with the coefficient of variation. Thus the range for plums is considerably wider than that for strawberries. In view of the fact, however, that the fruit content of a pulp cannot exceed 100 per cent., it is possible to restrict the upper limit of probable fruit content to this figure, which will considerably shorten the range.

If it is possible from manufacturing considerations to assign a maximum upper limit to the proportion of ingredient in a particular food product, this additional information should theoretically reduce the range of probable proportions even where the maximum limit is not reached. The change produced in the range by restricting the permissible upper limit is usually very small in these cases, and an exact calculation of the range is not easy. For present purposes it is sufficient to calculate the range on the assumption that all values are possible and subsequently to remove from the range any values known to be impossible.

Some other examples in the analysis of food products where equations (3*a*) and (3*b*) would be applicable are the determinations of the shell content of cocoa, the coffee content of coffee essence or the water content of milk.

EFFECT ON THE RANGE OF PROBABLE COMPOSITION WHEN ONLY PART OF THE CONSTITUENT DETERMINED IS DUE TO THE INGREDIENT

In the previous discussion it has been assumed that the amount of the chemical constituent due to the ingredient, in the food product, was known exactly. This is not so when the constituent that is being determined occurs not only in the ingredient itself but also in some component of the diluent in the food product. In this event the portion of the constituent due to the ingredient is ascertained by subtracting the amount contributed by the diluent from the total percentage of constituent in the foodstuff. In general, the proportion of the constituent in the diluent is not known exactly, as different specimens of the diluent may possess different percentages of the constituent. In fact, the same sort of uncertainty applies to the value of the constituent in the diluent as to the value in the ingredient itself. Thus, in the ratio used to calculate the proportion of ingredient, both numerator and denominator become variable quantities. This is, therefore, a generalisation of the simpler case already dealt with.

In order to prevent the argument from becoming too abstract the method of treating this general problem will be developed for the particular example of the meat content of sausages. To calculate the lean meat content it is necessary to know that part of the total protein of the sausage which is due to the meat. In other words, allowance has to be made

for the percentage of protein contributed by the cereal, which is then subtracted from the total percentage of protein. In the present procedure it is assumed that the cereal protein is calculated from the carbohydrate content of the sausage by multiplying this by the value of the ratio protein/carbohydrate found in cereals. This ratio is variable and introduces an element of uncertainty into the calculation of the cereal protein and, therefore, of the meat protein also. The values of the ratio protein/carbohydrate occurring in different specimens of cereals will constitute a population whose frequency distribution can be determined by analysis.

Let z_T = total percentage of constituent (protein) in the food product (sausage).

z_D = percentage of constituent (protein) in food product due to diluent (cereal).

z = percentage of constituent (protein) in food product due to ingredient (lean meat).

$\bar{x}$ = mean of all percentages x of the constituent (protein) in the ingredient (lean meat).

σ_x = standard deviation of the percentages of the constituent (protein) in the ingredient (lean meat).

u = percentage of carbohydrate in the sausage.

$\bar{q}$ = mean of all values q of the protein/carbohydrate ratio in cereals.

σ_q = standard deviation of the protein/carbohydrate ratio population.

P = proportion of ingredient (lean meat) as a fraction of the whole in the food product (sausage).

For a particular sample of sausages analysed the percentage of carbohydrate u and of total protein z_T are both known and therefore are fixed. There will then be a hypothetical population comprising the different percentages of the cereal protein in all possible sausages containing the percentage u of carbohydrate.

These possible protein percentages are given by

$$z_D = u\,q \qquad (4a)$$

and will be distributed about a mean $u\bar{q}$ with standard deviation $u\sigma_q$. Also in the population of all sausages containing the percentage z_T of total protein (as well as the percentage u of carbohydrate) the possible percentages of meat protein given by

$$z = z_T - z_D = z_T - uq \qquad (4b)$$

will have a mean value $\bar{z} = z_T - u\bar{q}$ and standard deviation $\sigma_z = u\sigma_q$ (since z_T is fixed). If the original population of protein/carbohydrate ratios is normal, the population of differences $z_T - uq$ will also be normal. The proportion of meat in the sausage is then calculated as the ratio z/x of two quantities each of which is distributed normally.

Though this discussion has related to the special example of sausages, the argument is perfectly general. Whenever part of the chemical constituent determined in the food product arises from a component of the diluent, and has to be determined from the value of some other constituent, the proportion of ingredient will be given by the ratio of two variable quantities as above.

The problem now reduces to that of finding the probable limits to the value of the quotient of two normally distributed variables. Unfortunately the quotient, not being a linear function of the variables, is not itself normally distributed. It has been shown,[3] however, that if P = z/x, where x and z are independent normal variables, and x is unlikely to be negative, then the quantity w is normally distributed about zero with standard deviation equal to unity, where

$$w = \frac{P\bar{x} - \bar{z}}{\sqrt{P^2\sigma_x^2 + \sigma_z^2}}. \qquad (5)$$

It follows that probable limits to the value of w, which will not be exceeded more than 5 times in 100, are $\pm 1{\cdot}96$. Hence, if P_1 and P_2 are the lower and upper limits to the probable proportion of ingredient, then

$$P_1\bar{x} - \bar{z} = -1{\cdot}96\sqrt{P_1^2\sigma_x^2 + \sigma_z^2}$$

and

$$P_2\bar{x} - \bar{z} = +1{\cdot}96\sqrt{P_2^2\sigma_x^2 + \sigma_z^2}.$$

TABLE IIa.—PROBABLE RANGE *above* AN APPARENT INGREDIENT CONTENT OF 100 PER CENT.* WITHIN WHICH THE TRUE PERCENTAGE OF INGREDIENT LIES (PROBABILITY = 0·95)

V_x \ V_z	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0	0·0	2·0	3·9	5·9	7·8	9·8	11·8	13·7	15·7	17·6	19·6	21·6	23·5	25·5	27·4	29·4	31·4	33·3	35·3	37·2	39·2
1	2·0	2·8	4·4	6·2	8·1	10·0	12·0	13·9	15·8	17·8	19·7	21·7	23·6	25·6	27·6	29·5	31·5	33·4	35·4	37·3	39·3
2	4·1	4·5	5·7	7·2	8·9	10·7	12·6	14·4	16·3	18·2	20·2	22·1	24·0	26·0	27·9	29·8	31·8	33·7	35·7	37·6	39·6
3	6·2	6·6	7·4	8·7	10·2	11·8	13·5	15·3	17·1	19·0	20·8	22·7	24·6	26·5	28·5	30·4	32·3	34·2	36·2	38·1	40·1
4	8·5	8·7	9·4	10·5	11·8	13·2	14·8	16·5	18·2	20·0	21·8	23·6	25·5	27·4	29·2	31·1	33·0	35·0	36·9	38·8	40·7
5	10·9	11·1	11·6	12·5	13·6	14·9	16·4	17·9	19·6	21·3	23·0	24·8	26·6	28·4	30·3	32·1	34·0	35·9	37·8	39·7	41·6
6	13·3	13·5	14·0	14·7	15·7	16·9	18·2	19·7	21·2	22·8	24·5	26·2	27·9	29·7	31·5	33·3	35·2	37·0	38·9	40·8	42·6
7	15·9	16·0	16·4	17·1	18·0	19·0	20·3	21·6	23·0	24·6	26·1	27·8	29·5	31·2	32·9	34·7	36·5	38·3	40·2	42·0	43·9
8	18·6	18·7	19·1	19·7	20·4	21·4	22·5	23·8	25·1	26·5	28·1	29·6	31·3	32·9	34·6	36·4	38·1	39·9	41·7	43·5	45·3
9	21·4	21·5	21·8	22·4	23·1	24·0	25·0	26·1	27·4	28·8	30·2	31·7	33·2	34·9	36·5	38·2	39·9	41·6	43·4	45·2	47·0
10	24·4	24·5	24·8	25·2	25·9	26·7	27·6	28·7	29·9	31·2	32·5	34·0	35·5	37·0	38·6	40·2	41·9	43·6	45·3	47·1	48·9
11	27·5	27·6	27·8	28·3	28·9	29·6	30·5	31·5	32·6	33·8	35·1	36·5	37·9	39·4	40·9	42·5	44·1	45·8	47·5	49·2	50·9
12	30·8	30·8	31·1	31·5	32·0	32·7	33·5	34·5	35·5	36·7	37·9	39·2	40·6	42·0	43·5	45·0	46·6	48·2	49·9	51·5	53·2
13	34·2	34·3	34·5	34·9	35·4	36·0	36·8	37·7	38·7	39·7	40·9	42·1	43·5	44·8	46·3	47·8	49·3	50·9	52·5	54·1	55·8
14	37·8	37·9	38·1	38·4	38·9	39·5	40·2	41·1	42·0	43·0	44·1	45·3	46·6	47·9	49·3	50·7	52·2	53·8	55·3	56·9	58·6
15	41·6	41·7	41·9	42·2	42·7	43·2	43·9	44·7	45·6	46·6	47·6	48·8	50·0	51·2	52·6	54·0	55·4	56·9	58·4	60·0	61·6
16	45·7	45·7	45·9	46·2	46·6	47·2	47·8	48·6	49·4	50·3	51·3	52·4	53·6	54·8	56·1	57·5	58·9	60·3	61·8	63·3	64·9
17	50·0	50·0	50·2	50·5	50·9	51·4	52·0	52·7	53·5	54·4	55·3	56·4	57·5	58·7	59·9	61·2	62·6	64·0	65·4	66·9	68·5
18	54·5	54·6	54·7	55·0	55·4	55·8	56·4	57·1	57·8	58·7	59·6	60·6	61·7	62·8	64·0	65·3	66·6	68·0	69·4	70·8	72·3
19	59·3	59·4	59·5	59·8	60·1	60·6	61·1	61·8	62·5	63·3	64·2	65·2	66·2	67·3	68·5	69·7	71·0	72·3	73·7	75·1	76·5
20	64·5	64·5	64·7	64·9	65·2	65·7	66·2	66·8	67·5	68·3	69·1	70·1	71·0	72·1	73·2	74·4	75·7	76·9	78·3	79·7	81·1

* Probable range above any other apparent ingredient percentage I_A is obtained by multiplying the figure in the table by the factor $I_A/100$.

TABLE IIb—PROBABLE RANGE *below* AN APPARENT INGREDIENT CONTENT OF 100 PER CENT.* WITHIN WHICH THE TRUE PERCENTAGE OF INGREDIENT LIES (PROBABILITY = 0·95)

V_x \ V_z	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0	0·0	2·0	3·9	5·9	7·8	9·8	11·8	13·7	15·7	17·6	19·6	21·6	23·5	25·5	27·4	29·4	31·4	33·3	35·3	37·2	39·2
1	1·9	2·7	4·3	6·2	8·0	9·9	11·9	13·8	15·8	17·7	19·7	21·6	23·6	25·5	27·5	29·4	31·4	33·4	35·3	37·3	39·2
2	3·8	4·2	5·4	6·9	8·6	10·4	12·2	14·1	16·0	17·9	19·9	21·8	23·7	25·6	27·6	29·5	31·5	33·4	35·4	37·3	39·3
3	5·6	5·9	6·7	8·0	9·5	11·1	12·8	14·6	16·4	18·3	20·2	22·1	24·0	25·9	27·8	29·7	31·6	33·5	35·5	37·4	39·4
4	7·3	7·5	8·2	9·2	10·5	12·0	13·6	15·2	17·0	18·8	20·6	22·4	24·3	26·1	28·0	29·9	31·8	33·7	35·6	37·6	39·5
5	8·9	9·1	9·7	10·6	11·7	13·0	14·4	16·0	17·6	19·3	21·1	22·9	24·7	26·5	28·3	30·2	32·1	33·9	35·8	37·7	39·6
6	10·5	10·7	11·2	11·9	12·9	14·1	15·4	16·9	18·4	20·0	21·7	23·4	25·1	26·9	28·7	30·5	32·4	34·2	36·1	38·0	39·8
7	12·1	12·2	12·6	13·3	14·2	15·2	16·4	17·8	19·2	20·7	22·3	24·0	25·6	27·4	29·1	30·9	32·7	34·5	36·4	38·2	40·1
8	13·6	13·7	14·0	14·6	15·4	16·4	17·5	18·7	20·1	21·5	23·0	24·6	26·2	27·9	29·6	31·3	33·1	34·9	36·7	38·5	40·3
9	15·0	15·1	15·4	15·9	16·7	17·5	18·6	19·7	21·0	22·3	23·8	25·3	26·8	28·4	30·1	31·8	33·5	35·2	37·0	38·8	40·6
10	16·4	16·5	16·8	17·2	17·9	18·7	19·7	20·7	21·9	23·2	24·6	26·0	27·5	29·0	30·6	32·3	33·9	35·6	37·3	39·1	40·9
11	17·7	17·8	18·1	18·5	19·1	19·9	20·7	21·7	22·9	24·1	25·4	26·7	28·2	29·7	31·2	32·8	34·4	36·0	37·7	39·5	41·2
12	19·0	19·1	19·4	19·8	20·3	21·0	21·8	22·8	23·8	25·0	26·2	27·5	28·9	30·3	31·8	33·3	34·9	36·5	38·2	39·8	41·5
13	20·3	20·4	20·6	21·0	21·5	22·1	22·9	23·8	24·8	25·9	27·0	28·3	29·6	31·0	32·4	33·9	35·4	37·0	38·6	40·2	41·9
14	21·5	21·6	21·8	22·2	22·6	23·2	24·0	24·8	25·7	26·8	27·9	29·1	30·3	31·6	33·0	34·5	36·0	37·5	39·1	40·7	42·3
15	22·7	22·8	23·0	23·3	23·8	24·3	25·0	25·8	26·7	27·7	28·7	29·9	31·1	32·3	33·7	35·1	36·5	38·0	39·5	41·1	42·7
16	23·9	23·9	24·1	24·4	24·9	25·4	26·0	26·8	27·6	28·5	29·5	30·6	31·8	33·0	34·3	35·7	37·1	38·5	40·0	41·5	43·1
17	25·0	25·0	25·2	25·5	25·9	26·4	27·0	27·7	28·5	29·4	30·4	31·4	32·5	33·7	35·0	36·3	37·6	39·0	40·5	42·0	43·5
18	26·1	26·1	26·3	26·6	27·0	27·4	28·0	28·7	29·4	30·3	31·2	32·2	33·3	34·4	35·6	36·9	38·2	39·6	41·0	42·4	43·9
19	27·1	27·2	27·3	27·6	28·0	28·4	29·0	29·6	30·3	31·1	32·0	33·0	34·0	35·1	36·3	37·5	38·8	40·1	41·5	42·9	44·3
20	28·2	28·2	28·4	28·6	29·0	29·4	29·9	30·5	31·2	32·0	32·8	33·8	34·8	35·8	36·9	38·1	39·4	40·7	42·0	43·4	44·8

* Probable range below any other apparent ingredient percentage I_A is obtained by multiplying the figure in the table by the factor $I_A/100$.

TABLE III—LOWER LIMIT OF APPARENT PERCENTAGE OF INGREDIENT BELOW WHICH NOT MORE THAN ONE IN FORTY SAMPLES SHOULD LIE (SPECIFIED VALUE = 100 PER CENT.*)

V_x \ V_z	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0	100·0	98·1	96·2	94·4	92·7	91·1	89·5	87·9	86·4	85·0	83·6	82·3	81·0	79·7	78·5	77·3	76·1	75·0	73·9	72·9	71·8
1	98·0	97·3	95·8	94·1	92·5	90·9	89·3	87·8	86·3	84·9	83·5	82·2	80·9	79·6	78·4	77·2	76·1	75·0	73·9	72·8	71·8
2	96·1	95·7	94·6	93·3	91·8	90·3	88·8	87·4	86·0	84·6	83·2	81·9	80·6	79·4	78·2	77·0	75·9	74·8	73·7	72·7	71·6
3	94·1	93·8	93·1	92·0	90·8	89·4	88·1	86·7	85·4	84·0	82·7	81·5	80·2	79·0	77·8	76·7	75·6	74·5	73·4	72·4	71·4
4	92·2	92·0	91·4	90·5	89·5	88·3	87·1	85·9	84·6	83·3	82·1	80·9	79·7	78·5	77·4	76·2	75·2	74·1	73·1	72·0	71·1
5	90·2	90·0	89·6	88·9	88·0	87·0	85·9	84·8	83·6	82·5	81·3	80·1	79·0	77·9	76·8	75·7	74·6	73·6	72·6	71·6	70·6
6	88·2	88·1	87·8	87·2	86·4	85·6	84·6	83·6	82·5	81·4	80·3	79·3	78·2	77·1	76·0	75·0	74·0	73·0	72·0	71·0	70·1
7	86·3	86·2	85·9	85·4	84·8	84·0	83·2	82·2	81·3	80·3	79·3	78·3	77·2	76·2	75·2	74·2	73·2	72·3	71·3	70·4	69·5
8	84·3	84·2	84·0	83·6	83·0	82·4	81·6	80·8	79·9	79·0	78·0	77·1	76·2	75·2	74·3	73·3	72·4	71·5	70·6	69·7	68·8
9	82·4	82·3	82·1	81·7	81·2	80·7	80·0	79·3	78·5	77·7	76·8	75·9	75·0	74·2	73·3	72·4	71·5	70·6	69·7	68·9	68·0
10	80·4	80·3	80·2	79·8	79·4	78·9	78·3	77·7	77·0	76·2	75·4	74·6	73·8	73·0	72·1	71·3	70·5	69·6	68·8	68·0	67·2
11	78·4	78·4	78·2	78·0	77·6	77·2	76·6	76·1	75·4	74·7	74·0	73·3	72·5	71·7	71·0	70·2	69·4	68·6	67·8	67·0	66·3
12	76·5	76·4	76·3	76·1	75·7	75·3	74·9	74·4	73·8	73·2	72·5	71·8	71·1	70·4	69·7	69·0	68·2	67·5	66·7	66·0	65·3
13	74·5	74·5	74·4	74·2	73·9	73·5	73·1	72·6	72·1	71·6	71·0	70·3	69·7	69·0	68·4	67·7	67·0	66·3	65·6	64·9	64·2
14	72·6	72·5	72·4	72·2	72·0	71·7	71·3	70·9	70·4	69·9	69·4	68·8	68·2	67·6	67·0	66·3	65·7	65·0	64·4	63·7	63·1
15	70·6	70·6	70·5	70·3	70·1	69·8	69·5	69·1	68·7	68·2	67·7	67·2	66·7	66·1	65·5	64·9	64·3	63·7	63·1	62·5	61·9
16	68·6	68·6	68·5	68·4	68·2	67·9	67·6	67·3	66·9	66·5	66·1	65·6	65·1	64·6	64·1	63·5	62·9	62·4	61·8	61·2	60·7
17	66·7	66·7	66·6	66·5	66·3	66·1	65·8	65·5	65·2	64·8	64·4	63·9	63·5	63·0	62·5	62·0	61·5	61·0	60·4	59·9	59·4
18	64·7	64·7	64·6	64·5	64·4	64·2	63·9	63·7	63·4	63·0	62·6	62·3	61·8	61·4	61·0	60·5	60·0	59·5	59·0	58·5	58·0
19	62·8	62·7	62·7	62·6	62·4	62·3	62·1	61·8	61·5	61·2	60·9	60·5	60·2	59·8	59·4	58·9	58·5	58·0	57·6	57·1	56·6
20	60·8	60·8	60·7	60·6	60·5	60·4	60·2	60·0	59·7	59·4	59·1	58·8	58·5	58·1	57·7	57·3	56·9	56·5	56·1	55·7	55·2

* Lower limit corresponding to any other specified percentage of ingredient I_s is obtained by multiplying the figure in the table by the factor $I_s/100$.

Either of these equations on squaring gives

$$P^2(\bar{x}^2 - 1{\cdot}96^2\sigma_x^2) - 2P\bar{x}\bar{z} + \bar{z}^2 - 1{\cdot}96^2\sigma_z^2 = 0,$$

where $P = P_1$ or P_2. The two values of P that satisfy this quadratic equation must, therefore, correspond to the respective values P_1 and P_2. Solving the equation to obtain these values gives

$$P = \frac{\bar{x}\,\bar{z} \pm \sqrt{\bar{x}^2\bar{z}^2 - (\bar{x}^2 - 1{\cdot}96^2\sigma_x^2)(\bar{z}^2 - 1{\cdot}96^2\sigma_z^2)}}{\bar{x}^2 - 1{\cdot}96^2\sigma_x^2}.$$

$$\therefore\ P = \frac{\bar{z}}{\bar{x}}\left[\frac{1 \pm 1{\cdot}96\sqrt{\left(\frac{\sigma_x}{\bar{x}}\right)^2 + \left(\frac{\sigma_z}{\bar{z}}\right)^2 - 3{\cdot}84\left(\frac{\sigma_x\,\sigma_z}{\bar{x}\,\bar{z}}\right)^2}}{1 - 3{\cdot}84\left(\frac{\sigma_x}{\bar{x}}\right)^2}\right] \quad \ldots \quad \ldots \quad (6)$$

Writing V_x and V_z for the coefficients of variation of x and z in the two populations, the lower and upper limits to the probable percentages of ingredient will be given by

$$I_L = I_A\left[\frac{1 - 0{\cdot}0196\sqrt{V_x^2 + V_z^2 - 0{\cdot}000384\,V_x^2\,V_z^2}}{1 - 0{\cdot}000384\,V_x^2}\right] \quad \ldots \quad \ldots \quad (7a)$$

and

$$I_U = I_A\left[\frac{1 + 0{\cdot}0196\sqrt{V_x^2 + V_z^2 - 0{\cdot}000384\,V_x^2\,V_z^2}}{1 - 0{\cdot}000384\,V_x^2}\right], \quad \ldots \quad \ldots \quad (7b)$$

where I_A, $= 100\bar{z}/\bar{x}$, is the apparent, or most likely percentage of ingredient. V_x is the coefficient of variation of the percentages of the constituent in the ingredient population. V_z has to be calculated from the coefficient of variation V_q of the ratio of the value of the given constituent to that of some other constituent in the diluent population.

$$i.e.,\ V_z = \frac{100\,\sigma_z}{\bar{z}} = \frac{100\,u\sigma_q}{z_T - u\bar{q}} = \frac{u\bar{q}}{z_T - u\bar{q}}\,V_q \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (8)$$

A comparison of equations (7*a*) and (7*b*) with equations (3*a*) and (3*b*) shows that the effect of the variation in z is to widen the range of probable values of the ingredient content. If $V_z = 0$ the present equations reduce to the earlier ones. Values of the upper and lower limits corresponding to an apparent ingredient content of 100 per cent. ($I_A = 100$) have been calculated for various values of V_x and V_z. These are shown in Tables II*a* and II*b* as the ranges above and below 100 per cent. The limits above and below the most likely value corresponding to any other apparent percentage of ingredient are obtained in simple proportion by multiplying by $\bar{z}/\bar{x}$. For example, for $V_x = 10$ and $V_z = 4$, the probable ranges above and below an apparent ingredient content of 100 per cent. are $+ 25{\cdot}9$ and $- 17{\cdot}9$, respectively, by Tables II*a* and *b*. If the apparent ingredient content were only 60 per cent. the corresponding ranges above and below 60 would be $+15{\cdot}5$ and $-10{\cdot}7$, giving a total range of 49·3 to 75·5 per cent.

