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A MONTHLY JOURNAL DEVOTED TO THE ADVANCEMENT OF ANALYTICAL CHEMISTRY

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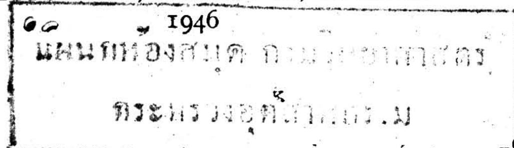

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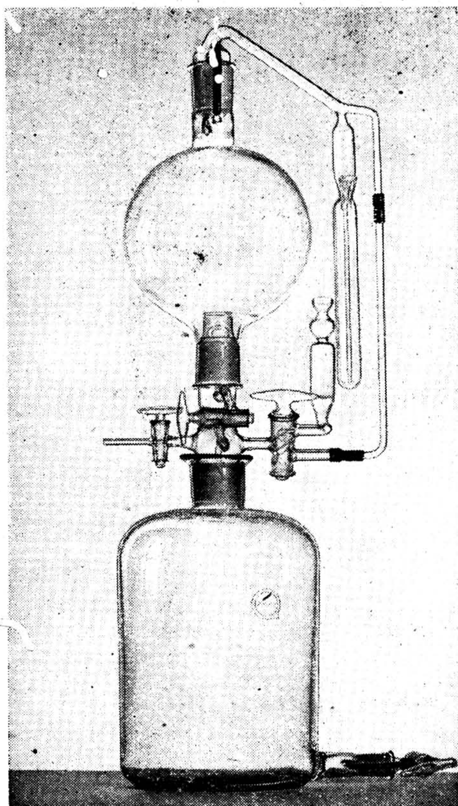

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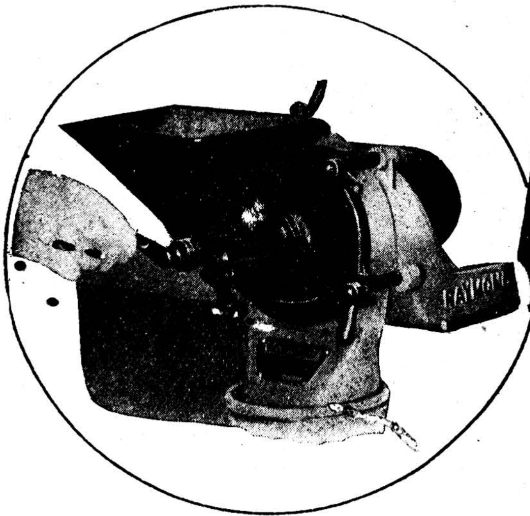

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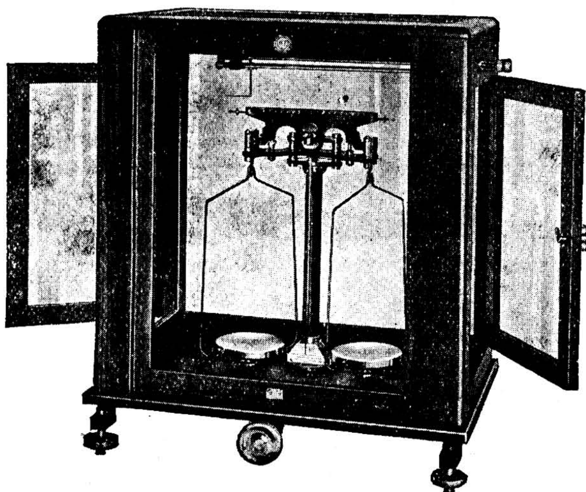

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A JOINT Meeting of the Society with the Food Group of the Society of Chemical Industry was held at 6.15 p.m., on Wednesday, December 5th, 1945, at the Chemical Society's Rooms, Burlington House, Piccadilly, London, W.1. The general subject of the meeting was "New Routine Tests and their Application in Modern Food Industry."

Dr. J. R. Nicholls, Vice-President, opened the proceedings on behalf of the Society, and the Minutes of the meeting of November 7th were read and confirmed. The chair was then taken by Mr. T. Rendle, Chairman of the Food Group, and the following papers were read:—"New Routine Tests in Examining Wheaten Products," by A. J. Amos, Ph.D., B.Sc., F.R.I.C.; "New Routine Tests in the Dairy Industry," by J. G. Davis, Ph.D., D.Sc., F.R.I.C.; "The Analysis of Cured Meats and Curing Brines," by J. C. Morpeth, B.Sc., A.R.I.C.; "Physical and Chemical Methods for Moisture Determination," by D. W. Grover, B.Sc., F.R.I.C. (read by Dr. L. E. Campbell). The discussion which followed was opened by Mr. Jan Straub, Chem.Ing., Public Analyst of Amsterdam, who gave some particulars of work there during the German occupation.

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The Theory of Certain Analytical Procedures, with particular reference to Micro-biological Assays

BY ERIC C. WOOD

(Read at the Meeting of the Society on October 3rd, 1945)

INTRODUCTION—If one attempts to classify analytical procedures with reference not to the substances analysed nor to the ingredients determined, but to the fundamental principles involved—the strategy underlying the attack, as it were—one soon finds that many methods, which vary widely from the former view-point, can be broadly described in the same terms. First, some physical quantity must be found—it may be the weight of an animal, the volume of a reagent, the reading on the dial of an instrument—the magnitude of which depends on, and varies regularly with, the amount of the substance it is desired to estimate, and which I shall refer to as "Factor X." Second, the quantitative relation between the amount of "factor X" and the magnitude of the effect it causes is determined by performing parallel sets of operations with various known amounts or "doses" of the factor and measuring the result, which we may call the "response." The relation between the dose and the response may be concisely expressed either diagrammatically in the form of a graph, or algebraically in the form of an equation. Finally, a known amount of the material to be analysed is put through an identical series of operations, the response is measured, and the amount of factor X present is deduced from the graph or the equation.

This description will be found to apply to such diverse methods as the absorptiometric determination of trace-elements in alloys; the dielectric constant method of determining

moisture in powders; and practically all biological methods of estimating vitamins or other essential nutrients in foods, whether they be on the macro-scale and employ rats in cages or on the micro-scale and employ bacteria in test-tubes. I have recently given much thought to the theory of these related methods; and this paper summarises the conclusions at which I have arrived. After discussing certain considerations of quite general applicability, I propose to deal with the special case of micro-biological assays more closely.

The Fundamental Hypotheses

Whenever we adopt the general plan I have outlined we make two implicit assumptions. These are (*a*) that the response supposed to be produced by the known amounts of "factor X" is actually due to the factor itself and not to some other substance associated with it, *e.g.*, an impurity; and (*b*) that the response produced by the material to be analysed is also due solely to the presence in it of "factor X," without augmentation, diminution, or modification by any other substance also present. In other words, if we use the terms "Standard Preparation" and "Test Preparation" to denote respectively the solution of allegedly pure "factor X" and the solution prepared from the material to be analysed, we assume that the Std. Prep. contains no substance, other than factor X itself, contributing to the response we measure, and that the Test Prep. behaves for the purposes of the analysis so similarly to the Std. Prep. that it may be regarded simply as a dilution of the Std. Prep. in a completely inert diluent. This latter assumption is referred to below as the "hypothesis of similarity." If in any particular assay this hypothesis is not valid, then the result obtained will clearly be inaccurate.

There is nothing original or subtle about these statements, for they are obvious to anyone who thinks about the matter at all; but one finds that the validity of the hypothesis of similarity is often *assumed* by the analyst, and while this may sometimes be justifiable it is by no means always so. If one is determining the amount of manganese in a steel, using the Spekker absorptiometer, the method employed may have previously been shown to give accurate results without interference from any of the limited number of other elements that may be present. In such a case it may be unnecessary, in view of the constancy and reproducibility of the experimental conditions, to test the validity of each individual analysis. But, on the other hand, the bio-assayist who is determining the amount of some vitamin contained in a foodstuff can never be sure, merely because all previous assays have been satisfactory, that the present one will be; and macro-biologists have learnt from bitter experience to use such experimental designs, and to apply such statistical tests, as will enable the truth of the hypothesis of similarity to be examined separately each time an assay is performed, and solely on the internal evidence of the experiment itself. In the case of *micro*-biological assays, however, this question has not been given the attention it deserves. I shall return to this point.