In Table III are shown the probable lower limits of *apparent* percentage of ingredient for different values of V_x and V_z where the specified standard is 100 per cent. These are calculated from Table II*a* by multiplying the apparent ingredient percentage of 100 by the factor $100/(100 + R_U)$, where R_U is the range above 100. The apparent percentage of ingredient, in Table III, may alternatively be calculated directly from equation (7*b*) by putting $I_U = 100$. The lower limits corresponding to any other specification of percentage of ingredient I_s per cent. will be obtained by multiplying these values by $I_s/100$. These limits give the lowest permissible values for the apparent percentage of ingredient found in food products below which the samples would be rejected as deficient. Actually, as already stated, about one in forty samples that were in fact up to standard would be expected to show an apparent percentage of ingredient below the limit.

Application to the lean-meat content of sausages

To illustrate the preceding theory some numerical values for the probable limits to the lean-meat content of pork sausages are given, calculated in the way already indicated. The protein percentage due to the meat is found from the total protein and carbohydrate percentages in the sausage, making use of the protein/carbohydrate ratio for cereals. This is then divided by the mean percentage of protein in fat-free pork and multiplied by 100

to give the apparent percentage of lean meat in the sausage (no account is taken of the presence of any beef, which would here be reckoned as pork).

The frequency distribution of the protein/carbohydrate ratio has been determined from 150 values selected at random from a large amount of data for rusks of five different manufacturers over a period of three years (1943 to 1945 inclusive).* These ratio values formed a normal population with mean 0·174 and standard deviation 0·0165 (coefficient of variation = 9·5 per cent.). Also an examination of the analytical data for various cuts of pork* has shown that the protein percentage in the fat-free portion of the meat is distributed approximately normally with mean value 21·9 per cent. and standard deviation 2·76 per cent. (coefficient of variation = 12·6 per cent.). Thus, in the calculation of the probable limits to the lean-meat content of pork sausages, $\bar{x} = 21{\cdot}9$, $V_x = 12{\cdot}6$, $\bar{q} = 0{\cdot}174$, $V_q = 9{\cdot}5$. In Table IV are shown the apparent percentages of lean meat, with corresponding probable limits, for some different values of the percentages of total protein, z_T, and of carbohydrate, u. These limits have been obtained from Table II by calculating V_z in each instance from the relation

$$V_z = \frac{u\bar{q}}{z_T - u\bar{q}} V_q = \frac{1{\cdot}653\,u}{z_T - 0{\cdot}174\,u}$$

The range shown by Table II*a* and *b* is then multiplied by the apparent proportion of lean meat given by $P = \bar{z}/\bar{x} = (z_T - 0{\cdot}174\,u)/21{\cdot}9$.

TABLE IV

PROBABLE RANGE OF LEAN-MEAT CONTENT OF PORK SAUSAGES CONTAINING DIFFERENT PERCENTAGES OF PROTEIN AND CARBOHYDRATE

(Figures in brackets show the apparent percentages from mean values only)

Percentage of carbohydrate = u	Percentage of total protein = z_T		
	6 per cent.	9 per cent.	12 per cent.
5 per cent.	(23·4) 19 to 31	(37·1) 30 to 49	(50·8) 41 to 66
10 per cent.	(19·5) 15 to 26	(33·2) 27 to 44	(46·8) 38 to 62
15 per cent.	(15·5) 12 to 21	(29·2) 23 to 39	(42·9) 34 to 57

Examples where a similar general treatment would apply arise in the analysis of meat and fish pastes. The determination of the amount of egg in lemon curd from the phosphorus content,[4] after allowing for the phosphorus contributed by the flour, could also be treated in the above manner. This would involve the distribution of the phosphorus/starch ratio in flours and that of the phosphorus percentage in egg.

EFFECT OF ANALYTICAL AND SAMPLING ERRORS ON THE PROBABLE RANGE

A second source of variation which prevents an exact knowledge of the true percentage of the chemical constituent in the food product due to the ingredient arises from experimental errors in the determination. These may be due both to errors encountered in the chemical process of estimation and an error in sampling if the material is not homogeneous and only a part is taken for analysis. All these errors will act together as a single "experimental" error. In general, for any given value of the percentage of constituent due to the ingredient, the observed (experimental) values of the constituent in replicate experiments may be expected to conform to a normal distribution with the true value as the mean. If the standard deviation of experimental error (*i.e.*, of the observed experimental values) is known, the limits to the probable value of an observed percentage of constituent can be calculated. The converse problem of calculating limits to the probable value of the true percentage of constituent, given the observed value, may similarly be solved provided that the standard deviation of experimental error is constant (*i.e.*, independent of the percentage of constituent present). In practice this will be largely true for any one food product within the likely range of values of the percentage of constituent.

The estimation of the standard deviation of experimental error must be made from the final calculated values of the percentage of constituent due to the ingredient, obtained on

* Data obtained from a private communication.

replicate analyses. The error may be a composite effect made up of sampling errors and errors arising from the analysis of both ingredient and diluent constituents. As these errors might not be independent a computation of the over-all error from the separate effects would be difficult.

In practice the necessary data usually take the form of a number of duplicate or triplicate analyses on different samples, rather than a large number of replicate analyses on the same sample. In general there may be n different samples analysed and different numbers of replicate determinations made on the different samples. An estimate of the standard deviation of errors can be made from each of the n samples in the usual way. Each estimate will be made on a number of degrees of freedom equal to one less than the number of replicate determinations carried out on that sample. A pooled estimate of the standard deviation (assumed constant) over all n samples is then calculated by adding together the sums of squared deviations for each sample, dividing by the total number of determinations minus the number of samples n, and extracting the square root.

The standard deviation of experimental error, σ_ϵ, may be combined with the standard deviation of the constituent values in the diluent (in the general case). This will give an over-all standard deviation of possible observed percentages of the constituent determined in the food product due to both sources of variation. If this is done the range is still given by equations (7*a*) and (7*b*), but V_z has now to be calculated from the relation

$$V_z = \frac{100\sqrt{u^2\sigma_q^2 + \sigma_\epsilon^2}}{z_T - u\bar{q}}, \quad \ldots \quad (10a)$$

or in the simple case where no component other than the ingredient itself contributes to the percentage of constituent

$$V_z = \frac{100\,\sigma_\epsilon}{\bar{z}}. \quad \ldots \quad (10b)$$

The value of V_x is given as before by

$$V_x = \frac{100\,\sigma_x}{\bar{x}}. \quad \ldots \quad (11)$$

Tables II*a* and II*b* can thus be used to obtain the range of probable values for the percentage of ingredient, provided that V_z is calculated from (10*a*) or (10*b*). Strictly, σ_q or σ_x used in evaluating V_z and V_x should be the natural standard deviation of the constituent values without the superposition of experimental error, which will increase the apparent variation without affecting the mean values $\bar{q}$ and $\bar{x}$. The analytical error in the determination of the percentage of constituent in the ingredient or diluent itself, however, will in general be small compared with the natural variation and can probably be ignored.

The introduction of experimental error complicates the calculation of the range of probable ingredient contents. It is clear from Tables II, however, that unless σ_ϵ appreciably affects the value of V_z its effect on the probable range can be ignored. In the general case where the value of z is already subject to natural variation from the diluent the value of V_z is unlikely to be much altered. The greatest difference in V_z is likely to occur where V_z is otherwise zero, that is, where the ingredient alone contributes to the determined constituent. In this case, however, it is quite likely that the experimental error will be actually proportional to the percentage of ingredient, for the estimation of the percentage of constituent in the food product will be by a direct experiment (*i.e.*, not dependent on the diluent) of the same type as for the ingredient itself. If this is so the correct range will be given by equations (3*a*) and (3*b*), using the observed standard deviation of the constituent percentages in the ingredient and ignoring experimental error. This does not apply if the experimental errors in the analysis of the food product are of a different type from those of the ingredient (so that the proportionality does not hold). Nor does it apply in the general case where the determination of the constituent due to the ingredient is a complex affair dependent on the diluent analysis also. In these cases, if the experimental error is large, equations (10*a*) or (10*b*) should be used.

Effect on the range of probable composition when the true mean and standard deviation of the constituent populations are unknown

In arriving at the limits to the probable percentage of ingredient in a food product (equations (3*a*) and (3*b*)), it has been supposed that the true values of the mean and standard

deviation of the constituent population are known. Although the exact values of these two quantities cannot be known without analysing every member of the ingredient population, a sufficiently close estimate may be made by an analysis of fifty or more specimens of the ingredient selected at random. It may happen with some food products that only a small number of analyses have been carried out on the ingredient itself. If, for example, only ten specimens of the ingredient have been analysed, the mean value for the constituent calculated from ten values may differ appreciably from the value for the whole population.

If the values of the mean and standard deviation of the constituent population are uncertain, then, to achieve the same degree of probability in fixing the range within which a constituent value is expected to lie, the limits must be set farther apart. In these circumstances the limiting values can be calculated by making use of Student's t-distribution, which gives the distribution of the ratio of a variable, normally distributed about zero, to its *estimated* standard deviation.

Let $\bar{x}$ and σ_x be estimates of the true values based on m observations,

$\bar{q}$ and σ_q be estimates of the true values based on n observations,

σ_ϵ be an estimate of the true value based on k observations,

also let t denote the value of Student's ratio on the 0·05 probability level as given by tables of the distribution[5] for n′ degrees of freedom, where n′ is the least of the numbers n − 1, m − 1, k − 1. Then it may be shown that Tables II*a* and II*b* can again be used to find the probable range provided that V_x and V_z are now calculated from the relationships

$$V_x = \frac{100\,\sigma_x}{\bar{x}}\left(\frac{t}{1{\cdot}96}\sqrt{\frac{n+1}{n}}\right) \quad \ldots \quad (12)$$

$$V_z = \frac{100\sqrt{u^2\sigma_q^2 + \frac{m}{m+1}\sigma_\epsilon^2}}{z_T - u\bar{q}}\left(\frac{t}{1{\cdot}96}\sqrt{\frac{m}{m+1}}\right) \quad \ldots \quad (13)$$

As before, σ_x and σ_q should strictly be the *natural* standard deviations of the constituent values (not including experimental errors).

The limits so arrived at will not necessarily include 95 per cent. of all possible values for the proportion of ingredient in a food product in any given case, owing to the uncertainty in the mean and standard deviation. The use of the t-distribution, however, does ensure that over a large number of occasions the probability of the proportions of ingredient lying within the given limits averages 0·95.

In order to illustrate the magnitude of the effect of the t-distribution, the probable ranges for different values of n and V, in the case where the constituent value in the food product is known (*i.e.*, $V_z = 0$, $V_x = 100\,t\,\sigma_x/1{\cdot}96\,\bar{x}$), are given in Table V. The apparent percentage of ingredient (I_A) is assumed to be 50 per cent.

TABLE V

RANGE OF PROBABLE INGREDIENT CONTENT WHERE APPARENT PERCENTAGE OF INGREDIENT IS 50 PER CENT.

(Value of constituent in foodstuff known exactly)

Number of observations from which V_x is calculated = n	Estimated coefficient of variation in ingredient population = V_x		
	5 per cent.	10 per cent.	15 per cent.
5	43 to 59	38 to 72	34 to 92
10	45 to 57	40 to 66	37 to 78
15	45 to 56	41 to 64	38 to 75
25	45 to 56	41 to 63	38 to 73
50	45 to 56	42 to 63	38 to 72
∞	46 to 55	42 to 62	39 to 71

It is apparent from Table V that the probable upper limit increases rapidly as n diminishes where less than 10 observations are available for the estimation of the coefficient of variation. For coefficients of variation less than 5 per cent. the introduction of the t-distribution usually makes practically no difference. The limits when n = ∞ correspond to conditions where the true coefficient of variation of the constituent population is known (equations

(3*a*) and (3*b*)), and are substantially the same as when n = 50. Thus, as stated previously, fifty or more observations may be regarded as sufficient for estimating the true coefficients of variation in the ingredient (or diluent) populations.

Conclusion

The limits within which 95 per cent. of the possible values of the proportion of ingredient in any food product lie may seem alarmingly wide. The whole range is of the order of four or more times the coefficient of variation in the constituent population multiplied by the apparent proportion of ingredient. This may lead to a variation of 50 per cent. or more in the probable values of the percentage of ingredient when the coefficient of variation is large. Though all percentages in the calculated range are regarded as "probable" values, they are certainly not equally probable. The values become less probable towards either end of the range, the "most probable" one being the apparent ingredient percentage calculated in the usual way. However, no value in the range can be considered unlikely, and therefore the upper limit to the probable percentage of ingredient should provide the necessary criterion for deciding whether a food product conforms to a required specification. As already pointed out, the limit given by the statistical procedure suggested here ensures that a product will not be wrongly rejected more than one in forty times. This seems a reasonable safeguard and any attempt to reduce the range would lead to an increase in the number of products wrongly condemned.* If the latitude allowed by the range appears large, it is simply a consequence of the wide variation in the values of the analysed constituent in the ingredient (or diluent) itself. No improvement in analytical technique can overcome this source of error, which is fundamental to estimations of this type. The only chance of lessening this variation is by the possible use of more than one chemical constituent, to form a combination which shows less variation in the ingredient population than any of them taken separately.

These considerations bring out strongly the undesirability of judging a particular food product from one sample alone. Since the standard deviation of the mean of N values varies inversely as the square root of N, the probable range calculated from the mean of the percentages of constituent found in each of (say) four random samples would be reduced by about half. Similarly, if nine samples were analysed and the constituent percentages averaged, the probable range would be reduced to about one-third of the range calculated from one sample. If the mean percentage of the constituent due to the ingredient is calculated from the analysis of N random samples of a food product, the probable range for the percentage of ingredient will be given by the formulae already developed if the coefficients of variation V_x and V_z are divided by the square root of N.

Finally, if Tables II*a* and II*b* are to be applied, it is necessary that analytical data for the percentages of the constituents determined in foodstuff ingredients be accumulated, so that the standard deviations can be calculated. This calculation would not be valid if the results on any one constituent were determined by widely different methods; hence the desirability of standardising the analytical procedures must be emphasised.

Summary

By a statistical treatment of the analytical data it is possible to assign probable limits to the percentage of any particular ingredient in a food product where this percentage is estimated from some chemical constituent characteristic of the ingredient. These limits are calculated so that there is only one chance in twenty of the percentage composition actually lying outside the given range and, in general, depend on two coefficients of variation. One of these measures the natural variation encountered in the values of the chemical constituent determined in different samples of the ingredient. The other expresses a variation in the amount of the chemical constituent actually due to the ingredient in the food product. This latter variation may arise either from uncertainty as to the amount of the chemical constituent contributed by some other ingredient present or from experimental error in the determination. By use of the t-distribution the probable limits can be calculated when only small numbers of observations are available for estimating the coefficients of variation.

The author is indebted to the Council of the British Food Manufacturing Industries Research Association and the Department of Scientific and Industrial Research for permission to publish this paper.

* See Appendix.

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APPENDIX

PROPORTION OF FOOD PRODUCTS DEFICIENT IN INGREDIENT LIKELY TO BE PASSED AS UP TO STANDARD

Reference has been made to the fact that by widening the probable limits within which the percentage of ingredient is asserted to lie the chance of accepting a deficient product as correct is increased. Although the probability of making this kind of error in general cannot be assessed, it may be calculated for various *specified* deficiencies in ingredient as follows.

Consider a population of food products which are deficient in ingredient by " a " per cent., *i.e.*, the percentage of ingredient present is actually the specified percentage multiplied by $(100 - a)/100$.

If prepared to the specified standard, then, with the notation already used, the values of the ingredient constituent will be distributed about a mean value $\bar{x}$ with standard deviation σ. A deficient product can be regarded as prepared from a "diluted" ingredient, specimens of which give rise to a population of constituent values with mean $\bar{x}_a = \bar{x}(100 - a)/100$ and standard deviation $\sigma_a = \sigma(100 - a)/100$.

The lower limit below which 1 in N constituent values in undiluted specimens of the ingredient will be expected to lie is given by $x_1 = \bar{x} - k\sigma$, where the value of k depends on the probability 1/N. Thus, for the proportion 1 in 40 adopted, the value of k is 1·96. A deficient product will be passed if the value, x_a, of the constituent in the "diluted" ingredient used is greater than or equal to this lower limit x_1. The probability of this occurring in the "diluted" ingredient population will be equal to that with which a variable normally distributed about zero with unit standard deviation exceeds the deviation $(x_1 - x_a)/\sigma_a$, and may be obtained from tables of the normal distribution. The value of the deviation to be exceeded can be expressed in terms of a, k, and V, where V is the coefficient of variation in the constituent (undiluted) population. It follows by simple algebra that

$$\frac{x_1 - \bar{x}_a}{\sigma_a} = \frac{100}{100 - a}\left(\frac{a}{V} - k\right)$$

The value of k in turn depends on the proportion of correct food products likely to be wrongly condemned (*i.e.*, on the probability level chosen in fixing the probable limits of ingredient content). Assuming for the coefficient of variation average values of 10 and 15 per cent. respectively, Appendix Tables I and II show the proportions of food products, deficient by various amounts, that are likely to be passed as up to standard when different probability levels are used in fixing the limits of ingredient content.

APPENDIX TABLE I

PROPORTION OF DEFICIENT FOOD PRODUCTS LIKELY TO BE PASSED AS SATISFACTORY OUT OF EVERY 100 EXAMINED (ASSUMING V = 10 PER CENT.)

Percentage deficiency of ingredient in food product	Proportion of correct food products likely to be wrongly condemned out of every 100 examined			
	10	2·5	1	0·1
10	62	86	92	99
20	18	48	66	91
30	0·7	7	17	55
40	< 0·001	0·03	0·3	6

Appendix Table II

Proportion of deficient food products likely to be passed as satisfactory out of every 100 examined (assuming V = 15 per cent.)

Percentage deficiency of ingredient in food product	Proportion of correct food products likely to be wrongly condemned out of every 100 examined			
	10	2·5	1	0·1
10	75	92	97	100 (99·6)
20	48	79	89	99
30	15	48	68	94
40	1	12	29	76

These tables bring out the danger of allowing too wide a latitude in the probable limits to the percentage of ingredient in a food product. Even with the chance adopted of wrongly condemning a correct product 1 in 40 times, practically half of the 20 per cent. deficient products would be passed if the coefficient of variation concerned is 10 per cent. The coefficient of variation in most cases is not likely to be much less than this and may often be more, in which case a still larger proportion of deficient products would be passed. If a serious attempt is made to reduce the probability of a false condemnation, say to 1 in 1000 times, practically no protection is offered against products deficient by as much as 20 or 30 per cent. It is on these grounds that the probability level of 0·05 has been regarded as the most suitable for adoption here.

One further point may be noted. The probability adopted leads to the expectation that out of forty *correct* products examined, one will be falsely condemned. In view of the fact that a large number of deficient products will presumably be examined also, it does not follow that out of forty prosecutions one might be expected to be in error. In practice an erroneous prosecution would occur less frequently than one in forty times, though it is impossible to say how much less.

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Discussion on the Preceding Four Papers

Mr. Philip Lyle mentioned several uses to which statistical methods had been successfully applied in the sugar-refining industry, *e.g.*, in examining variation of crystal size and characters and in the control of factory efficiency and costs. Graphical methods, using specially ruled probability paper, enabled means and standard deviations to be estimated rapidly from cumulative frequency tables. He had usually found that methods of regression analysis gave more easily understandable results than the calculation of correlation coefficients. A "significant" correlation coefficient did not necessarily imply a direct causal relation between the variables.

The need for caution in interpreting correlation coefficients was stressed also by Dr. H. Liebmann and Mr. D. H. F. Clayson. The latter expressed some doubt of the advisability of attempting to correlate phenomena between which there was no apparent connecting chain of cause and effect, or various intermediate causes and effects, and instanced the apparent correlation, mentioned by Mr. Adam, between rainfall and the incidence of gumming in fruit trees. Other workers had attributed gumming to insect damage and bacterial infection. Mr. Adam replied that the results cited by him formed only part of the information obtained, and not all gumming could be attributed to bacterial infection. But he agreed that a "significant" correlation coefficient did not necessarily prove a direct causal connection, though the method was a useful means of exploring a problem.

Dr. J. G. A. Griffiths asked whether it was really justifiable to use a normal distribution to represent the distribution of a value the range of variation of which was limited. He was particularly concerned about the probabilities of extreme values, corresponding to the tails of the normal curve, and described the difficulty of using a statement of probability as the basis for legal action concerning the composition of a food product. The assertion that a sample analysed had only a small probability of occurrence in random sampling from a product that was up to standard might be countered in court by the claim that a rare chance could occasionally happen. He would prefer to argue on impossibility rather than on low probability and therefore would like to make use of a distribution with finite range.

Mr. Finney replied that even where it was known that the range of possible values from random sampling was limited the satisfactory determination of the extremes was not easy. In many instances the normal distribution did provide a satisfactory approximation, and it was often impracticable to obtain positive evidence of any other distribution that would fit the material better than the normal. Moreover, the distribution of means of several determinations was usually much closer to normality than the distribution of individual determinations, and usually it was on means that decisions were taken.