The Assessment of Validity

The problem of finding the correct method of testing the validity of an assay can be solved in quite general terms. If a "dose" x_s of Std. Prep. produces a "response" y_s (Fig. 1), then the relation between the two may be written as

$$y_s = F(x_s) \quad \ldots \quad (1)$$

i.e., in mathematical terms y_s is a function, F, of x_s.

The nature of the function may vary widely according to the type of assay; in most chemical and many micro-biological assays there is a linear relationship between response and dose, so that

$$y_s = a + b.x_s \quad \ldots \quad (2)$$

where a and b are constants (Fig. 2). In chemical analyses a is frequently zero, the line passing through the origin. On the other hand, macrobiological assays often conform fairly closely to a linear relationship between response and the *logarithm* of the dose, *i.e.*,

$$y_s = a + b.\log x_s \quad \ldots \quad (3)$$

Note that in all such cases a is the value of the intercept of the line on the vertical or y axis while b is the "slope" of the line, that is, the increase in the response y per unit increase in x, or $\log x$, as the case may be.

Now, going back to Fig. 1 once more, if the hypothesis of similarity is valid, and the Test Prep. behaves exactly like a dilution of the Std. Prep., then the response produced by a

dose x_t of Test Prep. will equal the response produced by a dose $R.x_t$ of the Std. Prep. R is here the quantity by which x_t must be multiplied in order to obtain the equivalent dose of Std. Prep. It thus denotes the potency of the Test Prep. relative to that of the Std. Prep. and may be called the Potency Ratio. Hence, y_t must be related to $R.x_t$ exactly as y_s is related to x_s, or, in algebraic language,

$$y_t = \mathrm{F}(R.x_t) \qquad \ldots \qquad (4)$$

and this must be true whatever may be the form of $\mathrm{F}(x)$. Thus, the two curves which connect response with dose either of Std. Prep. or of Test Prep. are identical except that the one is plotted to a dose scale R times that of the other. This leads at once to two important conclusions:

(1) The best method of testing the validity of such an assay is to examine the identity in form of the Std. and Test regression equations, as the statistician calls them.

(2) The best method of estimating the "factor X" content of the Test Prep. is to determine the Potency Ratio R.

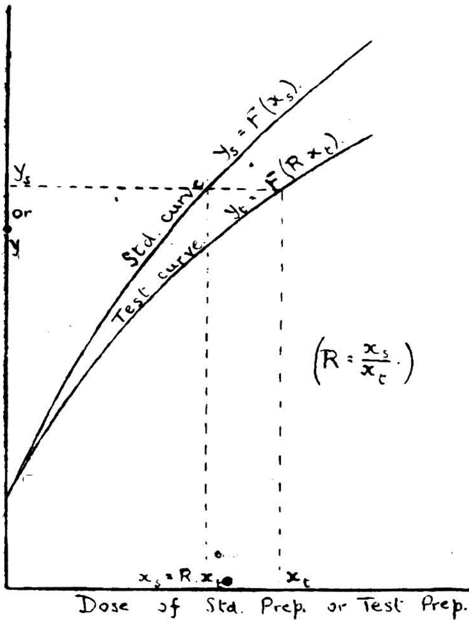

Fig. 1. The identity in form of the standard and test curves.

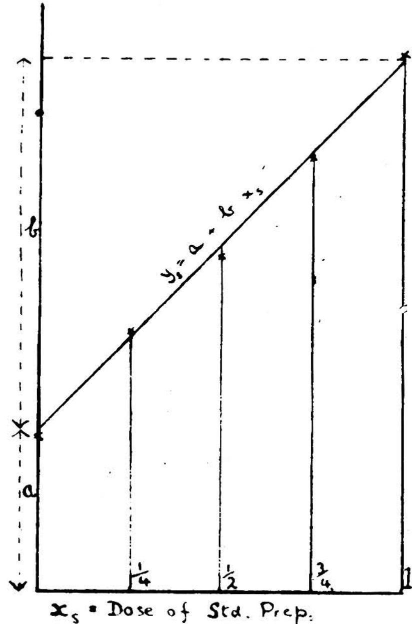

Fig. 2. The linear relationship between 'dose' and 'response'.