Mr. A. L. BACHARACH suggested that evidence in terms of probabilities might perhaps be avoided in court by a statement that a sample in question gave a more extreme value than, in the analyst's experience, had ever been found for a sample from a similar product *known* to be satisfactory.

Dr. J. R. NICHOLLS doubted if that would be satisfactory. It would tend to give undue weight to extreme values found for exceptional samples recorded in the literature. What was expected of a witness in court was a statement whether a particular sample was or was not deficient in some ingredient. He asked if there was a possibility of statistically combining experimental data for two or more constituents, which together might provide a more definite criterion than data for one constituent only. Mr. STEINER suggested that a combined index might be formed that would be more sensitive to discrepancies than any one of the determinations used in forming it.

Dr. E. C. WOOD urged analysts not to grudge the time spent on statistical analysis of their results when more reliable and informative conclusions could be obtained thereby. The time necessary was usually much less than that spent on the experimental work itself, especially if this had been well designed. Much of the time spent on the experimental work would have been wasted if the fullest information was not obtained from the results. The importance of making duplicate analyses is now generally recognised but the duplication is not always done to the best advantage; the duplicates should be made on different group samples rather than on the same final sub-sample, so that the effect of sampling errors might be included. He suggested that a probability level of significance suitable for control purposes in a factory might not be sufficiently stringent where possibilities of legal action depended on it.

Mr. K. A. BROWNLEE pointed out that the variability of results in replicate tests by a given analytical technique can for certain purposes be accurately assessed only by co-operation between different workers in different laboratories, for the same analyst using the same apparatus will usually obtain closer agreement.

The Determination of Organic Phosphorus

BY C. H. MANLEY AND H. LOBLEY

(*Read at a meeting of the North of England Section in Manchester, on April* 19, 1947)

As is well known, the determination of the percentage of organic phosphorus, expressed as phosphorus pentoxide, provides a useful means of ascertaining the proportion of dried egg present in a preparation containing it, owing to the much smaller percentage of organic phosphorus found in the other foods, in particular the cereals, with one or other of which the dried egg is often mixed.

Some interesting information on this subject is furnished by Beach, Needs, and Russell[1] who apparently used hot alcohol in the initial stage of the extraction, and then saponified in the resulting alcoholic solution. Cox[2] refers both to the successive use of ether and alcohol and to the use of chloroform prior to oxidation of the resulting extract with a mixture of sulphuric and nitric acids. Any advantage accruing from the ether - alcohol method has doubtless been accounted for in the belief that the ether, by first removing the fat, enables the alcohol subsequently to extract the organically combined phosphorus present in the form of phosphatides. Brooks and Hawthorne,[3] in their study of the lipins of fresh and spray-dried whole egg, used, amongst other solvents, chloroform (400 ml.) at room temperature in a 500-ml. flask containing 10 g. of dried egg, the resulting solution being employed for the determination of total lipoid, phosphorus and nitrogen.

With the introduction of soya flour as well as that of dried egg into certain foods marketed in the year 1941, we thought it desirable to obtain, if possible, definite data for the percentages of organic phosphorus in the various ingredients used. Accordingly, three separate methods of extraction were used, *viz.*, (*a*) with chloroform, (*b*) with alcohol, and (*c*) with ether followed by alcohol. Data were thus obtained for dried egg, soya flour and several cereals and applied to the calculation of the percentage of dried egg in various products claimed to contain it, amongst them certain self-raising flours, Yorkshire pudding mixtures and a so-called egg extract (a product in powder form). With these substances the increase in the fat content affords a useful indication of the percentage of dried egg present, as, with the exception of oatmeal, the cereals contain but little fat, and the result obtained in this way can be compared with that yielded by the organic phosphorus figure. Admixture of soya flour in appreciable proportion with dried egg and cereal tends to complicate the issue, although in such circumstances the sucrose figure should be a guide to the percentage of soya flour present.

EXPERIMENTAL—

From 3 to 10 g. of the powdered material were extracted for 2 to 3 hours with 40 ml. of the solvent in a Manley - Wood apparatus[4]; chloroform and ether extracts were dried to

constant weight to ascertain the fat content. Either the whole or a part of the dry extract was then oxidised by heating with 2 to 5 ml. of sulphuric acid and the requisite amount of nitric acid. The phosphorus was then precipitated as phosphomolybdate and subsequently weighed as magnesium pyrophosphate.

The following results were obtained:—

ORGANIC PHOSPHORUS CONTENTS, AS P_2O_5 PER CENT.

Material	Found via Chloroform	Alcohol	Ether and alcohol
Dried egg—			
No. 1	1·31	—	—
No. 2	0·99	1·15	1·12
No. 3	1·22	—	—
No. 4	1·20	—	—
No. 5	1·15	—	—
Soya flour	0·14	0·23	0·18
Wheat flour (white)	0·028	0·102; 0·102	0·038
Wheatmeal flour	0·020	0·115	0·051
Barley	0·013	0·076	0·038
Oatmeal	0·038	0·076	0·026
Cornflour	Nil	0·010	0·024

The corresponding figures for sago and tapioca were not determined, because the fat contents of these are negligible, nor was that for rice, for which Beach, Needs, and Russell gave a figure of 0·022 per cent. These workers obtained figures of 1·20, 1·27 and 1·28 per cent. for the three samples of dried egg they examined, and Cox, presumably after chloroform extraction, quotes 1·18, 1·25, 1·30 and 1·37 per cent. Brooks and Hawthorne's figure of 4·56 mg. of phosphorus per gram of dried egg, corrected for a 6·1 per cent. water content, corresponds to 1·05 per cent. P_2O_5 content; this was after using chloroform at room temperature. They, moreover, state that there is general agreement that the total extractable phosphorus in fresh yolk of egg corresponds to a calculated value for dried whole egg of 5·7 to 6·3 mg. per gram, this representing an average figure of 1·33 per cent. of P_2O_5.

Our own figures tend to show that extraction with chloroform in a warm state is sufficient for the purpose required, and there is therefore no need to resort to the longer ether - alcohol procedure. Also, except for oatmeal, the chloroform figures for foods other than dried egg are lower than the ether - alcohol ones, a fact which makes for increased accuracy in calculating the dried-egg content of a mixture. Incidentally, with one exception and contrary to expectation, the alcohol figures are all higher than the ether - alcohol ones.

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A Simple Colorimetric Method for the Determination of Copper in Photographic Developers*

BY G. I. P. LEVENSON

THE presence of traces of copper in a photographic developer containing hydroquinone may often result in very heavy aerial fogging, *e.g.*, in a recent instance of copper-sensitised aerial fogging, a fog density of about 2 resulted from the presence of 2 parts of copper per million of developer. When investigating the causes of chemical fogging it is therefore useful to know at the outset whether or not copper is present in significant amounts.

Traces of copper can be determined at the dropping mercury electrode in a straightforward manner, but no method hitherto described is suitable for use in the field. The method here described involves no more apparatus than can be carried in the pocket, and it is sufficiently accurate for the purpose for which it was designed. Used with greater refinement, however, the method should give much more accurate results.

* Communication No. 1147 H from the Kodak Research Laboratories, Harrow, Middlesex.

Sodium diethyldithiocarbamate is well known as a sensitive reagent for copper, with which it gives a brown coloration (a fine suspension). Iron also gives a brown colour with the reagent, and in order to determine copper it is first necessary to ensure that iron is not present or, if present, is rendered inactive. For colorimetry the brown copper diethyldithiocarbamate can be extracted with an organic solvent. Aluminium, lead, mercury, tin and zinc give white suspensions with the reagent.

A. Marriage, of these laboratories, pointed out that a solution of zinc diethyldithiocarbamate in butyl acetate could be used with advantage as reagent in this test. The colourless butyl acetate solution of zinc diethyldithiocarbamate is shaken with the solution suspected of containing copper. Any copper diethyldithiocarbamate formed is at once taken up in the butyl acetate, which can then be separated for colorimetry. The procedure finally adopted was based on this technique. Iron did not interfere.

Experimental

Preparation of a 0·1 per cent. solution of zinc diethyldithiocarbamate in butyl acetate—

Zinc diethyldithiocarbamate was precipitated by mixing aqueous solutions of zinc sulphate and sodium diethyldithiocarbamate. It was first necessary to free the reagents from any trace of copper. Distilled water from an all-glass still was used in this work. Distilled water from a general-purpose tinned-copper still was found to contain about one part of copper in 5 millions.

0·9 Gram of sodium diethyldithiocarbamate was dissolved in 50 ml. of water. Any copper diethyldithiocarbamate that formed was removed by extracting with 10-ml. portions of butyl acetate until the extracts were colourless. Excess of zinc sulphate (1·0 gram AnalaR) was dissolved in 25 ml. of water, and 2 ml. of a 0·1 per cent. aqueous solution of sodium diethyldithiocarbamate were added. The treated zinc sulphate solution was extracted with 5-ml. portions of butyl acetate until the extracts were colourless. The copper-free zinc sulphate solution was added to the copper-free sodium diethyldithiocarbamate solution. The zinc diethyldithiocarbamate which was precipitated was extracted with five 50-ml. portions of butyl acetate and the combined extracts were diluted with the same solvent to make a 0·1 per cent. solution.

Preparation of a set of standard comparator tubes—

Solutions of copper sulphate were prepared containing 0, 1, 2, . . . 10 parts of copper per million.

25 Ml. of each of these solutions were well shaken with 10 ml. of the 0·1 per cent. zinc diethyldithiocarbamate solution in a 100-ml. separating funnel After settling, the aqueous layer was run off and the butyl acetate layer was filtered through a rapid paper into a thin-walled test tube of 12·5-mm. internal diameter. The tubes were sealed and labelled in parts per million from 0 to 10.

Determination of copper in developers—

In order to obtain the correct determination on an Elon-hydroquinone developer (Kodak D 19b) to which 3 parts of copper per million had been added, it was found necessary first to make the 25-ml. test sample strongly alkaline by adding 2 ml. of 50 per cent. sodium hydroxide solution. Without any addition of copper the developer showed about 0·2 part per million. To measure concentrations of copper less than 1 part per million the volume of the test sample was increased sufficiently to give a brown tint deeper than that of the 1 part per million standard. None of the coloured oxidation product formed from the developer as a result of increasing the alkalinity passed into the organic layer in the extraction.

Added ferric and ferrous salts did not interfere with the determination. Silver salts did not affect the determination in developers because the developer reduced them to metallic silver. Ammonium ion was added in the form of ammonium carbonate but had no influence on the determination.

Permanence of standard tints—

The original standard tubes were 8-months old when this paper was written. They were checked by using the original 0·1 per cent. solution of zinc diethyldithiocarbamate and a fresh solution of copper (1 in 100,000) prepared from copper sulphate. A sample of D.19b developer was used which on testing showed a copper concentration of less than 1 part in 8 millions. Additions of copper sulphate equivalent to 1, 4, 6, 8 and 10 parts of copper per

million were made to 25 ml. lots of D.19b and were successfully determined. The extracts from the new samples under test matched identically with the 1 and 4 parts tubes but were somewhat lighter than the standards at the higher end of the range. It is generally desirable to work on the low end of the range, where tint differences are more apparent. Where a first test shows a concentration of copper greater than 5 parts per million, a repeat test on smaller samples would act as a useful check, appropriate adjustments being made in the calculation.

PROCEDURE TO BE FOLLOWED WHEN USING THE METHOD

Prepare suitable standard tubes in the way described. The standards are conveniently based on a sample volume of 25 ml. Make the 25-ml. sample of developer strongly alkaline by addition of 2 ml. of a 50 per cent. sodium hydroxide solution and shake the resulting solution vigorously with 10 ml. of the 0·1 per cent. solution of zinc diethyldithiocarbamate in a 100-ml. separating funnel. When the contents of the separating funnel have separated, draw off and discard the aqueous (lower) layer and run the organic extract through a filter paper into a test tube of suitable size. The filter retains water droplets. Then compare the tube with the standards.

Should the concentration of copper shown by the first test be outside the range of concentrations represented by the standards, repeat with a different volume of sample of developer and/or a different amount of the zinc diethyldithiocarbamate solution, and correct for the changes in volume.

SUMMARY—

A simple method for the determination of traces of copper in a developer is based on the formation of brown copper diethyldithiocarbamate when a sample of the developer is shaken with a butyl acetate solution of zinc diethyldithiocarbamate. The brown tint is compared with standard tints calibrated in parts per million of copper. The method is suitable for field use.

May, 1947.

Ministry of Food

STATUTORY RULES AND ORDERS*

1947—No. 2709. The Labelling of Food Order, 1946 (Amendment No. 3) Order, 1947. Dated December 18th, 1947. Price 1d.

This amending Order—

(1) *permits custard powders and blancmanges to be sold without a declaration of ingredients;*

(2) *requires sauces other than thick mixed fruit sauces, thin sauces of the Worcester type and tomato sauce or ketchup to be labelled with a declaration of ingredients;*

(3) *permits the sale of Advocaat (a mixture of eggs, spirit, sugar and flavouring) containing not less than* 30 *per cent. proof spirit;*

(4) *requires spa waters and certain soft drinks to comply with the provisions of the main Order (S.R. & O.,* 1946, *No.* 2169; 1947, *Nos.* 757 *and* 2001) *except as regards the declaration of ingredients and minimum quantity of contents;*

(5) *requires unfermented apple juice to comply with the provisions of the main Order.*

Except in relation to certain of the amendments referred to in paragraphs (2), (4) *and* (5) *above, which come into force at stated intervals, this Order came into force on December* 21*st*, 1947.

—No. 2756. The Soft Drinks Order, 1947. Dated December 22nd, 1947, Price 2d.

This Order replaces the Soft Drinks Order, 1946 (*No.* 945), *as amended; and provides*

(1) *that no soft drink shall be manufactured or packed except under licence;*

(2) *that no soft drink shall be sold unless it complies with certain provisions as to ingredients. (Undiluted fruit juice and unsweetened drinks other than soda water are exempted from this provision);*

(3) *conditions as to price and records;*

(4) *that caterers may, in connection with their catering business, manufacture (but not pack) soft drinks without a licence and sell soft drinks that do not comply with the provisions as to ingredients.*

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* Italics signify changed wording.

The principal differences between this Order and the replaced Order are that—

(*i*) *the definition of soft drink has been altered and, in particular, now includes only liquid soft drinks. Water from natural springs is also specifically excluded;*

(*ii*) *the definition of "sale by retail" now excludes sales to caterers;*

(*iii*) *certain other definitions have been deleted and new ones added;*

(*iv*) *there is no requirement that soft drinks shall be sold under particular descriptions;*

(*v*) *there are no labelling restrictions, either requiring anything to appear or prohibiting anything from appearing on a container;*

(*vi*) *there are no restrictions as to sizes of containers;*

(*vii*) *the classification of soft drinks for the purpose of prescribing ingredients has been altered, as have the provisions relating to the ingredients themselves. No acid content is prescribed; the quantities of sugar and saccharin have been changed; and a minimum sugar content and maximum saccharin content are now laid down.*

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Vitamin A in Fish-liver Oils with Activated Glycerol Dichlorohydrin. A. E. Sobel and H. Werbin (*Anal. Chem.*, 1947, **19**, 107–112)—Activated glycerol dichlorohydrin has been used to determine vitamin A in the unsaponifiable fraction of fish-liver oils, and in a few whole oils. The results have been compared with those obtained by the antimony trichloride and ultra-violet absorption methods on the same oils. The average deviations from the values obtained by the glycerol dichlorohydrin method were: on the unsaponifiable fractions, antimony trichloride, − 4·11 per cent., ultra-violet absorption $E^{1\%}_{1 cm.} \times 2000$, + 26·25 per cent.; and on the whole oils, antimony trichloride, − 1·6 per cent., and ultra-violet absorption, + 17·11 per cent. The mean errors in the estimation of known amounts of vitamin A added to fish-liver oils were ± 2·80 per cent. on whole oils and ± 1·88 per cent. on unsaponifiable fractions, compared with ± 3·11 and ± 2·11 per cent., respectively, by the antimony trichloride method.

The main advantages of the new method over the antimony trichloride method are: the colour produced is stable for 2 to 10 min.; the reagent is not affected by traces of moisture; no film of antimony oxychloride is left on the cells; the reagent is practically non-corrosive; and a photo-electric spectrophotometer may be used to measure the absorption of the violet colour.

Apparatus—A Coleman Universal Spectrophotometer, Model II, was used with 1·3-cm. absorption cells. The blank was set at 100 per cent. transmission.

Reagents—1. *Chloroform* A.R. dried over sodium sulphate, distilled, and stored over sodium sulphate. 2. *Activated glycerol dichlorohydrin.* To 1 litre of glycerol dichlorohydrin (1:3-dichloro-2-hydroxypropane), add 2 per cent. by weight of antimony trichloride dissolved in chloroform. Remove the chloroform by distillation and then distil the residue at 86° to 92° C. at 30 to 40 mm. pressure. The reagent prepared thus should be colourless and should give an $E^{1\%}_{1 cm.}$ value (550 mμ.) of 1150 to 1250 in the Coleman instrument. Stored in glass-stoppered bottles at room temperature, it is stable for at least 2 months. 3. *Standard vitamin A solution.* Dissolve vitamin A or a vitamin A concentrate of known strength in chloroform to give a solution containing 2 to 5 μg. per ml. The standard solution should not be used after more than 2 days.

Saponification—The procedure described by Oser *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 717) was used.

Procedure—To 4·0 ml. of the activated glycerol dichlorohydrin in a 10-ml., glass-stoppered graduated flask, add 1·0 ml. of a chloroform solution of the sample containing 2 to 5 μg. of vitamin A per ml., mix, and immerse in a water-bath at 25° C. for about 1·5 min. Transfer to the absorption cell and, 2 minutes after addition of the sample, measure the absorption at 550 mμ. Construct an optical density - concentration curve from the standard solution with each new batch of reagent and, from the curve, calculate the vitamin A content of the samples being tested.

Tables comparing the values obtained by the three methods for about 20 different oil samples are given, together with a table showing the recovery of vitamin A added to fish-liver oils.

G. R. Primavesi

Modified Method for the Determination of Monoglyceride in Fats and Oils by Oxidation with Periodic Acid. E. Handschumaker and L. Linteris (*J. Amer. Oil Chemists' Soc.*, 1947, **24**, 143)—The periodic acid procedure described by Pohle, Mehlenbacher, and Cook (*Oil and Soap*, 1945, **22**, 115; Abst. Analyst, 1945, **70**, 338), has been modified to produce a rapid, precise, and easily reproducible method for a routine control of monoglycerides in shortening blends. The oxidising agent consists of 5 g. of periodic acid dissolved in 200 ml. of distilled water and 800 ml. of glacial acetic acid, and must be stored in a glass-stoppered bottle. The analysis is carried out in an inert solvent prepared by mixing 2 parts of glacial acetic acid with 1 part of chloroform.

Procedure—Weigh duplicate samples, of weights chosen so that the titration for a sample is greater than 80 per cent. of that for the blank determination (see below), into 16-oz., wide-mouthed bottles, and add 15 ml. of solvent to each. If necessary, heat the samples and solvent carefully on a steam-bath until the fats are completely dissolved. Cool to room temperature.

Pipette 25 ml. of periodic acid reagent into each bottle and agitate in a mechanical shaker for 2 min. In absence of a shaker, 1 min. shaking by hand followed by 10 min. standing gives good results. Wash down the inside of the bottles with 5 ml. of glacial acetic acid, add 15 ml. of potassium iodide solution (150 g. per litre), shake, and dilute with 100 ml. of distilled water. Titrate the liberated iodine with 0·1 N sodium thiosulphate, using starch as indicator, and read the burette to 0·01 ml. At the same time and in the same manner, conduct a blank determination on the reagents.

The percentage of monoglyceride is given by

$$\frac{(x - y) \times C \times 100}{20,000 \times W}$$

where x = vol. of 0·1 N sodium thiosulphate used in blank titration; y = vol. of 0·1 N sodium thiosulphate used in test; C = molecular weight of the monoglyceride; W = weight of fat taken, in grams.

[Abstractor's Note: Pohle *et al.* (*loc. cit.*) give the average molecular weights of the monoglycerides of the fatty acids of a number of fats and oils as follows: coconut oil, 281·8; cottonseed oil, tallow, palm oil, and soya-bean oil may be taken as 354·5.]

A. H. A. ABBOTT

Determination of Crude Lipoids in Citrus Juices. L. J. Swift (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 389–395)—Since deterioration of the flavour of orange juice on storage is accompanied by rancidification of the lipoid fraction (Nolte *et al.*, *Food Res.*, 1942, **7**, 236), a routine method for determination of lipoid matter in fruit juice is required, and would serve also as a quantitative first step in the study of fatty acids, waxes, sterols, carotenoid pigments, hydrocarbons, *etc.*, occurring in citrus juices. Citrus juice is not conveniently or thoroughly extracted by liquid - liquid extraction, either in continuous extractors or in separating funnels. Most of the lipoid matter is associated with the suspended matter and it seemed feasible to confine the extraction to this. Filter-paper pulp proved the best filtering medium and, with acetone as the extracting solvent, the pulp need not be dried before extraction.

Procedure—Mix the juice by vigorous stirring and, while suspended matter is still uniformly distributed, transfer 500 ml. to an 800-ml. beaker. Disintegrate 8 g. of filter paper by mixing it with water in a Waring Blendor, transfer the pulp to a 11-cm. Buchner funnel containing a filter paper, and remove the water by suction. Remove the pad of paper pulp, replace the filter paper circle in the funnel, and suspend the pulp in the sample. Disintegrate 4 g. of filter paper in the same way, transfer it to the funnel containing the original filter paper circle, remove the water by suction and, after discarding the drained water, filter the mixture of sample and pulp, pouring back the first portions of the filtrate until it is perfectly clear, and maintaining the suction until drainage is complete.

Separate the filter pad from the filter paper circle, collect any material adhering to the funnel with the filter paper, and place the crumpled paper at the bottom of a Soxhlet extractor at least large enough to accommodate a 33 by 80-mm. thimble (no thimble is used). Tear the filter pad into small fragments and place them in the extractor without packing tightly. Extract with 150 to 200 ml. of acetone for at least 9 hr. with the solvent siphoning every 2 or 3 min. Transfer the acetone extract to a separating funnel and re-extract the filter pad fragments for 4 hr. with 150 to 200 ml. of low-boiling light petroleum. Transfer the petroleum extract to the same separating funnel adding, if necessary, more petroleum until its volume is equal to that of the acetone extract. After shaking the funnel, draw the separated aqueous layer into a smaller separating funnel. Wash the aqueous layer with three, 25-ml. portions of light petroleum. Emulsions can be readily broken by addition of 1 to 3 g. of common salt before shaking. Dry the combined extracts by shaking with a few grams of anhydrous sodium sulphate, and filter into a tared flask containing a few glass beads or carborundum chips. Remove the solvent by evaporation and dry the flask by heating it for 1 hr. over boiling water under 1 inch pressure, and finally weigh the crude lipoids.

To estimate the total carotenoids, dissolve the crude lipoid residue in light petroleum, filter, wash the filter with light petroleum, and finally dilute the filtrate and washings to 500 ml. Dilute a 10-ml. aliquot to 100 ml. and determine the colour density in an Evelyn colorimeter with a 440-mμ filter. The colour densities of the extracts from different samples are a measure of their relative contents of total carotenoids.

The crude lipoid extract consists approximately of two-thirds saponifiable fatty acid esters and one-third unsaponifiable matter.

A comparison of the two general methods used for extracting juice from citrus fruits showed that a reaming operation, in which a rotating burr is pressed into the halved fruit, yields juice of higher lipoid content than does a pressing operation, in which the halved fruit is pressed against a metal boss. Total carotenoids were 50 per cent. more abundant in the reamed juice, but the crude lipoid matter was only 25 per cent. greater. This suggests that reamed juice contains more provitamin A than does pressed juice, but caution must be exercised in applying the results of these laboratory experiments to commercial processes. A. O. JONES

Method for the Determination of Total Alkaloids in Belladonna and Stramonium. M. Roberts and W. O. James (*Quart. J. Pharm.*, 1947, **20**, 1–16)—This method has been evolved for the accurate, rapid routine determination of alkaloids in experimentally grown plants, and is designed for use with small samples.

Procedure—Evenly wet about 1 g., accurately weighed, of the dried plant material with about 6 drops of 0·5 N sulphuric acid, and extract the excess of pigment with two, 15-ml. quantities of ether. Drive off the solvent still remaining with the powder by gently warming. Then mix the powder with 6 drops of 10 per cent. ammonia solution, w/w, and extract the alkaloids with 60 ml. of benzene in a miniature percolator. The preliminary ether extraction is unnecessary with root

or stem powders. Pour the benzene extract on to a column of activated alumina, 2·5 cm. by 1 cm., arranged immediately above a column of activated silica gel, 12 cm. by 1 cm. When the benzene solution has passed through the columns, pipette 30 ml. of absolute alcohol on to the alumina, and when the alcohol has run through, remove the short alumina column, and add 5 ml. of acetone to the silica. Now de-activate the latter with 4 ml. of 20 per cent. ammonia solution, w/w, and elute the alkaloids with 50 ml. of chloroform. Remove the solvent from the percolate by evaporation under slightly reduced pressure on a water-bath at 60° C. Dissolve the residue in a few drops of alcohol and 1 ml. of 0·02 *N* sulphuric acid, and titrate back with 0·01 *N* sodium hydroxide, using a Rehberg-type microburette (*Biochem. J.*, 1925, **19**, 270), and methyl red as indicator. The titration should be conducted in about 15 ml. of carbon-dioxide-free water and in a carbon-dioxide-free atmosphere. One ml. of 0·01 *N* acid or alkali is equivalent to 2·892 mg. of alkaloid as *l*-hyoscyamine or *dl*-atropine. Before use, the alumina and the silica gel are washed with 50 per cent. acetic acid and with distilled water, and are activated by heating for 24 hr. in a silica dish at a red heat.

The positions of the alkaloids and pigments in the course of this procedure are shown in the Table below.

	Alumina column	Silica column	Eluted solution
I. After adsorption of benzene percolate	*alkaloids* and all pigments except carotenoids	carotenoids (weakly adsorbed)	carotenoids
II. After elution with alcohol	decomposition products of chlorophyll	*alkaloids*	chlorophyll, xanthophyll, etc.
III. After elution with ammonia solution and chloroform	—	—	*alkaloids*

A. H. A. Abbott

Biochemical

Differentiation between Ribose-3-phosphate and Ribose-5-phosphate by means of the Orcinol - Pentose Reaction. H. G. Albaum and W. W. Umbreit (*J. Biol. Chem.*, 1947, **167**, 369–376)—When equal quantities of ribose-3- and ribose-5-phosphate are heated with the orcinol reagent, the final colours developed are identical, but the rates of colour development are very different. The rate for ribose-3-phosphate is virtually identical with that for free ribose, owing, apparently, to the rapid removal of the phosphorus from ribose-3-phosphate by the strong acid in which the tests are carried out. Ribose-5-phosphate reacts more rapidly than ribose or ribose-3-phosphate, whilst xylose and arabinose develop colours more slowly than ribose.

Procedure—To 3 ml. of a solution containing 10 to 30 μg. of pentose, add 3 ml. of ferric chloride - hydrochloric acid reagent and 0·3 ml. of alcoholic orcinol solution. Mix and evaluate the colour in an Evelyn colorimeter with filter 660. Immerse the tubes in boiling water for 8 min., cool, and read the colour again. Repeat the readings at the end of 15, 25, 35, and 45 min., and plot the curve relating colour to time. With ribose-5-phosphate, maximum colour often develops at the end of 25 min. and certainly at the end of 35 min.; at the end of 7 min., the amount of colour developed is 65·5 per cent. of the maximum. With ribose-3-phosphate, on the other hand, colour development is much slower, maximum colour being produced after 45 min. heating, and only 26·5 per cent. of the maximum colour after 7 min. To distinguish between the two compounds, therefore, the readings after 7 and 45 min. heating should be compared with the corresponding readings obtained with pure specimens of the two compounds. By this means a distinction can readily be made between yeast adenylic acid and guanylic acid on the one hand, which contain ribose-3-phosphate, and muscle adenylic acid and adenosine triphosphate on the other hand, which contain ribose-5-phosphoric acid.

F. A. Robinson

Determination of Tryptophan. J. D. Hauschildt, T. L. Isaacs, and W. B. Wallace (*J. Biol. Chem.*, 1947, **167**, 331–337)—The method of Eckert (1) (*Ibid.*, 1943, **148**, 205) is compared with the chemical method of Shaw and McFarlane (2) (*Canad. J. Res.*, *B*, 1938, **16**, 361) and the microbiological method of McMahan and Snell (3) (*J. Biol. Chem.*, 1944, **152**, 83). Various modifications are described.

Preparation of samples—For method 1, 100 to 300 mg. of protein were hydrolysed in sealed tubes for 6 hr. at 15 lb. per sq. in. with 5 ml. of 5 *N* sodium hydroxide, neutralised to *p*H 7, filtered, and treated with enough extra hydrochloric acid to give 1·2 *N* acid after dilution for analysis. Samples for method 3 were treated similarly except that no excess of hydrochloric acid was added after neutralisation. Racemisation was assumed to be complete and the micro-organisms were assumed to utilise only the natural form of tryptophan. For method 2, 100 to 300 mg. were dissolved in 5 ml. of 20 per cent. sodium hydroxide solution. Diluted aliquots were analysed.

Method 1—Two modifications of the original method were made. A wavelength of 560 mμ. was used for the absorptiometer readings and diazotisation was continued for 60 instead of 30 min., as this gave greater accuracy. Standards ranged from 0 to 40 μg. of tryptophan. It was found necessary to plot a standard curve for each determination.

Method 2—A wavelength of 560 mμ. was used. Analytical-grade sulphuric acid was found to be satisfactory without distillation. No difference in tryptophan content was observed between hydrolysed and non-hydrolysed samples. Unhydrolysed human serum albumin gave turbid solutions. All standards and unknowns were analysed in triplicate. In each complete determination at least two levels, about 50 and 100 μg., were used.

Method 3—The medium of McMahan and Snell was used, tryptophan and hydroxyproline being omitted from the basal medium and 1 μg. of folic acid per tube added. *l* (−)-Tryptophan was used as reference standard with a range from 0 to 1·2 μg. Fresh cultures of *Lactobacillus arabinosus* were used for the inoculum, the tubes were incubated for 3 days at 37° C., diluted with 4 ml. of water, and determined turbidimetrically at 540 mμ. All values given as trustworthy were the average of at least three complete determinations which were calculated from five levels of unknown.

Conclusions—Comparison of the three methods shows certain factors in favour of each. Method 1 gave results lower than methods 2 or 3, whilst recovery of added tryptophan was poor with gelatin but excellent with casein hydrolysate, suggesting that recovery experiments should be carried out on all new proteins tested. Reproducibility was good, no interfering colours developed, and fairly small samples could be used for analysis. The method was simple and rapid. Method 2 gave results in good agreement with those of other workers and was dependable. Hydrolysis of proteins was not usually necessary. Method 3 compared in accuracy and magnitude with 2. It was more selective, but more time-consuming than 1 or 2. Its greatest advantage was the small amount of sample required for analysis. J. S. Harrison

Colorimetric Determination of Uric Acid with Alkaline Ferricyanide. H. Silverman and I. Gubernick (*J. Biol. Chem.*, 1947, **167**, 363–368)—*Reagents—Ferric iron reagent*—Fill a 1-litre cylinder to the mark with water, suspend 20 g. of gum ghatti on copper or galvanised iron gauze just below the surface, allow to stand for 24 hr., remove, and strain the liquid through double towelling. Dissolve 5 g. of anhydrous ferric sulphate, by heating in 75 ml. of 85 per cent. syrupy phosphoric acid and 100 ml. of water. Cool and mix with the gum solution; add 1 per cent. potassium permanganate solution to the mixture, 2 or 3 ml. at a time, until a faint pink colour remains for at least 5 min.

Uricase powder—Remove superficial fat from 5 lb. of fresh beef kidney, mince, place in a wide-mouthed bottle, wash with tap water by decantation, homogenise small portions with equal weights of benzene in a Waring Blendor, and combine the portions in the bottle. Add two volumes of cold acetone, allow protein to settle, filter through cheese-cloth, squeeze dry, re-suspend in three times the weight of acetone, allow to settle, and filter. Repeat until dehydrated and defatted, dry overnight in air, grind to a fine powder in a mortar, and store in a vacuum desiccator. Uricase powder is available from the Arlington Chem. Co., Yonkers 1, N.Y.

Uric acid stock solution—Dissolve 9 g. of anhydrous disodium hydrogen phosphate in 500 ml. of hot water, pour on to 200 mg. of pure uric acid suspended in a few ml. of water in a litre flask, mix, cool, and dilute to 1 litre (1 ml. ≡ 0·2 mg. of uric acid).

Procedure—Take three 50-ml. Erlenmeyer flasks and add: to *A* 1 ml. of serum or plasma and 10 mg. of uricase powder, and swirl gently to mix; to *B*, 1 ml. of serum or plasma; and to *C*, 1 ml. of water and 10 mg. of uricase powder. *C* is used to determine the uricase blank, and only a few runs are necessary for each batch of uricase. Place *A* and *C* in an incubator at 37·5° C. for 2 hr. Prepare a Folin-Wu filtrate by adding 8 ml. of *N*/12 sulphuric acid and 1 ml. of 10 per cent. sodium tungstate solution to each flask, shake thoroughly, and transfer to 16-ml. tubes. Centrifuge for 5 min. and pipette 5 ml. of the supernatant liquids into test tubes. Into a fourth tube *D*, pipette 5 ml. of water to serve as a reference solution for all untreated sera. Place all four tubes in an ice-brine bath, preferably a Dewar flask, with sufficient ice to maintain freezing temperatures for at least 90 min. Pipette 3 ml. of cold, alkaline ferricyanide solution (prepared by mixing equal parts of a 0·4 per cent. potassium ferricyanide solution and a 1·6 per cent. sodium carbonate solution) into each tube, mix immediately, promptly replace the tubes in the ice-bath and allow oxidation to proceed for exactly 60 min. Temperature control throughout the experiment is essential. After 60 min., add 2·5 ml. of ferric iron reagent to each tube, mix, place the tubes immediately in a water-bath at 25° C., allow to stand for 20 min. at this temperature, and measure photometrically at 540 mμ. Read flask *B* against *D*, and *A* against *C*.

Preparation of working standards—Uric acid working standards are prepared in a range equivalent to 2 to 16 mg. of uric acid per 100 ml. of serum. To prepare the standard equivalent to 4 mg. per 100 ml. serum uric acid, dilute 5 ml. of the stock solution to 50 ml. with 0·01 per cent. sodium carbonate solution, pipette 1 ml. of this solution into a test tube and add 4 ml. of a uric-acid-free filtrate prepared by treating 10 ml. of serum with 100 mg. of uricase powder and preparing a Folin-Wu filtrate. Cool, add 3 ml. of alkaline ferricyanide solution and continue as described under *Procedure*. Other standards are prepared similarly so that 1 ml. contains the requisite amount of uric acid to which 4 ml. of uric-acid-free filtrate can be added.

Calculation of results—Beer's law holds and a straight line relationship can be obtained by plotting the photometric densities against known uric acid concentrations. Serum uric acid values are obtained from the graph by subtracting the non-uric acid reducing value from the total reducing value. For clinical purposes, when the uric acid values are expected to fall in the normal range, treatment of the serum with uricase powder may be omitted and 0·6 mg. per 100 ml. subtracted from the total reducing value. Similarly, the uricase

step can be omitted in cases of urinary retention, where the non-uric acid value is 10 to 20 per cent. of the total, and in cases of elevated uric acid content due to other causes, where the non-uric acid remains about 0·6 mg. per 100 ml. as in normal serum.

J. S. HARRISON

Determination of Aromatic Amidines in Plasma and Urine. D. P. Jackson, W. J. Kuhl, jun., and J. L. Irvin (*J. Biol. Chem.*, 1947, **167**, 377–386)—The method can be used for the determination of aromatic amidines such as stilbamidine, propamidine, pentamidine, phenamidine, and *p*-carbethoxybenzamidine, used as chemotherapeutic-agents. Fluorescent glyoxalidone derivatives are produced by reaction with glyoxal and benzaldehyde in alkaline solution.

Reagents—*Glyoxal solution*—Dissolve glyoxal-bisulphite addition product in water to give 20 mg. per ml. *Benzaldehyde solution*—Dissolve 8 ml. of benzaldehyde in 100 ml. of ethyl alcohol. *Alcohol - ether mixture*—Mix 10 ml. of ethyl ether with 90 ml. of ethyl alcohol. *Dialysed iron*—Use a colloidal suspension equivalent to 5 per cent. of ferric oxide.

Procedure—The following procedure may be applied to pure aqueous solutions of aromatic amidines, to protein-free centrifugates and extracts of plasma, and to urine. Put 10 ml. of an aqueous solution of an aromatic amidine into a 125-ml. Erlenmeyer flask, add 0·4 ml. of glyoxal solution and 0·5 ml. of benzaldehyde solution, shake, add 1 ml. of 10 *N* sodium hydroxide dropwise with constant agitation, shake mechanicaly for 45 min. at room temperature, and add 10 ml. of the alcohol - ether mixture. Leave for 15 min. and measure the fluorescence. If a turbidity develops, as may happen with plasma, filter through a sintered-glass filter funnel with gentle suction, avoiding excessive evaporation of alcohol and ether.

Preparation of standard solutions—Either direct comparison with the fluorescence produced by known amounts of stilbamidine (or other aromatic amidine), or calculation from a standard calibration curve, can be used. The former is more accurate, but the latter can be used for routine purposes. If a calibration curve is used, the standard of fluorescence should be quite stable, *e.g.*, mepacrine in dilute sulphuric acid. Standards, ranging from 0·5 to 10 µg. for stilbamidine, are prepared by allowing 10-ml. portions of suitable aqueous dilutions of a salt of the amidine to react with glyoxal and benzaldehyde. A blank is prepared containing all the reagents except the amidine. Solutions are diluted with the alcohol - ether mixture, and the fluorescence is measured, using a mercury vapour lamp with a primary filter transmitting light of wave length either 410 or 365 mµ., and a secondary filter transmitting wavelengths greater than 490 mµ.

Preparation of plasma or serum for analysis—Two alternative methods may be used; the first (A) is quicker, but is limited to higher concentrations of the aromatic amidines.

Method A—*Preparation of protein-free centrifugate*—Put 5 ml. of plasma or serum into a 50-ml. flask and add 15 ml. of water, immerse the flask in boiling water for 3 min. or longer until the diluted plasma shows definite turbidity, and add 3 ml. of dialysed iron dropwise with constant agitation. Heat for a further 2 min., add 2 ml. of 22 per cent. sodium sulphate solution dropwise with agitation, cool, transfer to a centrifuge tube, and centrifuge for 5 to 10 min. Do not filter, as this causes loss by adsorption on the paper. The centrifugate should be colourless but may be faintly turbid; if so, filter through sintered-glass after the reaction with glyoxal and benzaldehyde. Allow 10 ml. to react with glyoxal and benzaldehyde, and prepare a blank by taking a 10-ml. aliquot from the protein-free centrifugate into a 125-ml. Erlenmeyer flask, adding 1 ml. of 10 *N* sodium hydroxide and placing the flask in boiling water for 15 min. to hydrolyse the amidine. Evaporation is minimised by covering the flask with an inverted funnel. Cool, add 0·4 ml. of glyoxal solution and 0·5 ml. of benzaldehyde solution, shake for 45 min., dilute with 10 ml. of the alcohol - ether mixture, filter if necessary, and measure the fluorescence after 15 min.

Method B—*Extraction with butyl alcohol*—Put 5 ml. of plasma into a 50-ml. centrifuge tube with a constricted neck, add 25 ml. of *n*-butanol, stopper, shake for 2 min., centrifuge, and decant the extract. Again extract the plasma protein with 25 ml. of butanol, combine the extracts, acidify to Congo red with dilute sulphuric acid, and evaporate to dryness by distillation *in vacuo* at 40° to 60° C. Dissolve the residue in the flask in 12 ml. of water, extract with 10 ml. of chloroform to remove lipoids and bilirubin, and remove 10 ml. of the aqueous phase by a pipette into a flask. Allow to react with glyoxal and benzaldehyde, add alcohol - ether mixture, and measure fluorimetrically. The amount of amidine in the final solution corresponds to that contained in 4·17 ml. of the plasma sample. Carry out a blank determination on a second extract that has been boiled in alkaline aqueous solution before the addition of glyoxal and benzaldehyde.

Calculation—Standard and unknown should be in the range where intensity of fluorescence bears a linear relationship to the concentration of amidine. Then

$$x = \frac{S(I_x - I_b)}{(I_s - I_r)}$$

where x = amidine in the final solution, S = amidine in the final standard solution, and I_x, I_b, I_s, and I_r are the intensity readings of the unknown, the combined plasma and reagent blank, the standard, and the reagent blank, respectively.

Determination in urine—Put a measured volume of urine, containing 2 to 10 µg. of stilbamidine, preferably less than 5 ml., into a 125-ml. Erlenmeyer flask, dilute to 10 ml., add 0·8 ml. of glyoxal solution and 1 ml. of benzaldehyde solution, mix, and add 2 ml. of 10 *N* sodium hydroxide dropwise with constant agitation. Shake mechanically for 45 min. at room temperature, extract with 20 ml. of *n*-butanol, separate by centrifuging, discard the aqueous layer, and wash the butanol extract once with 10 ml. of 0·1 *N* sodium hydroxide. Centrifuge, discard the aqueous phase, and to 15 ml. of the extract add 5 ml. of ethyl ether. Leave for 15 min., filter through a Whatman No. 42 paper, and measure

the fluorescence in the usual manner. If it is found necessary to use more than 5 ml. of urine, proportionally more of the reagents should be used. Prepare a blank, using an equal volume of urine which, after dilution to 10 ml., is treated with 2 ml. of 10 N sodium hydroxide and boiled for 15 min. before addition of the other reagents. Standards are prepared by adding to an equal volume of urine a known amount of amidine and carrying out the reaction and extraction as above.

Calculation—

$$x = \frac{s(I_x - I_b)}{(I_s - I_x)}$$

where x = amidine in the original sample, s = amidine added to the standard sample, and I_x, I_s, and I_b are the intensity readings of the original sample, the standard sample with added amidine, and the urine blank, respectively. For greater accuracy, graphical computation may be used with a series of standards added to the urine. The accuracy of the method as applied to pure aqueous solutions is about ± 4 per cent., whilst with plasma the average deviation of a single determination is about ± 6 per cent. J. S. HARRISON

Colorimetric Micro-determination of Urea-nitrogen by the Xanthydrol Method. M. G. Engel and F. L. Engel (*J. Biol. Chem.*, 1947, **167**, 535–541)—The xanthydrol method for the determination of urea-nitrogen has been re-investigated, a colorimetric technique being used. It is simple, selective, and accurate to about 1 per cent. at levels as low as 2 μg.

Reagents—*Wash solution A*—Methyl alcohol saturated with dixanthylurea. In each of four, 50-ml. centrifuge tubes place 5 ml. of a solution containing 0·6 mg. of urea, 5 ml. of glacial acetic acid, and 1 ml. of 5 per cent. alcoholic xanthydrol solution. Stir, allow to stand for 30 min., add 20 ml. of methyl alcohol, again stir, centrifuge, and decant the supernatant liquid. Wash with a further 20 ml. of methyl alcohol, centrifuge, and decant. The precipitate must saturate 2000 ml. of methyl alcohol at 20° C. Filter immediately before use. *Wash solution B*—Methyl alcohol-water mixture (3 : 1), saturated with dixanthylurea. Dilute 3 parts of A with 1 part of water; filter immediately before use.