In the special case of those macro-biological assays in which the response is linearly related to the logarithm of the dose, the implications of these general conclusions have been fully worked out. There is no space to go into details here: Bliss and Marks,[1] and Irwin,[2] have shown how to make the necessary calculations, while Gridgeman[3] in his recent monograph on the estimation of vitamin A has set out very lucidly the principles underlying the design of the assay. I wish only to note the fact that the well-known "4-point" design, with the animals divided equally among the 4 groups—two Std. doses and two Test doses—makes the most efficient use of a given number of animals both as regards the precision of the result and also as regards the sensitivity of the test for validity. The macro-biologists probably do not realise how lucky they are thus to be able to have it both ways, so to speak; as we shall see later, the micro-biologists are not so lucky.

The Desirability of a Symmetrical Design

One further point of general validity must be made. Whatever the form of relationship between dose and response, it will always be found that the evaluation of R, the Potency Ratio, is derived from the constants of both the Std. and Test regression lines in a quite symmetrical fashion, so that the accuracy of the final result depends equally on the accuracy of

the two lines. Thus, in the macro-biological case R depends on the mean slope of the Std. and Test lines, while in micro-biological assays it is the ratio of the slopes of the two lines which gives R. In either case, the error in R is contributed to equally by errors in the two slopes. Clearly then, once it has been decided what shall be the *total* number of rats or test-tubes to be used, they should be divided equally between Std. groups and Test groups if the maximum precision for a given amount of work is to be obtained. It is not logical to devote two-thirds of one's observations to establishing the Std. line with extra accuracy, for as a result the Test line will be less accurate than it could have been, and the accuracy of the final result cannot exceed that of the more inaccurate of the two slopes. Most macro-biological assays nowadays conform to this principle, but most micro-biological assays do not. One reservation must be made. It may be quite justifiable to devote special attention to establishing the Std. line as accurately as possible if the analytical method is such that once a line has been established it can be relied on to "stay put"—*i.e.*, it is of the nature of a permanent calibration curve, and in actual analyses only Test Prep. observations are made. This applies, for instance, to certain absorptiometric or electrometric techniques. Whenever the analyst finds it necessary to make simultaneous observations on the Std. and Test Preps., however, his experimental design must be symmetrical if maximum precision is sought.

Present Methods of Designing and Computing Micro-biological Assays

Now let us consider in more detail the design and computation of micro-biological assays. The experimental technique usually employed has obviously been developed out of typical chemical, rather than biological, procedures. From five to seven different dosage-levels of Std. Prep. are used, each in duplicate or triplicate, and the mean response at each level is plotted against the dose (Fig. 3). If the resulting points are found to lie on or close to a straight line, this is then drawn as accurately as possible by eye. Similar groups of observations are made at 3 (or sometimes 4) dosage levels of the Test Prep. From the mean response at each of these levels, a result is read off from the graph of the Std. line, the Test Prep. points not being plotted, which is why I have called this the "single-curve" method. If the results obtained at the 3 or 4 different levels of Test Prep. agree within 10%, *i.e.*, the highest of them is not more than 110% of the lowest, this is regarded as sufficiently good agreement; and the mean of the several results is then taken as the best final estimate.

I have pointed out elsewhere[4] some of the objections to this method of computation. Inspection of the agreement between the three or four results is admittedly a rough test of validity, and is improved by Barton–Wright's observation[5] that if they show a progressive "drift" from the lowest to the highest dosage-level, the assay is suspect; even so, the test is not very sensitive in detecting an invalid assay, and it is possible for a set of figures which appear satisfactory on these counts to give an inaccurate final result. One reason for this is that, in taking as one's final estimate the arithmetic mean of the individual results at the 3 or 4 different dose-levels, one is in effect giving equal weight to all of them; whereas those at the higher levels are worth more, and should be given more weight. This point is automatically taken care of in the slope-ratio method described below. Again, the quantitative estimation of the precision of the final result in the usual form of the standard error is laborious and difficult, so that if two or more assays are to be combined to obtain a pooled estimate of the final result, or compared to see if there is any significant difference (*e.g.*, if the relative merits of different techniques are being investigated) the statistician's task is made almost impossible. The only advantage of the single-curve method is that the few calculations required are child's-play to even the most non-mathematical of workers.