Procedure—Two to 30 μg. of urea-nitrogen can be determined in 0·1 to 1 ml. of blood or serum. Tungstate filtrates may be used, 1 : 10 dilution for ureas in the normal range and 1 : 50 for higher levels. Place 1 ml. of filtrate in a 15-ml. centrifuge tube, add 1 ml. of glacial acetic acid, stir with a glass rod, and add 0·2 ml. of a 5 per cent. solution of xanthydrol in absolute alcohol; avoid touching the side of the tube or rod as the solution tends to creep up glass. Stir vigorously, allow to stand for 10 min., leaving the rod in the tube, and put in the refrigerator for 6 to 24 hr. to allow the dixanthylurea precipitate to form. Allow the suspension to reach room temperature, add 4 ml. of wash solution A, rinse the sides of the tube and the rod, mix, allow to stand for 15 min., stirring frequently, and remove the rod. Wash the tube and rod with about 0·5 ml. of wash solution, centrifuge for 5 min. at 2500 r.p.m., decant, and discard the supernatant liquid. Wash the precipitate with 4 ml. of wash solution B, centrifuge, and decant. Repeat the washing with solution B. Check the completeness of washing by adding 50 per cent. sulphuric acid to the discarded wash; a yellow colour indicates that further washing is required. Wash again if necessary, drain the solution from the tubes, add 10 ml. of 50 per cent. sulphuric acid, mix, and after 20 min. read in a colorimeter with a filter transmitting at 420 mμ., using 50 per cent. sulphuric acid as blank. A series of standards is prepared to give a standard curve ranging from 2 to 25 μg. of urea-nitrogen. A straight line passing through the origin is obtained by plotting colour density against urea-nitrogen. The maximum error of eight determinations was 1·6 per cent. The average recovery of added amounts of urea-nitrogen, varying from 0·43 to 13·8 μg., was 97·5 per cent. Thiourea interfered with the method and allantoin reacted slightly at concentrations greater than 1·5 mg. per ml. None of the many other substances tested caused interference. J. S. HARRISON

Some Problems in the Titration of Streptomycin. J. R. May, A. E. Voureka, and A. Fleming (*Brit. Med. J.*, 1947, i, 627–630)—Streptomycin is usually assayed on the Gram-negative bacilli *B. coli* or Friedländer's bacillus, of which the strain Klebsiella 41 has been widely used in America. The paper gives an account of difficulties encountered in the titration of streptomycin in aqueous solution or in serum. The influence of the following factors has been investigated—nature of the culture medium; dilution of the culture medium; nature of the test organism; size of the inoculum; access of air. For the estimation of streptomycin in blood the recommended procedure is given below.

Procedure—Prepare serial dilutions of the serum to be tested in physiological saline, and to each dilution add an equal volume of infected medium. Use 0·025-ml. volumes and incubate the mixture in capillary tubes open at both ends and stuck flat on a microscopic slide with plasticine. Larger volumes in test tubes can be used, if desired. Using serum water as the medium and an infection with Klebsiella 41 or staphylococcus, the end-point is at a dilution of 1 in 4 to 6 million. In blood, about a 1 in 1 million concentration of streptomycin is required to inhibit growth completely, so that using serum water the method will indicate levels of streptomycin below the therapeutic range, whereas if broth is used this is not so. Since so many factors affect the titration, a control titration of a known concentration of streptomycin (1 in 10^6) in human serum should be set up with the test so that comparison of the end-point of this test can be made directly with that of the serum under examination.

The infected medium is prepared as follows:—Take a dilution of 1 part of serum to 4 parts of distilled water and add glucose to 1 per cent. and sufficient of a saturated, aqueous solution of phenol red to give a definite red colour. The medium

should be boiled or steamed, and 1 ml. inoculated with 10 ml. of a 24-hour broth culture of the test organism. The test organism may be staphylococcus or Friedländer's bacillus (Klebsiella 41). The former is almost as sensitive as the latter, and there is no need to inactivate the serum. If the patient has had penicillin, staphylococcus cannot be used unless penicillinase is added to the medium.

A chart giving the serum levels of streptomycin after intramuscular injections of the drug is reproduced.

Attention is directed in an addendum to the cumulative error inherent in the usual method of making serial dilutions, which becomes serious if more than 5 or 6 are set up. R. H. THORP

Organic

Determination of Carbon and Hydrogen by Combustion. Unitised Dual Apparatus and Improved Procedure. D. D. Tunnicliff, E. D. Peters, L. Lykken, and F. D. Tuemmler (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 710–718)—The apparatus described gives accurate results on macro-samples, and is so designed that two determinations can conveniently be conducted side-by-side in one compact dual combustion unit. This feature, together with the replacement of rubber connections by commercial compression metal fittings, a special metal-to-glass fitting, and standard taper glass joints; the provisions made for adequate combustion control; and the well-defined procedure, all combine to give accurate results in the minimum of time. Other important features of the method are the control and indication of all gas flow rates; the adequate and convenient pyrometer temperature indication; the addition of extra oxygen between the sample and the catalyst to ensure completeness of oxidation; the use of carefully determined combustion and operating conditions; and a method for the accurate analysis of volatile samples.

Two procedures are detailed; the one, a long, precision method in which a skilled operator can make two determinations in an 8-hour day with a precision of ± 0·008 per cent. of hydrogen and ± 0·009 per cent. of carbon, and a probable accuracy of 0·011 and 0·015 per cent., respectively; and the other, a short, routine procedure in which an experienced operator can perform 8 determinations a day with a precision of ± 0·02 per cent. of hydrogen and ± 0·05 per cent. of carbon, with probable accuracies of 0·05 per cent. for both elements. Each procedure is applicable in presence of sulphur, halogen, or nitrogen.

The paper contains details of the design of the apparatus, the fillings of the tubes, the electrical working of the dual combustion apparatus, and the analytical procedure, and data showing the precision obtainable in weighing the absorbers, the time required for removing combustion products during purging, the time required for equilibrium to be eached in the absorber, the effect of oxygen flow-ate and copper oxide catalyst temperature on ompleteness of combustion, the reproducibility and ccuracy of the routine and precision procedures, nd the time schedule. For these, however, the riginal must be consulted. M. E. DALZIEL

Analysis of Naphthalene - Tetralin - Decalin Mixtures. W. J. Cerveny, J. A. Hinckley, jun., and B. B. Corson (*Anal. Chem.*, 1947, **19**, 82–86)—Two methods are described by which the mixtures of naphthalene, tetralin, and decalin formed by the hydrogenation of naphthalene can be analysed. In the first method measurements are made of the heat of reaction of the sample with nitrating acid (Bishop and Wallace, *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 563) and of the temperature at which the first crystals of naphthalene appear on cooling the stirred sample (the cloud point temperature). In the acid test the mixtures behave as two-component systems of naphthalene - tetralin and *cis*-decalin - *trans*-decalin. Decalin is determined by this test, naphthalene by the cooling test, using the values determined for decalin, and tetralin by difference. An alternative method is to separate the sample into two fractions by distillation and to measure the specific dispersion of the fractions, both of which are two-component systems in respect of specific dispersion.

Acid heat test—Apparatus—A pint Thermos flask is fitted with a rubber stopper carrying a fast stirrer, a funnel, and a thermometer. *Reagents*—Nitrating acid: Mix one volume of concentrated sulphuric acid with two volumes of concentrated nitric acid. *Cyclo*hexane; shake with nitrating acid, wash, distil, and store over a desiccating agent.

Procedure—Weigh 2 g. of sample into a 4-oz, glass-stoppered bottle, add exactly 50 ml. of *cyclo*hexane, shake, and suspend the bottle in a thermostat at 27° C. for 15 min. Pour into the Thermos flask exactly 100 ml. of nitrating acid warmed in the thermostat to 27° C. Insert the stirrer, thermometer and funnel, leave for 3 min., stir for 1 min., and read the temperature to ±0·02° C. Pour the solution of the sample through the funnel, stir for a standard time of about 10 min. and note the final temperature. Read the percentage of decalin from the graph, obtained as described below, relating the rise in temperature to the percentage of decalin present. Carry out the above procedure on 2-g. portions of purified naphthalene, tetralin, and decalin. If the rises in temperature for naphthalene and tetralin are the same, plot from the rises a straight-line graph relating rise in temperature to weight per cent. of decalin. If the condition is not satisfied, the nitrating acid may be too weak.

Cloud point temperature—Apparatus—A Pyrex test tube, 15 cm. by 1·8 cm., is fitted with an aluminium hand stirrer and a stopper carrying a thermometer.

Procedure—Place 10 ml. of the sample in the test tube, immerse the tube in a beaker of water maintained at 5° C. below the cloud point, stir until a thin haze of crystals appears, and read the temperature to ± 0·2° C. Take the average of three determinations. Read the weight percentage of naphthalene from the appropriate curve of the family of curves relating cloud point temperature, percentage of decalin, and percentage of naphthalene. This family of curves can be constructed from the data given in the Table.

Naphthalene-tetralin-decalin mixtures of known composition and containing from 12 to 100 per cent. of naphthalene were analysed. The deviations between the found and the correct percentages of naphthalene were not greater than ± 1 per cent.

Distillation - specific dispersion method—Apply this method only to samples containing at least 70 per cent. of tetralin and not more than 15 per cent. of naphthalene or decalin. Use a packed Fenske distillation column with a 200-ml. distilling flask, a total reflux head, and a 25-ml. distillate reservoir.

PER CENT. OF NAPHTHALENE CORRESPONDING TO VARIOUS CLOUD POINTS AND DECALIN CONCENTRATIONS

Decalin %	Cloud point, ° C.							
	10	20	30	40	50	60	70	80
0	22·1	28·0	35·1	43·6	54·0	66·7	81·9	100·0
10	20·8	26·5	33·5	42·0	52·4	65·2	80·6	—
20	19·3	25·0	31·9	40·4	50·6	63·6	79·4	—
30	17·9	23·5	30·3	38·5	48·9	61·7	—	—
40	16·7	22·0	28·6	36·7	47·0	59·7	—	—
50	15·4	20·5	26·9	34·8	44·8	—	—	—
60	14·1	19·0	25·1	32·8	—	—	—	—
70	12·8	17·5	23·3	—	—	—	—	—
80	11·3	15·9	—	—	—	—	—	—

Procedure—Weigh a 100-ml. sample into the distilling flask and heat the liquid until the head temperature is constant. Empty the distillate reservoir into a tared, 125-ml. glass-stoppered Erlenmeyer flask. Continue the operation of the column until the head temperature is again constant and empty the reservoir into the Erlenmeyer flask. Determine the specific dispersion of the combined overheads and of the bottoms and compute the composition of the original sample with the aid of specific dispersion - composition curves. The overheads contain tetralin and decalin and the bottoms, tetralin and naphthalene.

In the analysis of known mixtures the error in the percentage of tetralin found was up to ± 5 parts in 100.

B. ATKINSON

Determination of Mono- and Di-alkylacetylenes. C. D. Wagner, T. Goldstein, and E. D. Peters (*Anal. Chem.*, 1947, **19**, 103–105)—A method is described for the determination of mono- and di-alkylacetylenes of four and five carbon atoms, in presence of related paraffins, olefines, or diolefines. The acetylenes are allowed to react with methanol in presence of a mercuric oxide - boron trifluoride catalyst to produce ketals, which are hydrolysed to ketones. The ketones are distilled into hydroxylamine hydrochloride and the liberated acid is titrated with alkali.

Reagents—Pass boron trifluoride into methanol cooled in an ice-bath until the solution contains 45 to 50 per cent. of boron trifluoride by weight. Dissolve 0·1 g. of methyl orange and 0·14 g. of xylene cyanol FF in 500 ml. of 50 per cent. ethyl alcohol.

Procedure—Dissolve, with gentle heating, 0·1 g. of red mercuric oxide in 60 ml. of methanol containing 2 g. of the methanol - boron trifluoride mixture. Place in a 500-ml., round-bottomed flask, and immerse the flask in ice. Add 1 to 12 mg.-mol. of the alkylacetylene, from a pipette or in a glass ampoule. Stopper the flask, swirl, and allow to stand for 75 to 110 min. Cool in an ice-bath and add slowly 200 ml. of a cold, saturated sodium bicarbonate solution. Add a "boiling stone," connect the flask to a spray trap and vertical condenser, and distil until approximately 200 ml. of the distillate have been collected in 100 ml. of 2·5 per cent. hydroxylamine hydrochloride solution contained in a 500-ml., glass-stoppered Erlenmeyer flask cooled in ice. Stopper the receiving flask and shake well. Remove the stopper, warm the distillate to room temperature, pass a vigorous current of air through the solution for 10 min., add 0·7 ml. of the indicator solution, and titrate with 0·1 or 0·5 *N* sodium hydroxide to the colour of the blank. If carbonyl compounds are known to be absent, carry out the blank determination on a mixture of 60 ml. of methanol, 140 ml. of water, 0·7 ml. of indicator solution, and 100 ml. of hydroxylamine hydrochloride solution. If the carbonyl compounds present are known to be entirely volatile in the tests, add to the mixture used for the blank titration approximately the same amount of sample as was used in the determination. If relatively non-volatile carbonyl compounds are believed to be present, for the blank treat an appropriate amount of sample as for the determination, but omit the catalyst.

Samples of 2-butine, 1-pentine, and 3-methyl-1-butine, all of known purity, were used in the preparation of mixtures similar to those met in the routine analysis of crude *iso*prene. Analyses of these mixtures indicate a recovery of about 92 per cent. for samples containing 0·5 per cent. or more of the alkine. It is therefore necessary to multiply the results given by the above procedure by 1·09 to obtain the true values. Variations in the amount of catalyst have a marked effect on the results, the correction factor given being applicable only when the prescribed amount of catalyst is used.

Peroxides interfere both by oxidising the alkines and by forming carbonyl compounds. Large amounts of *cyclo*pentadiene precipitate the mercury catalyst.

B. ATKINSON

Test for *tert*-Butyl and *iso*-Propyl Alcohols with Denigès' Reagent. R. F. Robey and N. C. Robertson (*Anal. Chem.*, 1947, **19**, 310–311)—The scope of the mercuric sulphate method for the detection of impurities in, and the identification of,

iso-propyl alcohol has been investigated, and interference due to other compounds is reported. Pure *iso*-propyl alcohol yields a white precipitate with Denigès' reagent, but the yellow colour thought to be specific for *tert*-butyl alcohol may indicate a number of impurities, the tint depending on the type and concentration.

Preparation of reagent—Dissolve 50 g. of yellow mercuric oxide in 200 ml. of concentrated sulphuric acid, add the solution to 600 ml. of distilled water, and dilute to 1 litre with distilled water.

Procedure—Mix 1 ml. of the reagent with 1 ml. of water and add 2 ml. of the sample. Heat at 75° C. for 5 min. and, if no precipitate appears, repeat the test by heating at 100° C. for 10 min. The following Table indicates the coloured precipitates produced with undiluted substances, but when they are diluted to 1 per cent. or less with *iso*-propyl alcohol, they impart a yellow colour to the precipitate formed. Mesityl oxide, phorone, *tert*-butyl *iso*-propyl ether, and *tert*-butyl chloride give strong positive tests at much lower concentrations than that of *tert*-butyl alcohol required for an equivalent response.

REACTION OF VARIOUS SUBSTANCES WITH MERCURIC SULPHATE REAGENT

I. Substances giving a precipitate within 5 min. at 75° C.

Substance	*Colour*	*Character of precipitate*
iso-Propyl alcohol (87%)	white	moderate
tert-Butyl alcohol ..	bright yellow	heavy
sec-Butyl alcohol ..	bright yellow	light
Di-*iso*-propyl ether ..	dark yellow	light
Phorone	greyish	moderate
iso-Phorone	orange	light
tert-Butyl chloride ..	bright yellow	heavy
tert-Butyl *iso*-propyl ether	bright yellow	heavy
Polymer oil (plant) ..	dark yellow	heavy

II. Substances giving a precipitate within 10 min. at 100° C.

Substance	*Colour*	*Character of precipitate*
Ethyl alcohol (95%) ..	white	light
Mesityl oxide	grey needles	light
n-Butyl alcohol	bright yellow	light

Acetone, methyl ethyl ketone, diacetone alcohol, and di-*iso*-butylene give no precipitate under either set of conditions. A. H. A. ABBOTT

Determination of Allyl Groups in Polyallyl Ethers and Esters. H. M. Boyd and J. R. Roach (*Anal. Chem.*, 1947, **19**, 158–159)—The purpose of this work was to test the applicability of several known methods for the determination of unsaturation to the analysis of allyl ethers and allyl esters. The methods used were the 1-hr. Wijs (*Official and Tentative Methods of Analysis, Assoc. Off. Agric. Chem.*, 1940, p. 90), the Kaufmann and Hartwig (*Ber.*, 1937, **70B**, 2554), the Rosenmund and Kuhnhenn (ANALYST, 1924, 105), the rapid Wijs, and a bromine method.

Rapid Wijs—Prepare the usual Wijs reagents and the mercuric acetate catalyst, and proceed by the method of Hoffman and Green (*Oil and Soap*, 1939, **16**, 236).

Bromine method—Add 10 ml. of a 0·5 *N* solution of bromine in chloroform to a cold solution of 0·1 g. of sample in 10 ml. of chloroform in a flask. Place in a refrigerator at 4° C. for 10 min., seal the stopper with a few ml. of potassium iodide solution, and allow to stand at 4° C. for 1 hr. or longer, as desired.

Purified samples of allyl acetate, allyl phthalate, and triallyl glycerol were used in the tests. The rapid Wijs method gave iodine values less than 1 per cent. lower than the theoretical values, the 1-hr. Wijs, slightly lower values, and the Rosenmund - Kuhnhenn and bromine methods gave values within about ± 1·5 per cent. of theory. The Kaufmann method gave results about 10 per cent. low.

The methods were applied to the determination of the iodine values and hence the degrees of substitution of samples of allyl starch and allyl sucrose. Again there was good agreement between the values given by all the methods, except that of Kaufmann. B. ATKINSON

Analysis of Mixtures of Glycerol, Propylene Glycol, and Trimethylene Glycol. W. D. Pohle and V. C. Mehlenbacher (*J. Amer. Oil Chemists' Soc.*, 1947, **24**, 155)—These compounds may be found together in glycerol fractions or in sweet-water concentrate. The method of separation described takes advantage of the selective action of periodic acid (which oxidises glycerol to formic acid and aldehydes, propylene glycol to aldehydes only, and does not react with trimethylene glycol), together with a determination of the total acetylatable material. With pure samples, an accuracy of 0·8 per cent. for glycerol, within 1·7 per cent. for propylene glycol, and within 1·0 per cent. for trimethylene glycol, is attainable.

Reagents—(1) 2·0 per cent. periodic acid in distilled water: the solution must be filtered through a sintered-glass filter, stored in an amber-coloured, glass-stoppered bottle, and at no time allowed to come in contact with corks or rubber bungs. (2) Acetic anhydride - pyridine reagent prepared by mixing 1 volume of reagent-grade acetic anhydride with 6 volumes of pyridine; the mixture is unstable and must be discarded at the end of two weeks.

Procedure for glycerol—Weigh accurately about 0·5 g. of the sample into a 600-ml. beaker, add 50 ml. of distilled water, acidify with 0·2 *N* sulphuric acid, and neutralise with 0·05 *N* sodium hydroxide to *p*H 6·2, using a glass-electrode *p*H meter. Add 50 ml. of periodic acid reagent, allow to stand for 1 hr., dilute with 250 ml. of distilled water, stir mechanically, and titrate with 0·125 *N* sodium hydroxide to *p*H 6·2, using the *p*H meter. Conduct a blank determination, but titrate to *p*H 5·4 instead of *p*H 6·2. Reserve the test and blank solutions.

Procedure for propylene glycol—Make up the test solution to 500 ml. with distilled water and transfer 50 ml. to a 300-ml. Erlenmeyer flask. Add 10 ml.

of glacial acetic acid (99·5 per cent.), 10 ml. of 20 per cent. potassium iodide solution, mix, allow to stand for 2 min., and titrate with 0·08 N sodium thiosulphate, using starch indicator. Repeat with the blank solution; if the titration of the sample is not more than 80 per cent. of the blank, or if the difference between the titrations is less than 3 ml., repeat the whole of the analysis using smaller or larger samples, respectively.

Procedure for trimethylene glycol—Weigh accurately about 0·18 g. of the sample into a 300-ml., glass-stoppered flask, add 5 ml. of acetic anhydride - pyridine reagent, and set the loosely stoppered flask on a steam-bath. After 3 min., stopper tightly and heat for 30 to 40 min. Cool to room temperature, add 5 ml. of distilled water, re-stopper, and heat for a further 2 min. Cool to room temperature for 15 min., add 25 ml. of *iso*butyl alcohol (reagent quality) and titrate with 0·32 N to 0·35 N alcoholic potassium hydroxide, using phenolphthalein as indicator. Conduct a blank determination simultaneously and repeat the analysis with smaller or larger samples if the titration reading is less than 65 per cent. of the blank or if the difference in readings is small.

Calculation of results—The percentage of glycerol $= [(S - B) \times N \times 9{\cdot}209]/W = G$, where S = ml. of sodium hydroxide for sample, B = ml. of sodium hydroxide for blank, N = normality of sodium hydroxide and W = weight of sample in grams.

Propylene glycol: the total material reacting with periodic acid calculated as glycerol (D) is $[(B - S) \times N \times 23{\cdot}02]/W = D$ whence, the percentage of propylene glycol, $P = (D - G) \times 1{\cdot}6526$.

Trimethylene glycol: the total acetylated material calculated as glycerol (T) is $[(B - S) \times N \times 3{\cdot}070]/W = T$, where B = ml. of alcoholic potash for blank, S = ml. of alcoholic potash for sample, N = normality of alcoholic potash, and W = weight of sample for acetylation whence, the percentage of trimethylene glycol

$$= [(T - G) \times 1{\cdot}239] - P$$

A. H. A. Abbott

Determination of Beta-Dicarbonyl Compounds. W. Seaman, J. T. Woods, and E. A. Massad (*Anal. Chem.*, 1947, **19**, 250–251)—The method described for determining acetyl acetone, ethyl acetylpyruvate, and sodium ethyl acetylpyruvate may be applicable to β-dicarbonyl compounds in general. The compound is precipitated as a copper complex by adding an excess of copper acetate, the precipitate is filtered off, and the filtrate extracted with chloroform to remove the portion of complex remaining in solution. The excess of copper in the aqueous layer is determined iodimetrically. If the *p*H of the solution is too high, copper hydroxide is precipitated with the complex, whilst if the *p*H is too low, the keto form of the carbonyl is present and no complex is formed. For acetyl acetone, the optimum *p*H range for the precipitation is 5·2 to 6·0 and for ethyl acetylpyruvate, 4·8 to 6·5.