Moreover, whatever method may be chosen to work out the result, there is a major objection to the experimental *design* just described, namely, that it is inefficient. For a given number of observations—*i.e.*, for a given expenditure of experimental labour—the precision obtained from such a design is definitely less than the theoretical maximum; for, apart from any other reasons, approximately two-thirds of the total observations are in the Std. Prep. groups, a fault which has already been discussed.

Macro-biologists have been through this sort of thing before. In their case, the inherent and irreducible variability of their experimental animals is so great that they were driven by sheer necessity to adopt the most efficient experimental designs and to compute both the validity and the precision of their results by rigid statistical means. The much smaller variability of the bacterial aggregates used in micro-methods has made the need for a similar approach in this case much less urgent, and it is often possible to obtain a reasonably accurate

result without using the theoretically best methods; nevertheless micro-biological methods can be improved by the application of statistical theory, and the analyst who is content to accept the conclusions and the formulae without attempting to verify the theory will find that the necessary calculations do not take very long, and do not involve any higher mathematics.

THE "SLOPE-RATIO" METHOD OF COMPUTATION

First, then, let me briefly describe the method I advocate in principle for the computation of any assay, micro-biological or not, in which the experimental observations connecting

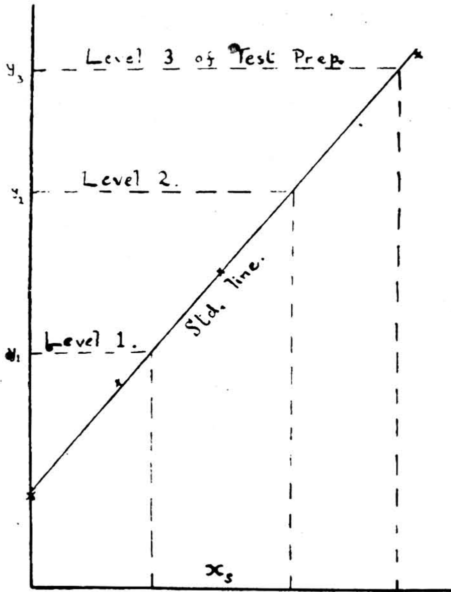

Fig. 3. The 'single curve' procedure.

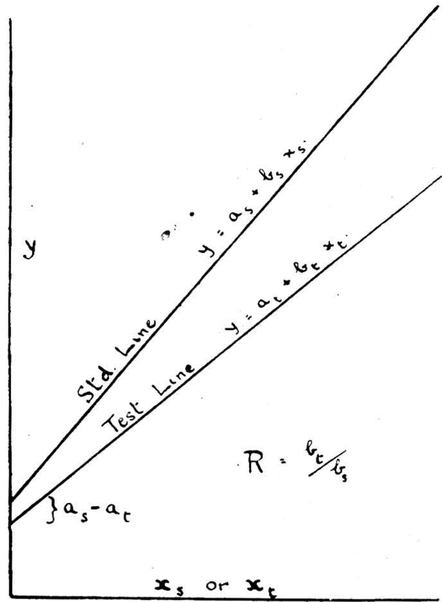

Fig. 4. The 'slope ratio' procedure (Theoretically, a_t should be equal to a_s).

dose of Std. Prep. with response lie, between certain limits at least, on or very close to a straight line (Fig. 4). Let the equation which represents this, the Std. line, be

$$Y = a_s + b_s . x_s \quad \text{(5)}$$

Then, as in the general case discussed earlier, if the hypothesis of similarity is valid, the Test Prep. observations must also lie on a straight line, identical with (5) except that the scale is different. Let the Test line be represented by the equation

$$Y = a_t + b_t . x_t \quad \text{(6)}$$

Since the dose x_t of Test Prep. must be exactly equivalent to a dose $R.x_t$ of Std. Prep. (it will be remembered that R is the potency ratio) the replacement of x_s by $R.x_t$ in (5) must make it identical with (6). It follows at once that

$$a_t = a_s \quad \text{(7)}$$

and

$$b_t = R.b_s$$

i.e.,

$$R = b_t / b_s \quad \text{(8)}$$

Thus, the theoretically correct method of evaluating R (from which the result of the assay in the usual form of "units of factor X per gram" follows immediately, of course) is to calculate the ratio of the slopes of the Test and Std. lines. It is natural, then, to call this the "slope-ratio" method of computation, and it involves either the drawing of the two lines on a graph, or the calculation of the regression equations (5) and (6) from the experimental data by the usual method as given by Fisher.[6] Moreover, the necessity for the identity of the constants a_t and a_s, as shown by equation (7), provides a criterion of validity having a sound theoretical basis, as I have previously[4] pointed out.