Method—Reagent—Dissolve 50 g. of cupric acetate monohydrate and 200 g. of sodium acetate trihydrate in 2 litres of water, and filter the solution. Standardise by titrating a 100-ml. portion with 0·1 N sodium thiosulphate as described below. This solution provides the correct *p*H for the determination of acetylacetone.

Procedure—If the sample is soluble in water, weigh 1·5 to 2 g. into a 250-ml., glass-stoppered flask containing 100 ml. of the cupric acetate solution. If the sample is not soluble in water, dissolve in a few ml. of ethanol and add the copper solution. Stopper the flask and shake frequently for 5 min. Filter through a 6-cm. Buchner funnel into a clean suction flask, and wash the flask and filter with 5-ml. portions of distilled water. Transfer the filtrate to a 250-ml. separating funnel, add 20 ml. of chloroform, and shake for 1 min. Run the chloroform layer into another separating funnel containing about 40 ml. of water and shake for 30 sec. Discard the chloroform layer. Repeat the procedure with three or four more 20-ml. portions of chloroform, washing each with the same 40-ml. portion of water. Transfer the water layers to a 500-ml. iodine flask, rinsing each funnel with several 5-ml. portions of water. Add 15 ml. of diluted hydrochloric acid (1 + 1) and 8 g. of potassium iodide, stopper the flask and shake several times over a period of 10 min. Titrate the liberated iodine with 0·1 N sodium thiosulphate, adding starch near the end-point. Subtract the titration figure from the figure obtained in the standardisation to obtain the volume of thiosulphate equivalent to the amount of complex formed. The equivalent weight of a β-dicarbonyl compound is twice its molecular weight.

Under the conditions given, the precipitated copper complexes of acetylacetone and sodium ethyl acetylpyruvate contain the correct percentages of copper. The error due to incomplete extraction of the complex is probably less than 1 part in 1000. The results are shown to be reproducible, but the accuracy of the method has not been determined.

B. Atkinson

Determination of Water in Phenol. L. R. Pollack (*Anal. Chem.*, 1947, **19**, 241–242)—A rapid cryoscopic method for the determination of water in phenol is described.

Procedure—Introduce about 25 g. of phenol into a 1-inch by 6-inch test tube and heat until the phenol has melted. Insert a cork stopper carrying an accurate thermometer and a loop stirrer and suspend the tube in an empty 500-ml. Erlenmeyer flask by means of a stopper. Stir at a fast and constant rate and record the steady temperature (T_1° C.) established after freezing has commenced. Place a fresh 25-g. sample in a 125-ml. Erlenmeyer flask and boil until the refluxing vapours reach the top of the flask, and for one minute more. Insert a stopper carrying a soda-lime tube, cool the flask, remove the stopper, and pour the liquid into a 1-inch by 6-inch test tube. Determine the freezing point as before (T_2° C.). The percentage of water in the phenol $\rightleftharpoons 0{\cdot}27\ (T_2 - T_1)$.

The results obtained show that the phenol is effectively dehydrated by boiling. For a water content of up to 1 g. of water per 100 g. of phenol the errors in the results are not greater than $\pm$ 0·01 g. of water per 100 g. of phenol. If the

percentage water content is over 2 per cent., the errors are high. The accuracy of the method is not affected by the presence of 1·1 per cent. of cresol in the phenol. B. ATKINSON

Colorimetric Determination of Acetophenetidine. E. F. Degner and L. T. Johnson (*Anal. Chem.*, 1947, **19**, 330–331)—The authors describe a rapid colorimetric method for the determination of acetophenetidine (phenacetin) based on Ritsert's reaction, in which a highly coloured compound is formed by the action of chromic acid on the *p*-phenetidine obtained by the acid hydrolysis of the drug, the sensitivity and reproducibility being greatly improved by performing the test in presence of a high concentration of ammonium citrate.

Procedure—Dissolve a weighed sample of dry, finely ground powder (from tablets, etc.), containing 0·15 to 0·20 g. of acetophenetidine, in chloroform, or if the solid is not completely soluble, extract with several small quantities of chloroform, and make up to 100 ml. with the same solvent. Transfer 3 ml. to a 10-ml., narrow, flat-bottomed flask graduated to contain 1 ml., add 3 ml. of concentrated hydrochloric acid, heat gently to remove the chloroform, and then boil until 1 ml. of solution remains. Add 8 ml. of water and cool. Transfer to a 50-ml. flask and make up to 50 ml. with water. Pipette 2 ml. of this solution into a test tube, add 8 ml. of 50 per cent. aqueous ammonium citrate solution, mix, and add 0·1 ml. of 1·0 per cent. aqueous chromic acid (chromium trioxide), mix, and begin timing. Transfer to an absorption tube, and read in a spectrophotometer at 543 mμ. after 7 min., the spectrophotometer being set at 100 per cent. transmission, and the blank tube containing a solution made by mixing 2 ml. of diluted hydrochloric acid (1 in 100), 8 ml. of the ammonium citrate solution, and 0·1 ml. of the chromic acid solution. The readings are taken at 23° to 24° C., and the quantity of acetophenetidine is determined from a standard curve, prepared from determinations on known amounts of pure acetophenetidine. The quantities prescribed are optimal when a Beckman spectrophotometer is used, but much smaller amounts of acetophenetidine can be used. Of compounds related to acetophenetidine, only acetanilide is likely to interfere appreciably since it produces a brown compound that reaches maximum intensity after several hours. When equal amounts of acetanilide and acetophenetidine are present, results may be up to 7 per cent. too high, but a correction can be made.

A. H. A. ABBOTT

Derivatives of Diphenylamine as Oxidation-Reduction Indicators in Alkaline Solution. H. H. Willard and G. D. Manalo (*Anal. Chem.*, 1947, **19**, 167–170)—Various diphenylamine derivatives have been tested as indicators for oxidation-reduction titrations in alkaline solution. Diphenylamine sulphonic acid and 2-aminodiphenylamine sulphonic acid-4 are satisfactory for the titration of arsenite with hypobromite in alkaline solution. For use when tervalent arsenic, antimony, or chromium, or hydrazine sulphate is oxidised with an excess of potassium ferricyanide and the excess is titrated with vanadyl sulphate, one of the following indicators is recommended; diphenylamine sulphonic acid, 2-carboxy-2′-methoxydiphenylamine, 2-carboxy-2′-methyldiphenylamine, 2-carboxydiphenylamine, 2:2′-dicarboxydiphenylamine, 2-carboxy-2′-bromodiphenylamine, or 2-carboxy-3′-ethoxydiphenylamine. The same indicators may be used in the titration of hydrogen peroxide into alkaline potassium ferricyanide solution. For the direct titration with hypobromite of thiosulphate, thiocyanate, and tervalent antimony, in alkaline solution, diphenylamine sulphonic acid and 2-aminodiphenylamine sulphonic acid-4 are satisfactory indicators. The colour change of the indicators on oxidation is from colourless to red, but diphenylamine sulphonic acid must be oxidised in acid solution to the green form before use as an indicator in alkaline solution.

Hypobromite titrations—Hypobromite solutions must be standardised every time they are used, and for this purpose arsenic trioxide is a convenient standard. When hypobromite is titrated into antimonite, thiocyanate, or thiosulphate solutions, using diphenylamine sulphonic acid as indicator, the indicator end-point coincides with the potentiometric end-point.

Potassium ferricyanide - vanadyl sulphate titrations—Potassium ferricyanide, dried overnight at 100° C., can be used as a primary standard, and a 0·05 *N* solution is stable for at least a month. Vanadyl sulphate is stable in acid solution, but is easily oxidised in alkaline solution. 2-Carboxy-2′-methoxydiphenylamine is especially recommended for use with this system.

Procedure—Prepare an acid solution of vanadyl sulphate by reducing with sulphur dioxide a nearly boiling solution of ammonium vanadate in sulphuric acid. Remove the excess of sulphur dioxide by bubbling carbon dioxide through the solution. Make the potassium ferricyanide solution about 3 *N* with respect to sodium hydroxide, saturate with oxygen-free nitrogen, add the indicator, and while bubbling nitrogen through the solution titrate with the vanadyl sulphate solution. Perform a blank titration to determine the indicator correction.

Determination of arsenic, antimony, or chromium—Reduce the metal to the tervalent state, make the solution 3 *N* in respect of sodium hydroxide, and add an excess of 0·05 *N* potassium ferricyanide. Heat to 85° to 90° C., cool, saturate with nitrogen, and proceed as above.

Titration of hydrogen peroxide—Make a measured quantity of standard potassium ferricyanide solution 3 *N* with respect to sodium hydroxide, heat to 70° to 80° C., and titrate with hydrogen peroxide, using the desired indicator. B. ATKINSON

Effect of Sample Preparation on Analytical Values for Alpha-Cellulose, Copper Number, and Cuprammonium Viscosity. B. L. Browning (*Paper Trade J.*, 1947, **124**, April 10, *T.A.P.P.I. Sect.*, 158–159)—Comparison of various methods of disintegrating samples (*e.g.*, of cellulose pulp) shows that the values obtained in the above determinations vary considerably according to the method chosen. For the α-cellulose determination, the sample

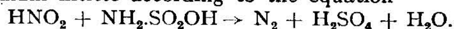
should be reduced in size by very mild treatment, *e.g.*, by tearing apart by hand. For the copper number, the best method of preparation is that which gives the lowest results, *i.e.*, slushing with water, or the use of an Abbé-type mill having a 4-inch exit screen. With the cuprammonium viscosity, the effect of disintegration is greatest with high-viscosity samples. The best method is wet disintegration, followed by formation into very thin sheets or pads, which are washed with the usual organic solvents to eliminate any "horniness"; hand disintegration gives high results. J. GRANT

Determination of Alpha-cellulose. P. F. Cundy and M. M. Beck (*Paper Trade J.*, 1947, **124**, May 1, *T.A.P.P.I.Sect.*, 194–195)—Existing alkali-extraction methods, in which a correction for the presence of lignin must be made, are lengthy and inaccurate, because the lignin must be determined separately. It is preferable to de-lignify the sample before the actual determination of α-cellulose.

Procedure—Stir the air-dry equivalent of 3 g. of moisture-free sample (*e.g.*, unbleached wood pulp, torn up by hand), in a 400-ml. beaker with 100 ml. of water and sufficient glacial acetic acid (usually 0·5 ml.) to produce a *p*H of 4 to 5 after thorough stirring. Add 1 g. of sodium chlorite, heat at 70° to 80° C. for 30 min. with agitation, filter on a sintered-glass crucible (Pyrex C grade), and wash thoroughly with water and then with acetone. Aspirate air through the sample until it is dry, and proceed with the α-cellulose determination in the usual way, using the same sintered-glass crucible. The method gives lower, but more accurate, results than does the conventional method, owing to the removal, after de-lignification, of hemi-cellulosic materials which are otherwise protected from dissolution in alkali by the lignin present. J. GRANT

Comparison of Methods of Sulphamate Determination. W. W. Bowler and E. A Arnold (*Anal. Chem.*, 1947, **19**, 336–337)—Of the available methods for determining sulphamic acid, the gravimetric method of Baumgarten and Krummacher (*Ber.*, **1934**, **67**, 1260) is too tedious for routine use, and the gas evolution method of Meuwesen and Merkel (*Z. anorg. Chem.*, 1940, **244**, 89) requires special apparatus. Direct titration with sodium nitrite as for primary amino-groups has been used for sulphanilamide and sulphapyridine determinations, and to standardise sodium nitrite solutions, and is here modified to determine sulphamate.

Procedure—Acidify 100 ml. of a solution containing between 0·15 and 0·2 g. of sulphamic acid with 10 ml. of 10 per cent. sulphuric acid solution, and titrate in an Erlenmeyer or iodine flask with 0·2 *N* sodium nitrite according to the equation



$$HNO_2 + NH_2.SO_2OH \rightarrow N_2 + H_2SO_4 + H_2O.$$

Shake vigorously after each 5 or 10 ml. of solution is added to assist in nitrogen liberation, and after each drop as the end-point is approached. Equivalence is identified by a blue discoloration of starch-iodide indicator solution used externally.

Results—Compared with figures obtained by titrating with sodium hydroxide to the phenolphthalein end-point, agreement is to 1 part in 50 on 0·02 to 0·1 g. of sulphamic acid, to 1 in 1000 on 0·1 to 0·2 g., and to 1 in 200 on larger amounts. The accuracy at low concentrations would probably be increased by using more dilute sodium nitrite solution. Four samples have been analysed by this and the two methods mentioned above and the values obtained agree well.

Addition of an excess of sodium nitrite and its determination with excess of permanganate and ferrous sulphate, or with potassium iodide and thiosulphate in an atmosphere of carbon dioxide, is not suitable.

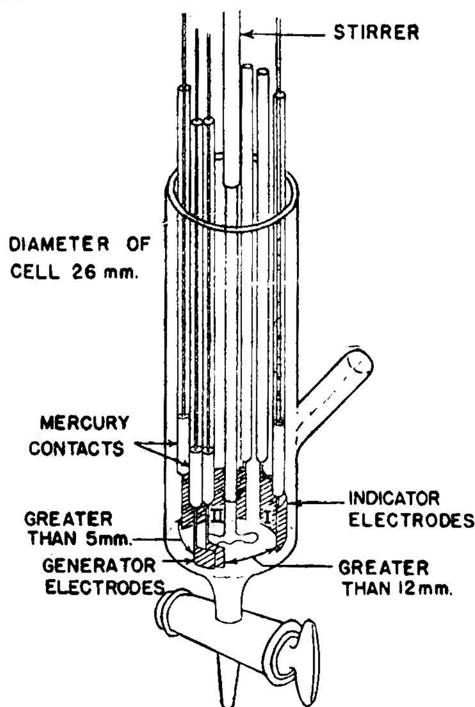
Naphthol blue-black gives a blue-to-purple colour change near the end-point, but the blank correction is too large for its use as an internal indicator in accurate work. It serves for obtaining approximate values, when used in conjunction with an external indicator. Ferric salts, ozone, hydrogen peroxide, and chlorine interfere when starch-iodide indicator is used, but Griess' diazo reagent is then effective. M. E. DALZIEL.

Polarographic Analysis of Mixtures of Maleic and Fumaric Acids. J. Warshowsky, P. J. Elving, and J. Mandel (*Anal. Chem.*, 1947, **19**, 161–164)—The simultaneous determination of maleate and fumarate ions is carried out by comparing the current readings at three applied potentials at a dropping mercury cathode, in an aqueous ammonia - ammonium chloride buffer of *p*H 8·2. The half-wave potentials of fumarate and maleate ions in this supporting electrolyte are − 1·67 v. and − 1·43 v., respectively, *versus* the saturated calomel electrode. If interfering substances are present, the fumarate and maleate ions are purified by precipitation of the barium salts, which are then redissolved in acid solution.

Procedure—Neutralise a sample of solution containing 0·10 to 0·15 g. of maleic and fumaric acids with 0·35 *N* barium hydroxide, using phenolphthalein as indicator, and add an equivalent amount of barium chloride solution, followed by four volumes of 95 per cent. alcohol. Allow the mixture to stand for 1 hr., filter off the barium salts on a sintered-glass crucible, and wash the precipitate with several small portions of 80 per cent. alcohol. Dissolve the salts immediately by adding 0·2 *N* hydrochloric acid and stirring until solution is complete, collect the solution in the filter-flask by applying suction, and wash the filter with the diluted hydrochloric acid. Neutralise the combined filtrate to phenolphthalein with concentrated aqueous ammonia, quantitatively decant it into a 100-ml. flask, and dilute it to 100 ml. with water. Pipette 10 ml. of this solution into a second 100-ml. flask, and dilute it to 100 ml. with the aqueous ammonia - ammonium chloride base solution, which is prepared by adjusting the *p*H of a 1·0 *M* ammonium chloride solution to *p*H 8·2 by the addition of concentrated aqueous ammonia. Examine the resulting solution polarographically and determine the concentrations of maleate and fumarate ions from a calibration curve. The values are obtained with an accuracy of ± 3 per cent. J. G. WALLER

Amperometric Titration of Thiodiglycol with Electrolytically Generated Bromine. J. W. Sease, C. Niemann, and E. H. Swift (*Anal. Chem.*, 1947, **19**, 197–200)—In order to adapt the titration of thiodiglycol with bromine to the determination of microgram quantities of this compound, the bromine is generated electrolytically, and the excess of it is detected by observing the current that passes between two platinum electrodes when a potential of 0·3 v. is applied across them.

The determination is carried out in a glass cell containing a stirrer and two pairs of platinum foil electodes. The generator electrodes each have an area of 0·5 to 1·0 sq. cm., and the indicator electrodes each an area of 4 to 9 sq. cm. The potential applied across the generator electrodes is supplied



by a 45-volt dry battery and can be varied by the use of resistances that are adjusted to give three rates of bromine generation, *viz.*, 0·8, 4·0, and 10·0 μg. per sec. These resistances are put in or out of circuit by means of a three-way switch. A constant potential of 0·3 v. is applied across the indicator electrodes.

Procedure—Pipette 10 ml. of the thiodiglycol solution in 50 per cent. acetic acid and 1 ml. of 1·0 formal [molar] potassium bromide into the cell, with the stirrer in motion. Set the circuit switches to give the desired rate of bromine generation, close the generating current switch, starting a stop-watch simultaneously, and note the current that passes in the indicator circuit. When the indicator current starts to rise, switch off the generating current, and note the time and rate of bromine generation. The maximum value of the indicator current should be at least 3 microamperes. If this is not reached some more bromine must be generated. After noting the final value of the indicator current, generate a further 10 μg. of bromine and again note the current. Since the amount of bromine present is proportional to the current passed, the necessary correction can be applied. The thiodiglycol content can be calculated with an accuracy of ± 2 per cent. from a knowledge of the corrected amount of bromine generated.

J. G. Waller

Inorganic

Test for Selenium based on a Catalytic Effect. F. Feigl and P. W. West (*Anal. Chem.*, 1947, **19**, 351–353)—Selenium has a catalytic effect on the reduction of chromates, picric acid, dichlorophenol-indophenol, cacotheline, and methylene blue by alkali sulphides. The reduction is caused by sulphide ions with the formation of polysulphides, and of selenosulphides, when selenium is present: the latter are the less stable and react more readily. Elemental sulphur has the same effect, but to a lesser degree. Solutions containing much polysulphide can be decolorised by heating with sulphur dioxide to form inactive thiosulphates, while selenosulphides are converted into selenosulphates, which retain their activity. Selenosulphates prepared directly by dissolving selenium in sodium sulphite are not active until some sulphide is added. Selenites can be identified by heating with an excess of sodium sulphide, which appears first to reduce selenite to selenium and then to dissolve the selenium, and the sulphur formed, as seleno- and polysulphides, respectively. This gives a solution that can be tested directly for selenium by the catalytic reduction of methylene blue.

Procedure A. Drop reaction—Place 1 drop (0·08 ml.) of 0·2 *M* sodium sulphide in a depression of a drop-reaction plate and a drop of a similar solution containing selenium in an adjacent depression. Add to each 1 drop of 0·01 per cent. methylene blue solution and compare the times required for decolorisation to take place. Under these conditions, the limit of identification is 0·08 μg. of selenium, and the limiting concentration 1 in 10^6.

B. Test tube reaction—Add 2 drops of 0·05 per cent. methylene blue solution to 2 ml. of the comparison solution (0·2 *M* sodium sulphide containing 5 per cent. of sodium sulphite), and to 2 ml. of the test solution (0·2 *M* sodium sulphide containing 5 per cent. of sodium sulphite and any selenium present). Mix by shaking, and compare the rates at which decolorisation takes place. It is possible to detect 0·3 μg. of selenium in a limiting concentration of 1 in $6·5 \times 10^5$ in this way.

Detection of selenium dioxide—Reduce solutions of alkali selenites by heating with 0·2 *M* sodium sulphide. Add solid sodium sulphite until the hot solution is decolorised. Cool and apply procedure *B*. The limit of identification is 0·4 μg. of selenium dioxide.

Detection of selenium in presence of sulphur—Heat the sample with an excess of sodium sulphide solution. Decolorise the solution by adding sodium sulphite and heating. Apply procedure *B*.

In such a test, the blank determination retained a blue colour for at least 20 min., while the solution

containing 0·5 μg. of selenium was decolorised within 1 min. One part of selenium in 48 × 10^3 of sulphur could be detected in this way.

Sodium hypophosphite, sodium phosphite, sodium nitrite, and tellurium do not interfere. In presence of thiocyanates, the normal colour of methylene blue changes to lavender colour, but the fading effect due to the presence of selenium can still be observed. Cyanides interfere by forming selenocyanides instead of selenosulphides.

M. E. DALZIEL

Precipitation of Mercuric Chloride with Dithiane. J. R. Schroyer and R. M. Jackman (*J. Chem. Educ.*, 1947, **24**, 146–148)—Dithiane precipitates the colourless, crystalline compound $HgCl_2.C_4H_8S_2$ from solutions of mercuric mercury in dilute hydrochloric acid. Concentrations as low as 0·01 mg. of mercury per ml. of 0·1 *N* hydrochloric acid can be detected, and the presence of mercury can be confirmed by treatment of the precipitate with sodium hydroxide solution to give yellow mercury oxide. After removal of insoluble chlorides, the only common metallic ion interfering is copper which, when present in quantity, gives a pink colour with the reagent, and is slightly co-precipitated with the mercury. This interference is most marked when the hydrochloric acid concentration is high. Precipitation of the mercuric chloride - dithiane compound is complete in 0·1 to 0·2 *N* hydrochloric acid. As the corresponding mercuric sulphate compound is sparingly soluble, sulphate must be absent in quantitative work.

The procedure proposed for the quantitative determination of mercury has been tested on only five samples and the results obtained indicate an error of ± 1 part in 100 for the method. Factors affecting the accuracy are the slow rate of formation of the precipitate and the loss in weight on drying the dithiane compound. When amounts of precipitate from 0·1 to 0·3 g. are dried at 100° C. for 2 hr. there is a loss in weight of up to 1 per cent. The chief value of the reagent lies in its selective detection of mercuric mercury.

Method—Reagent—A 2 per cent. solution of dithiane in 95 per cent. ethyl alcohol. The preparation of dithiane is described by Bouknight and Smith (*J. Amer. Chem. Soc.*, 1939, **61**, 28).

Procedure—Dissolve in a suitable manner, avoiding addition of sulphate, an amount of sample containing 50 to 100 mg. of mercury. Oxidise mercurous mercury, if desired, neutralise the solution with aqueous ammonia and hydrochloric acid, and filter off precipitated chlorides. Add a quantity of hydrochloric acid such that the solution will be 0·2 *N* when diluted to 100 ml. Dilute to 85 ml. and add a 30 per cent. excess of the dithiane solution. Allow to stand for 24 hr., transfer the precipitate to a filter crucible, wash with water, and dry at 100° C. for 2 hr.

B. ATKINSON

Determination of Mercury in Paints and Toxicological Material. J. W. Elmore (*J. Assoc. Off. Agric. Chem.*, 1946, **29**, 387–389)—Organic matter in paints and animal matter can be conveniently and completely destroyed with a mixture of fuming sulphuric acid and red fuming nitric acid by heating under refluxing conditions. This treatment also oxidises chlorides to chlorine, which passes through the condenser. Standard ammonium thiocyanate solution can then be used for titrating mercury in the final solution.

Procedure for paints—To a weighed amount of the thoroughly mixed sample, containing about 0·2 g. of mercury, in a 200-ml. Erlenmeyer flask connected to a water-cooled reflux condenser through a ground glass joint, add 10 ml. of 98 per cent. sulphuric acid, and mix. Introduce 30 to 40 ml. of fuming sulphuric acid (containing 30 per cent. of additional sulphur trioxide) through the condenser, heat the flask with a small flame, and add small portions of red fuming nitric acid through the condenser with continued heating until the residue is white and nitrogen peroxide fumes persist in the flask. Continue the heating for 15 min., or for 2 hr. if chlorine is present, with occasional additions of fuming nitric acid. Cool, and add 100 ml. of cold water through the condenser slowly as the cooling proceeds. Transfer the liquid to a 600-ml. beaker, dilute to 300 ml., boil for about 1 min. to expel most of the nitrogen peroxide, add an excess of saturated potassium permanganate solution and cool to 15° C., and then destroy the excess of potassium permanganate with ferrous sulphate solution. Add 10 ml. of 10 per cent. ferric alum solution, containing enough nitric acid to remove its original brown colour, and titrate the liquid with 0·1 *N* ammonium thiocyanate to the first appearance of a pink colour (1 ml. ≡ 0·01003 g. of mercury).

If large amounts of insoluble matter are present, filter the hot solution through a Gooch crucible before adding the potassium permanganate, wash the filter and residue with hot water, and finally digest it with nitric acid to dissolve small amounts of mercury. Filter through asbestos and treat the filtrate separately in the same manner as the main solution.

Procedure for plant and animal materials—To a sample containing 50 to 100 μg. of mercury in a 250- or 500-ml. Erlenmeyer flask connected as described above, add 30 to 40 ml. of fuming sulphuric acid. Heat gently and add red fuming nitric acid, a few drops at a time, through the condenser until the liquid is clear and continue to heat for 15 to 20 min. Cool, and add about 100 ml. of water through the condenser as the cooling proceeds. Dilute the liquid to 300 ml. in a 600-ml. beaker, boil for 1 min. to remove most of the oxides of nitrogen, add an excess of a saturated potassium permanganate solution and cool to 30° to 40° C. Decolourise the liquid with a 10 per cent. solution of hydroxylamine hydrochloride and add 3 ml. in excess. Cool, make alkaline to litmus paper with aqueous ammonia, neutralise with diluted nitric acid (1 + 1), and add 1 ml. in excess. Transfer the liquid, or a suitable aliquot to a separator and proceed as described in "*Methods of Analysis of the A.O.A.C,*" 6th Ed., 1945, XXIX, 56, beginning "Add 2 ml. of dithizone . . ." Blank determinations should be made on the reagents.

A. O. JONES

Determination of Calcium by Potentiometric Titration. N. Uri (*Anal. Chem.*, 1947, **19**, 192–193)—Ferrous iron and a small quantity of ferric iron are added to a solution of the calcium as chloride, and the whole is titrated with potassium fluoride solution. When all the calcium has been precipitated as fluoride, the excess of fluoride forms the ion FeF_6''' and the consequent fall in the oxidation - reduction potential can be observed potentiometrically. Owing to the appreciable solubility of calcium fluoride in water, it is necessary to precipitate the calcium in a 50 per cent. solution of ethyl alcohol in water, saturated with sodium chloride.

Apparatus—The electrodes are a saturated calomel reference electrode and a 1-sq. cm., bright platinum electrode. The e.m.f. of the cell is measured by means of a valve potentiometer (Hellige Roehrenpotentiometer).

Procedure—Dilute the solution of calcium chloride with ethyl alcohol and water until the concentration of calcium chloride is 0·05 to 0·2 *M* and that of alcohol, 50 per cent. by volume. Add 0·02 g. of ferrous chloride containing 0·4 mg. of ferric chloride, saturate with sodium chloride, and cool below 16° C. Titrate with *M* potassium fluoride solution, stirring, and waiting after each addition until the potential is constant. Towards the end of the titration, it may be necessary to wait for over 3 min. between additions. Ascertain by a graphical method the titration figure corresponding to the maximum rate of change of voltage with titration figure. One ml. of *M* potassium fluoride ≡ 20·04 mg. of calcium.

The determination of calcium is accurate to within ± 1 part in 200. If magnesium is present, the potential fall, which is less marked, occurs when the calcium has been precipitated as CaF_2 and the magnesium as $KMgF_3$. The error in the determination of the sum of calcium and magnesium in a solution, using the above procedure, does not exceed ± 1 part in 100. B. ATKINSON

Determination of Metallic Aluminium in Aluminium Pigments. A. K. Light and L. E. Russell (*Anal. Chem.*, 1947, **19**, 337–338)—The method described is based on the reduction of ferric sulphate by finely divided aluminium powder. The method has been reported as unsuccessful, but modification of conditions has given a method useful for routine work.

Procedure—Weigh about 0·2 g. of the well-mixed powder or paste into a small, tared weighing bottle with a ground-glass, outside stopper. Dry at 200° C. for 45 min., cover in the oven, remove it and cool, and then weigh accurately. Remove the lid, and lower the bottle and sample into a 500-ml., wide-necked flask. Add 100 ml. of acid ferric sulphate solution, prepared by dissolving 330 g. of ferric sulphate nonahydrate in 750 ml. of water and 75 ml. of concentrated sulphuric acid, and diluting to 1 litre when dissolution is complete. Close the flask with a rubber stopper fitted with a 25-ml. separating funnel and an outlet tube, the end of which is immersed in 10 per cent. sodium bicarbonate solution in a small flask. Add quickly, through the funnel, 50 to 75 ml. of 10 per cent. sodium bicarbonate solution, and shake gently. Bring the solution to the boiling point and maintain boiling for 5 min. Cool the flask to 10° to 15° C., keeping the outlet submerged in the alkaline solution. Remove the stopper, add 15 ml. of 85 per cent. ortho-phosphoric acid and titrate with 0·5 *N* potassium permanganate.

The precision of the method is ± 0·1 per cent. of aluminium, and the time occupied about 1 hr., exclusive of drying.

Aluminium oxide, silica, mica, and the polishing lubricant do not interfere; iron, zinc, and copper are usually not present in sufficient amount to affect results. If too high an acid concentration (more than 10 ml. per 100 ml. of solution) is used, low results are obtained owing to the liberation of hydrogen. The weighing bottle must be covered in the oven to prevent the ready absorption of atmospheric moisture. An inert atmosphere is attained in the flask by adding sufficient bicarbonate solution, 50 to 75 ml., to give a thick, persistent, orange foam before the flask is shaken. M. E. DALZIEL

New Indicator for Iodimetric Analysis. S. Peat, E. J. Bourne, and R. D. Thrower (*Nature*, 1947, **159**, 810–811)—A sodium salt of starch glycollic acid, in which the ratio of gycollic acid units to glucose units is about 1:10, is suitable as an indicator in iodine titrations. The compound is a non-hygroscopic, white powder, soluble in water to give a clear solution that may be kept for months without any sign of deterioration. As the glycollate does not form a water-insoluble compound with iodine, it may be added at any stage of a titration. The end-point is sharp and reproducible, the colour change at the end-point being similar to that of starch.

Preparation—Stir a dispersion of 10 g. of starch in 160 ml. of water with 30 ml. of 50 per cent. sodium hydroxide solution, warm to 50° C., and add slowly a solution of 5 g. of sodium monochloroacetate in 20 ml. of water. Neutralise with acetic acid and dialyse in a Cellophane bag against running water for 3 days. Precipitate the "sodium starch glycollate" by addition of an excess of alcohol, and purify by extraction in a Soxhlet apparatus with 90 per cent. alcohol. B. ATKINSON

Use of the Persulphate Method of Determining Manganese in Ores, Slags, and Ferro-manganese. J. Zeutzius (*Z. anal. Chem.*, 1938, **115**, 400–402)—*Procedure*—A. *For low manganese contents*—Dissolve a 5-g. sample in concentrated hydrochloric acid and treat as usual. Pipette 10 ml. of the filtered solution into a 250-ml. beaker, add 10 ml. of nitric acid (*d* 1·4), and then concentrate to a few millilitres. Rinse down the walls of the vessel with hot water, and boil the solution. Add 5 ml. of 0·35 per cent. silver nitrate solution, 5 ml. of 22 per cent. ammonium persulphate solution, and set aside for exactly 5 min. Dilute with 100 ml. of cold water and titrate the permanganate formed with standard arsenite solution. If the sample is soluble in nitric acid, weigh 0·1 g. into the beaker directly and proceed as above. The manganese

concentration should be adjusted to about 2 per cent. for the titration.

Comparison with results obtained by the Volhard method for substances containing up to 1 per cent. of manganese showed a maximum deviation of 1 in 8 on a 0·16 per cent. manganese content. For manganese contents up to 4 per cent., the maximum deviation was 1 part in 60.

B. For higher manganese contents—Take 0·5 g. of the sample and treat as described above. Take 10-ml. portions of the filtered solution, and add 2 ml. of nitric acid (*d* 1·4) and 10 ml. of diluted sulphuric acid (1 + 3), and concentrate to fuming. After cooling, rinse down the walls of the containing vessel, and boil the solution. Add 10 ml. of the silver nitrate solution and 10 ml. of the persulphate solution. Allow to stand for 10 min., dilute with 100 ml. of cold water, and titrate with arsenite.

Comparison with results from the Volhard method showed agreement to 1 in 240 on a 19·3 per cent. content, but on 3 values of about 50 per cent., the agreement was to 1 part in 600.

M. E. DALZIEL

Reduction of Quinquevalent Vanadium in the Silver Reductor. J. J. Lingane and L. Meites, jun. (*J. Amer. Chem. Soc.*, 1947, **69**, 277–279)—From the reduction potential of the system vanadic ion - vanadyl ion (Jones and Colvin, *Ibid.*, 1944, **66**, 1563) the equilibrium constant of the reaction $VO^{\cdot\cdot} + Ag + 2H^{\cdot} + Cl' \leftrightarrow V^{\cdot\cdot\cdot} + AgCl + H_2O$ is calculated to be 87·9 at 25° C. However, this reaction takes place so slowly that only very slight amounts of tervalent vanadium are formed when solutions of quinquevalent vanadium in 0·2 to 2 *N* hydrochloric acid are passed through the silver reductor at 25° C. Iron, cobalt, nickel, manganese, chromium, titanium, molybdenum, uranium, and tungsten, in amounts approximately equal to the amount of vanadium present, have no effect on the amount of tervalent vanadium formed. The proportion of quadrivalent vanadium reduced by the reductor increases with rising temperature and with increasing acid concentration, up to a reduction of 95 per cent. of the vanadium to the tervalent state when the acid concentration is high. Equilibrium in accordance with the calculated constant can be established by agitating the vanadium solution with silver for a number of hours, in an atmosphere of carbon dioxide. The observation of Fryling and Tooley (*Ibid.*, 1936, **58**, 826) that considerable amounts of hydrogen peroxide are formed when hydrochloric acid solutions containing dissolved oxygen are passed through the reductor is confirmed. The amount of peroxide formed in vanadium solutions is several times larger than the amount formed in iron solutions.

A procedure by which vanadium can be determined via reduction with the silver reductor to the quadrivalent state is as follows. Use the reductor as described by Walden, Hammett, and Edmonds (*Ibid.*, 1934, **56**, 350). Remove air from the reductor, the vanadium solution, and the wash liquid, by means of pure hydrogen. Pass the solution of vanadium, made *N* with respect to hydrochloric acid, through the reductor at the rate of 30 ml. per min., and wash the reductor four times with 25-ml. portions of *N* hydrochloric acid. Dilute with an equal volume of water and add 0·2 g.-mol. of sodium acetate to buffer the solution to *p*H 4·5. Allow the solution to stand in contact with the air for 15 min. to ensure re-oxidation of any tervalent vanadium to the quadrivalent state, add 2 ml. of saturated manganous sulphate solution, heat to 80° C., and titrate with potassium permanganate solution.

Results given by the above procedure agreed closely with those obtained when portions of the same vanadium solution were reduced by sulphur dioxide and titrated with permanganate.

B. ATKINSON

X-Ray Analysis of Chromium - Molybdenum and Chromium - Tungsten Alloys. W. Trzebiatowski, H. Ploszek, and J. Lobzowski (*Anal. Chem.*, 1947, **19**, 93–95)—The systems chromium - molybdenum and chromium - tungsten have been examined by the X-ray powder diffraction method, using a cylindrical camera of 57·6 mm. diameter. Lattice constants are given for the two systems.

In earlier work by various authors, pure materials had not been used, and results were therefore affected by the presence of impurities such as carbon or aluminium. In the investigation described in this paper, materials of a high degree of purity have been used, and the alloys have been made by a powder metallurgical method. Preliminary sintering was carried out at 1000° or 1100° C. for 2 hr. followed by a further treatment at 1430° C. for 5 to 8 hr. The samples were allowed to cool in the furnace by switching off the heating current. The alloys thus formed were then cut into sections and annealed under the following different conditions:

Annealing temperature ° C.	Time hours	Cooling
600	1500	Quenched in water
1000	350	,,
1200	240	,,
1430	7	,,
1700	7	Rapid cooling in a stream of hydrogen

Melting points were determined for separate samples, and for the chromium - molybdenum system a minimum melting point of 1700° C. was obtained with an alloy containing about 15 atomic per cent. of molybdenum.

Lattice constants were determined from the "back" reflections of the X-ray patterns, but owing to their diffuse appearance no greater accuracy than ± 0·005 A. was obtained.

X-ray analysis results—(1) *Chromium-molybdenum system*—The patterns of this system showed only one phase and this had the body-centred cubic structure. The lattice constants (A. ± 0·005) for this phase, for alloys containing 0, 10, 20 . . . 100 atomic percentages of molybdenum, when the samples were annealed at 1700° C., are respectively

as follows: 2·878, 2·913, 2·946, 2·974, 3·003, 3·030, 3·055, 3·080, 3·102, 3·122, 3·144. The lattice constants are also given for samples annealed at 1000° C. and 600° C. but the values appear to be the same, within the stated limits of experimental error, as those for the samples annealed at 1700° C.

(2) *Chromium-tungsten system*—At 1700° C., the two components form a series of unlimited solid solutions, but at lower temperatures two phases are found to occur. The lattice constants are as follows:

Composition of samples. atomic % W.	Lattice constants (A. ± 0·005) for samples annealed at various temperatures								
	1700° C.	1430° C.		1200° C.		1000° C.		600° C.	
0	2·878	2·878	—	2·878	—	2·878	—	2·878	—
10	2·916	2·913	—	2·912	—	2·900	—	2·889	—
20	2·948	2·944	—	2·925	—	2·906	—	2·895	3·125
30	2·979	2·975	—	2·931	3·123	2·904	3·132	2·897	3·128
40	3·014	2·992	—	2·928	3·125	2·903	3·132	2·898	3·130
50	3·043	2·996	3·084	2·927	3·124	2·902	3·127	2·898	3·131
60	3·072	2·991	3·086	2·925	3·127	2·906	3·125	2·897	3·128
70	3·095	—	3·092	—	3·128	—	3·126	2·900	3·125
80	3·114	—	3·116	—	3·130	—	3·128	—	3·127
90	3·135	—	3·136	—	3·137	—	3·138	—	3·139
100	3·160	—	3·160	—	3·160	—	3·160	—	3·160

E. G. STEWARD

Polarographic Determination of Vanadium in Steel and Other Ferro-Alloys. J. J. Lingane and L. Meites, jun. (*Anal. Chem.*, 1947, **19**, 159–161)—The method describes the polarographic analysis of vanadium based on the anodic wave produced by the oxidation of quadrivalent vanadium to the quinquevalent state. Iron and other interfering elements are removed by electrolysis of the solution in a phosphoric - sulphuric acid solution at a mercury cathode. This avoids losses due to co-precipitation which are inherent in the classical method for the removal of iron and other elements by sodium hydroxide precipitation.

Procedure—Dissolve 0·5 to 2·5 g. of the alloy in 20 ml. of 6·0 *N* hydrochloric acid containing 5 g. of disodium hydrogen phosphate dodecahydrate in a small Kjeldahl flask, adding 3 ml. of concentrated nitric acid drop by drop. When the reaction moderates, add 3 ml. of concentrated sulphuric acid, and evaporate until fumes of sulphur trioxide are evolved. Transfer the solution to an electrolytic cell, dilute it to 100 ml., and electrolyse the solution, using a current of from 3 to 5 amp. for a length of time corresponding to 3 ampere-hours per gram of sample. When the electrolysis is complete, transfer the solution to a Kjeldahl flask, add 2 to 5 ml. of 30 per cent. hydrogen peroxide and boil for 2 min. to oxidise any tervalent vanadium to the quinquevalent state, and then reduce it to the quadrivalent state by addition of 2·0 g. of sodium sulphite. Finally, concentrate the solution to about 75 ml., add 1·0 g. of sodium sulphite and dilute the solution to 100 ml. in a volumetric flask. Add an aliquot portion of this solution to a known volume of air-free, 1·0 *N* sodium hydroxide that is 0·1 *M* with respect to sodium sulphite, in a polarographic cell, such that the final vanadium concentration lies between 0·2 and 2·0 mg. per 100 ml. Measure the diffusion current of the anodic wave at − 0·25 v. *versus* the saturated calomel electrode, and calculate the amount of vanadium present by reference to a calibration curve.

Molybdenum interferes with the determination if the amount present is more than one hundred times the vanadium content, but it can be removed by prolonging the electrolysis by a period of time corresponding to 30 ampere-hours per gram of molybdenum present. Under optimum conditions, the precision is about ± 0·5 per cent. in terms of the average deviation from the mean. J. G. WALLER

Polarographic Characteristics of Chloro-Complexes of Quinquevalent Antimony. J. J. Lingane and F. Nishida (*J. Amer. Chem. Soc.*, 1947, **69**, 530–533)—Tervalent antimony is readily reduced at the dropping mercury electrode in hydrochloric, nitric, or sulphuric acid solutions, in sodium hydroxide solutions, and in acidic, neutral, or basic tartrate solution, but quinquevalent antimony is not (*cf.* Page and Robinson, *J. Soc. Chem. Ind.*, 1942, **61**, 91). Since, in strongly acid solution, quinquevalent antimony behaves as a fairly strong but slow oxidising agent, the conditions for polarographic reduction appear to be satisfied. The difficulty of obtaining a reduction wave is attributed to the slow rate of reaction, and to a very large overvoltage.

With the stannic ion a similar situation exists, and this was overcome by conversion of the aquo stannic ion to the chloro complex. The same technique has been applied to quinquevalent antimony and the authors have shown that the $SbCl_6'$ ion, in solutions that are 4·0 to 6·0 *N* with respect to hydrochloric acid is readily reduced at the dropping mercury electrode in two stages. It is first reduced to the tervalent state, and then to the metal. In 6·0 *N* hydrochloric acid, the half-wave potential is about − 0·26 v. *versus* the saturated calomel electrode, and 0·005 per cent. of gelatin is required to suppress the maximum on the second wave. The diffusion current in 6·0 *N* hydrochloric acid is proportional to the antimony concentration over the range 0·07 to 2·62 × 10^{-3} *M*. Standard quinquevalent antimony solutions are prepared by dissolving a known weight of antimony metal powder in hydrochloric acid, adding sufficient nitric acid to oxidise the metal completely, and evaporating off the excess of nitric acid before diluting to a known volume in 6·0 *N* hydrochloric acid.

In solutions that are less than 4·0 *N* with respect

to hydrochloric acid, the wave is ill-defined, and is completely absent in 0·2 *N* hydrochloric acid. The wave is produced in solutions containing only 0·2 *N* hydrochloric acid if the hydrogen ion concentration is maintained by making the solution 6·0 *N* with respect to perchloric acid. Solutions containing high chloride ion concentrations and low hydrogen ion concentrations will give only a small, ill-defined wave. J. G. Waller

New Titrimetric Methods for Thorium. C. V. Banks and H. Diehl (*Anal. Chem.*, 1947, **19**, 222–224)—Thorium is precipitated as the normal molybdate and, after dissolution of the precipitate, the molybdenum is reduced in the Jones reductor, and titrated with ceric sulphate. The conditions under which thorium can be separated as the molybdate from calcium and uranium are defined, and a method for the analysis of thorium - uranium alloys is given. A shorter method for determining the thorium, in which the thorium solution is titrated with ammonium paramolybdate solution and the end-point determined potentiometrically, is outlined. The thorium molybdate precipitation is made the basis of a procedure for the analysis of uranium - molybdenum alloys.

Determination of thorium—Weigh the sample containing 0·15 to 0·2 g. of thoria into a 250-ml. beaker, dissolve it in a suitable acid, evaporate nearly to dryness, dilute to 150 ml., and add 11 ml. of glacial acetic acid. Add 15 ml. of thick filter-paper pulp and 1 ml. of a solution of 0·5 g. of diphenylcarbazide in 200 ml. of 95 per cent. ethanol. Add from a burette, with stirring and until the indicator colour is deep pink, a solution containing 7·6 g. of ammonium paramolybdate per litre. Heat to boiling, filter the hot solution through an 11-cm., Whatman No. 42 paper and wash the precipitate 5 or 6 times with hot, diluted acetic acid (1 + 100). Transfer the filter paper and precipitate back to the 250-ml. beaker, add 25 ml. of concentrated hydrochloric acid, and stir until the paper is disintegrated. Add 75 ml. of water, heat to boiling, filter, and wash 5 or 6 times with hot, diluted hydrochloric acid (1 + 100). Cool the filtrate to room temperature, and pass the liquid through a Jones reductor into an excess of ferric alum solution containing 2 or 3 ml. of concentrated phosphoric acid. Titrate with 0·1 *N* ceric sulphate, using 2 drops of 1 : 10-phenanthroline - ferrous sulphate solution as indicator. Results for thorium in thorium nitrate obtained by this method are accurate to within ± 1 part in 400.

Separation of thorium from calcium—Thorium is separated quantitatively from calcium by the above procedure, provided that the amount of calcium in the sample used for the analysis does not exceed 0·4 g.

Separation of thorium from uranium—Ammonium acetate is used to prevent the precipitation of uranium molybdate and a larger excess of molybdate than that used above is then needed to precipitate the thorium quantitatively. Weigh the sample containing 0·15 to 0·2 g. of thoria and not more than 0·5 g. of uranium oxide into a 250-ml. beaker. Dissolve the sample and remove the excess of acid as above, add 5 g. of ammonium acetate and 11 ml. of acetic acid, and dilute to 150 ml. Proceed as for the determination of thorium, but add a 4- to 6-ml. excess of the molybdate solution and check that the amount added is sufficient to complete the precipitation. Values for the thorium in samples containing 0·2 g. of U_3O_8 with 0·17 g. of thoria are high by up to 6 parts in 1000.

Analysis of thorium - uranium alloys—Decompose the alloy with hydrochloric acid, dissolve by fuming with perchloric acid and proceed with the separation as described above. Determine uranium on separate samples.

Separation of thorium from the rare earths—Neither under the conditions described for the determination of thorium, nor at various other acetic acid concentrations and with other temperatures of precipitation is a quantitative separation from the rare earths obtained.

Potentiometric method for thorium—Use a 0·1 *N* calomel reference electrode and a molydenum wire indicator electrode. Titrate the ammonium molybdate solution into a 7 per cent. acetic acid solution of the thorium at 50° to 55° C. Standardise the ammonium molybdate against a standard thorium solution, using the same procedure. Calcium does not interfere.

Analysis of uranium - molybdenum alloys—Dissolve a portion of sample containing 0·1 to 0·15 g. of molybdenum trioxide in the minimum quantity of diluted hydrochloric acid (1 + 1), adding nitric acid if necessary. Add hydrogen peroxide, boil for 10 min. to remove the excess of peroxide, and dilute to 200 ml. Add 16 ml. of glacial acetic acid, about 1 g. of ammonium acetate, and 15 ml. of thick filter-paper pulp. Precipitate the molybdenum by adding, with stirring, a 25 per cent. excess of a thorium perchlorate solution, prepared by fuming purified thorium nitrate with perchloric acid to near dryness and diluting until 1 ml. is equivalent to about 5 mg. of molybdenum. For the molybdenum determination, proceed as described for the determination of thorium. After filtering off the molybdate, analyse the filtrate for uranium in the usual manner. If nitric acid is used in dissolving the sample, fume this filtrate with perchloric acid before reducing the uranium in the Jones reductor.

B. Atkinson

Agricultural

Polarographic Determination of Zinc in Soil. F. Takazawa and G. D. Sherman (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 182–186)—The method involves alkali fusion of the soil, followed by the extraction of the zinc from alkaline solution into a solution of dithizone in chloroform. The zinc is then washed out of the chloroform solution by hydrochloric acid, and determined polarographically.

Procedure—Mix 0·5 g. of soil, ground to pass a 100-mesh sieve, with 3 g. of sodium carbonate-potassium carbonate mixture in a platinum crucible, and heat until fusion is complete. Place the crucible in a 400-ml. beaker containing 10 ml. of distilled water, and add 10 ml. of concentrated

hydrochloric acid to dissolve the cake. Remove the crucible from the liquid, add a further 10 ml. of concentrated hydrochloric acid and, with the beaker covered, digest on a hot-plate until the sample is completely disintegrated. After removing and rinsing the cover glass, evaporate the solution to dryness, cool, and moisten the residue with 10 ml. of concentrated hydrochloric acid. Add 30 ml. of 2 *N* hydrochloric acid, boil for 5 min., decant the solution through an ashless filter paper moistened with 2 *N* hydrochloric acid, and dilute to 100 ml. with water.

Pipette 20 ml. of the solution into a 125-ml. separating funnel containing 40 ml. of ammonium citrate solution. This solution is prepared by dissolving 113 g. of citric acid in 1 litre of re-distilled water, adjusting the *p*H to approximately 8·5 by adding 40 to 42 ml. of concentrated aqueous ammonia, removing traces of zinc by extracting three times with dithizone reagent followed by three washings with chloroform, and finally, diluting to 4 litres.

Add 2 drops of phenolphthalein solution and sufficient concentrated aqueous ammonia to give the solution a strong colour, followed by 10 ml. of a 0·02 per cent. solution of dithizone in chloroform. After shaking the solution, draw off the chloroform layer into a second 125-ml. separating funnel, and extract the aqueous solution twice with 5-ml. portions of dithizone solution. To the chloroform solution add 30 ml. of 0·1 *N* hydrochloric acid, shake for 2 min., drain off the chloroform layer, and wash the aqueous solution with two 2-ml. portions of chloroform until the chloroform layer is no longer green. Evaporate 25 ml. of the aqueous solution containing the zinc to dryness in a 50-ml. beaker, and add exactly 10 ml. of the supporting solution, which is 0·1 *M* with respect to ammonium acetate and 0·05 *M* with respect to potassium thiocyanate. The zinc is determined by measuring the diffusion current obtained on electrolysis of the solution at the dropping mercury cathode, after removal of dissolved oxygen by passing hydrogen or nitrogen, and reading the value from a standard curve.

Reagents of high quality, purified, if necessary, from zinc, must be used. Glassware should be washed with concentrated hydrochloric acid and rinsed with distilled water before use. Satisfactory results are obtained from samples of soil containing 100 to 500 p.p.m. of zinc, with an accuracy of ± 2 per cent. J. G. WALLER

Water Analysis

Amperometric Methods in the Control of Water Chlorination. H. C. Marks and G. L. Bannister with J. R. Glass and E. Herrigel (*Anal. Chem.*, 1947, **19**, 200–204)—The construction of a rotating platinum micro-electrode and of a large rotating platinum electrode is described, each electrode being incorporated in a hypodermic syringe plunger. Satisfactory current - voltage curves were obtained with these electrodes for the reduction of hypochlorous acid and chloramine solutions, and the large electrode has been applied to the production of a continuous record of the chlorine content of water.

The electrodes—The micro-electrode (Fig. 1) consists of a platinum disc, 2 mm. in diameter, let into the surface of a glass bulb on one end of a hollow, hypodermic syringe plunger, the electrode being on the side of the bulb farthest from the axis of the plunger. The other end of the plunger is open to admit the electrical contact to a pool of mercury inside the glass bulb, the platinum disc being connected with the mercury by means of a platinum wire passing through the glass. The plunger is rotated at 60 r.p.m. by gearing to an electric motor, the syringe cylinder serving as a guide.

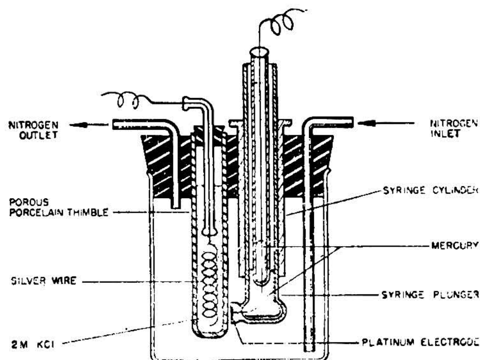


Fig. 1

The large electrode (Fig. 2) consists of a cylinder of platinum 29 mm. in diameter and 11·8 mm. high cemented into a Lucite holder which is mounted in a second syringe plunger. Connection to the electrode is made through the stainless steel shaft cemented into the plunger.

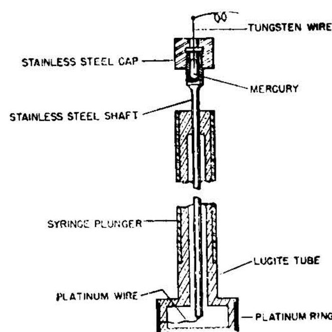


Fig. 2

These two electrode assemblies are interchangeable, and both fit into a syringe cylinder which passes through a rubber stopper into a glass jar. The second electrode is a silver - silver chloride electrode immersed in 2·0 *M* potassium chloride, contained in a porous platinum thimble, which is fitted through the bung.

Results—In solutions that are 0·1 *M* with respect to potassium chloride, as a supporting electrolyte, low concentrations of hypochlorous acid give satisfactory limiting currents, using either electrode.

With the large electrode, satisfactory curves are obtained for chlorine concentrations as low as those encountered in drinking-water samples, but in the absence of any supporting electrolyte, no limiting current is obtained although the current flowing at a given voltage is proportional to chlorine concentration. The large electrode can be used to obtain a continuous record of chlorine concentration, but changes in *p*H, temperature, ratio of chlorine to ammonia, the state of the electrode surface, and the oxygen content of the water all interfere with the record.

J. G. WALLER

Reviews

STATISTICAL METHODS IN RESEARCH AND PRODUCTION WITH SPECIAL REFERENCE TO THE CHEMICAL INDUSTRY. Edited by OWEN L. DAVIES. Pp. 292. London: Oliver and Boyd Ltd. (for Imperial Chemical Industries Ltd.). 1947. Price 28s.

This excellent book is written by a team of seven chemists, engineers and statisticians, who state in their preface that they were invited by Imperial Chemical Industries Ltd. to write a handbook for use within the Company's own organisation, and that it was recently suggested to, and accepted by, them that it would be helpful to British industry as a whole if the book were published. Lord McGowan has written a foreword which refers to the present work as the first of a series to be published by I.C.I. "with the aim of making generally available the important body of information accumulated as a result of the Company's manufacturing experience and research." (The whole of this foreword, by the way, deserves careful consideration and wide publicity; as a statement of a wise policy and an enlightened outlook it is, shall we say, highly significant.) Should the I.C.I. be as fortunate in the authors of subsequent publications as they have been in this one, a heavy debt of gratitude will be owing to them from both industrial and general members of the chemical profession.

Two introductory chapters are followed by an exposition of the kinds of statistical treatment most generally required. A good idea of the ground covered is conveyed by the headings of Chapters 3 to 10—Averages and Measures of Dispersion; Tests of Significance; Analysis of Variance; Regression and Correlation; Frequency Data and Contingency Tables; Sampling; Control Charts; Prediction and Specification. Each presents just so much of the theory as is necessary for a proper understanding of the practical examples which follow, all drawn from actual problems met with in industry. The computations are very clearly set out, and the authors have recognised the importance of discussing the circumstances in which each method is or is not applicable and the interpretation of the results obtained. There is a very readable glossary, a list of symbols and their meanings, some of the more commonly required tables and an adequate index. The whole book is eminently practical and workmanlike and is almost free from printers' errors.

The authors, in inviting criticisms and suggestions, foreshadow the writing of a sequel on the Planning of Experiments; the present book hardly refers at all to the value of the statistician *before* an experiment is begun. The more fundamental part of the chapter on Sampling could well be expanded, the concepts of "randomness" and "representative sampling" being developed from a wider viewpoint, and inserted before Chapter 4. Conclusions are all too often drawn from differences between the means of samples which differ in other respects besides the factor the effect of changes in which is being examined. Something should be said, perhaps in Chapter 6, about transformations of variates and the reasons for using them. An instance occurs on p. 193; ranking data can be normalised by the transformation given in Table XX of Fisher and Yates's well-known book, and are best so treated. There is room for mention, at or about p. 93, of the occasional necessity for testing the main fractions in an analysis of variance against appropriate interactions rather than against the error variance itself. But the fact remains that this is a book that ought certainly to be acquired by all whose work involves the drawing of conclusions from numerical data—and probably by many others as well.

ERIC C. WOOD

ELSEVIER'S ENCYCLOPAEDIA OF ORGANIC CHEMISTRY. Edited by E. JOSEPHY and F. RADT. Vol. 13. Tricyclic Compounds. Series III, Carboisocyclic Condensed Compounds. Pp. xx + 1265. New York and Amsterdam: Elsevier Publishing Co. London Distributors: Cleaver-Hume Press. 1946. Price £19 7s. to subscribers; £25 17s. as single volume.

The first volume (No. 14) of this monumental work appeared in 1940 but its advent was quite eclipsed by the momentous events of that year. With the arrival of the next, the present volume, the occasion is opportune to give an outline of the ambitious programme that the editors have set themselves. The aim is to present a comprehensive and complete account of the chemical, physical and physiological properties of organic compounds. The work is not a translation or paraphrase in English of "Beilstein"; it is claimed that with a very few exceptions, where both *Chemical Abstracts* and *Chemisches Zentralblatt* have been compared, the matter has been taken from the original papers. In this connection, it may be mentioned that the list of journals consulted is an imposing one.

Compounds are grouped in four series—Aliphatic (3 volumes), non-condensed Carbocyclic (8 volumes), condensed Carbocyclic (3 volumes) and Heterocyclic (4 volumes). Many of these volumes will be in more than one part, so it is expected that, with the two index (subject and formula) numbers, the work will run into about 40 parts. Purchases may be of a single volume, of a series or of the complete set, at progressively decreasing prices per volume. Until such time as the main indexes appear, some difficulty might be experienced in locating the proper volume for a particular compound; the position is eased as much as possible by the formulation of a few simple rules.

In general, the work will cover the periodical literature up to four years prior to publication. The present volume was practically ready for the press by 1940; this means that the subject matter is only covered in detail up to 1936. There are, however, occasional references, usually relating to structure, up to 1946. This lag will be remedied by the issue of supplements. In the issue of subsequent volumes, the gap will be gradually reduced to the normal period.

For all practical purposes, this volume deals with the fluorene (112 pp.), acenaphthene (58 pp.), anthracene (592 pp.) and phenanthrene (223 pp.) groups; among the several minor ring systems (95 pp.), there may be found di*cyclo*pentadiene, phenalene, tricyclic sesquiterpenes and their derivatives. The space allotted to different compounds naturally varies enormously, ranging from half a line (*e.g.*, just a record of melting point) to several pages. Various little points are indicative of much editorial forethought. For example, full references are grouped together at intervals, a practice that can be most exasperating; but here, each page carries a footnote giving the location of the relative references, which can be quickly determined from the shortened form used in the text. Again, most pages carry a skeleton showing the numbering of the particular ring system and such abbreviations as are used are practically self-evident. The two indexes (subject 64 pp., formula 119 pp.) are quite adequate, though it may be mentioned that simple derivatives such as esters, oximes, etc., generally appear only in the second, since they follow the parent compound in the text.

Search for a number of out-of-the-way compounds was made with success, subject to the limitations of time and place. The former qualification is inevitable, but the latter requires comment. The expressed intention being completeness, there is one serious gap which needs to be filled. Tucked away in the patent literature is mention, often with descriptive matter, of many compounds that may or may not reach the journals even though they attain commercial production. From the time angle, 3-hydroxy-2-anthroic acid should have appeared in the present volume; it does not, though both the acid and its *o*-toluidide should be picked up in the supplement, but various other arylides and at least one of the intermediates actually used in the manufacture of the acid will be missed except by mere chance in some other connection. Hiatus in sequence must inevitably occur; for example, 4-chloro-l-aminoanthraquinone is to be obtained from the commercial benzoyl derivative, a substance not otherwise mentioned. This further field of search might well be described as Operation Ossa, but, with Pelion surmounted, the ultimate summit is not so distant as might appear, since, to complete the spoiling of the simile, the two have much ground in common.

Printed in the Netherlands, the book is produced in a manner that can only be described as lavish on present-day standards. The price is inevitably high and such as to place it outside the reach of the individual chemist other than a specialist in a particular branch. With "Beilstein" out of action, at any rate temporarily, a work such as this could not have appeared at a more opportune moment and the promoters are doubtless applying themselves to take full advantage of the circumstances. B. A. ELLIS

AN INTRODUCTION TO QUANTITATIVE INORGANIC ANALYSIS. By R. BELCHER, F.R.I.C. and M. B. THOMPSON, B.Sc., Ph.D., A.R.S.M. Pp. xii + 160. London: Blackie & Son, Ltd. 1946. Price 5s. 6d.

Analytical chemistry being both a science and an art, the textbooks devoted to it show a natural tendency to emphasise either one aspect of the subject or the other. One type of book, written at length in well set terms, subordinates all else to teaching general theoretical principles, its practical exercises being specially selected to illustrate theory, regardless of their general utility or analytical worth. The other type, concise almost to the point of abruptness, places its main emphasis on the craftsmanship side of its subject and confines theoretical considerations to the minimum necessary for the work in hand, its sole object being to teach the art, the technique, of chemical analysis. It is seldom that both sides of the subject receive equal treatment in the same book. This is not surprising, for the double outlook and ability are but seldom to be found in the same writer.

This present book belongs to the second type. It treats analysis as an Art and regards accurate laboratory work by modern methods as a matter of prime importance. Manipulative details that conduce to speed combined with accuracy are minutely described and the essential principles of sound analytical practice are taught in the fewest possible number of words. Its 160 pages contain much that is new to this class of book, both in the selection of the methods described and in the inclusion of recent improvements to some of the older procedures. Among the former are to be found the titration of ferric iron by mercurous nitrate, dichlorofluorescein as an indicator in titrating halides, and the determination of potassium as dipotassium sodium cobaltinitrite and of sodium as the zinc-uranyl complex. Examples of the latter are the copper catalyst in standardising thiosulphate solution against dichromate, addition of potassium iodide to the starch indicator used in iodine titrations, and ammonium nitrate as a sensitiser in determining copper by means of ferrocyanide.

The book is almost free from typographical and textual errors. There is, however, one detail that calls for attention in the next edition. The figure on p. 10 purporting to show a filter paper, folded twice, with one corner torn off, appears to have two corners missing instead of only one; moreover, there is no mention of the effect of removing the corner, whereby air is prevented from passing into a filled funnel stem and reducing the filtration rate by breaking the column of liquid.

The authors have not had the requirements of any particular examination syllabus in view; they suggest that their book should meet the needs of students preparing for the London Intermediate or its equivalent and also certain pre-professional examinations; but a survey of its contents as a whole leaves the impression that it is better suited to fill the early needs of a student who intends to make analytical chemistry a main interest in life. F. L. OKELL

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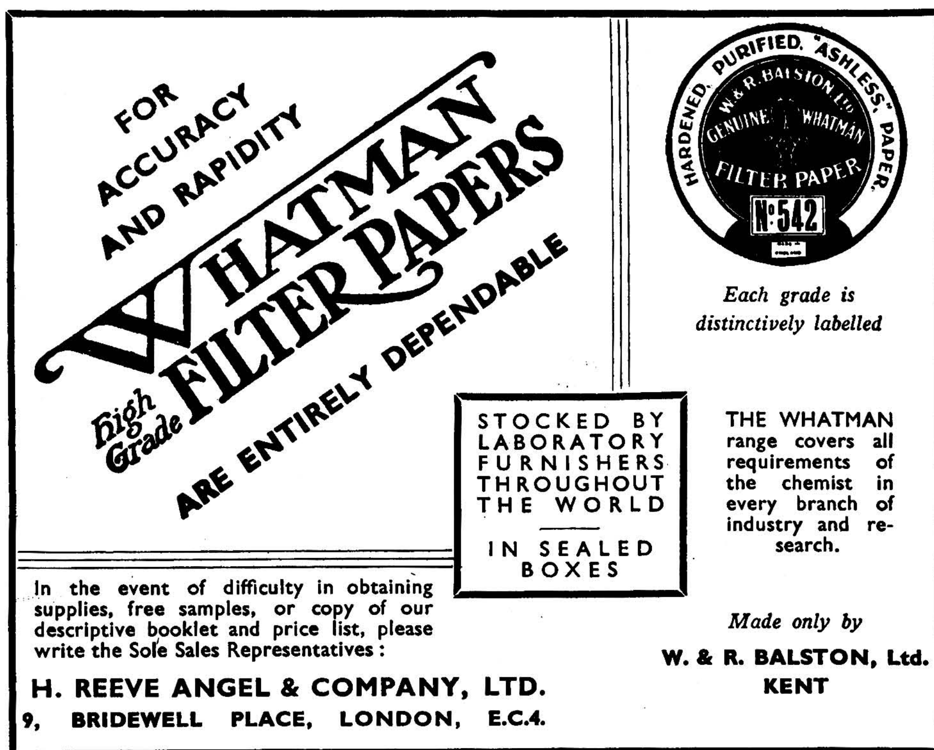

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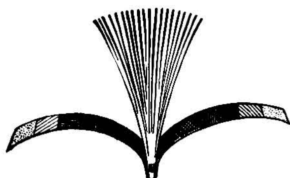

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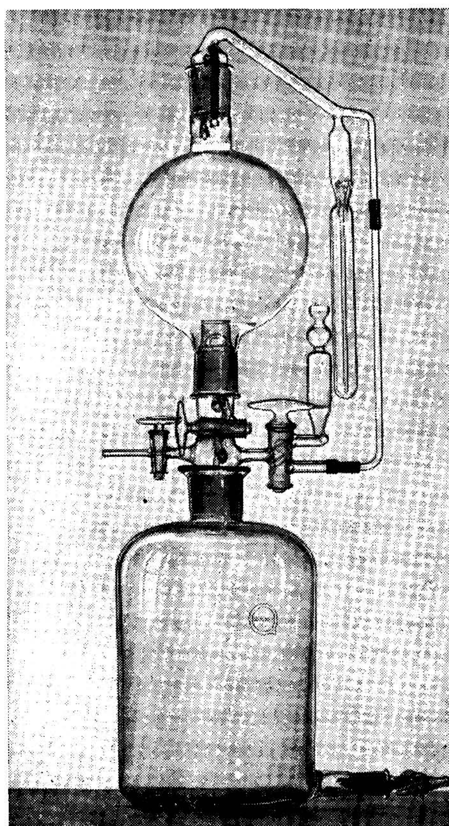

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