If the usual experimental design is adhered to, the calculation of the precision of the result in the usual form of the standard error involves considerable statistical work and a lengthy computation. Finney[7] has recently shown how it can be done. Seeing that the design is inefficient, however, the logical next step is to consider what is the best experimental design; after that has been settled we can discuss how to compute it.

PRINCIPLES OF SOUND DESIGN

Let us go back to equation (8). It is clear that for R to be established with maximum precision, we need to know the two slopes with maximum precision. Now it is easy to prove that if one wishes to establish the slope of a line as precisely as possible from a given number of observations, they should be divided into two equal groups, one at the lower and the other at the upper limit of linearity. To establish the slope of our Std. line, therefore, we ought to put all our eggs in two baskets, so to speak; if the line has been found experimentally to be straight from zero-dose up to 0·2 μg., say, then these are the two points at which we should concentrate all available test-tubes.

Before going further, however, one incidental point must be dealt with. It is the experience of many workers, including myself, that in riboflavine assays employing *L. helveticus* with any of the published basal media, the mean responses to doses between 0·05 μg. and 0·2 μg. are well fitted by a straight line, but the response to zero-dose, or "blank," is definitely off this line. This means that any observations made at this level are wasted; they cannot be used to contribute towards the determination of slope. I have made several series of observations with graduated doses of riboflavine between zero and 0·05 μg., in order to determine the lower limit of linearity. It seems to be somewhat variable, the response to as little as 0·005 μg. being at times on the line, while at other times the response to 0·025 μg. may be the lowest point on the line (Fig. 5 shows a typical Standard line of this kind). This is the highest value of the lower limit I have encountered. I now therefore deliberately incorporate sufficient riboflavine in the basal medium to provide 0·03 μg. in every tube. This increases my "blank" (or response to zero *additional* riboflavine) to about 2·5 ml., and this point is always on the line. The latter is, of course, straight as far as 0·20 μg. of *total* riboflavine, *i.e.*, doses of up to 0·17 μg. of riboflavine can be used (I actually now use in assays a maximum dose of 0·16 μg.).

This device has no disadvantages, for the *useful* range of dosage is not decreased (it is in fact slightly increased); in effect, one simply moves the vertical axis of one's graphs 0·03 μg. to the right. The advantages, as will be seen later, are a simplification in design and calculation, a lessening of experimental labour and a gain in precision. In what follows, therefore, it is assumed that any worker who finds his "blank" to be off the Std. regression line will add to his basal medium just enough of the factor being assayed to bring the new "blank" on to the line. The amount required may vary from one laboratory to another; each worker should find out for himself experimentally how much he needs to add.

THE SIMPLEST POSSIBLE DESIGN

Let us now go back to the problem of evaluating R with maximum precision. I have said that the slope b_s is most accurately established by making all observations either at the zero-dose level or at the upper limit of linearity. Now what is true of b_s is of course also true of b_t; we need the mean response at the two extreme ends of the line to be established as precisely as possible. Combining these two statements, we arrive at the simplest possible experimental design (Fig. 6). If the "blank" were off the line, we should need four sets of observations, *viz.*, a high and a low dosage-level on each line, and our observations would best be distributed equally between the four points—in other words, we should have the macro-biologists' 4-point assay design as previously described. But if, by good luck or good management, the "blank" is on the Std. line, it must also be on the Test line. Thus our two low-level points coincide, and we have a 3-point assay. I propose to call such a design, however, a "common-zero 3-point" design, because it will be necessary later to distinguish assay designs with 4 or more points and a common zero from the more familiar designs (such as the 4-point just mentioned) in which the points on one line are quite independent of those on the other. It should be added that the amount of Test Prep. taken should be chosen to give a response y_t as near as possible to the Std. response y_s without risk of exceeding it and thereby getting on to a non-linear part of the response curve. If insufficient is known about the Test Prep. to allow a shrewd forecast of the correct amount to take, it might be necessary

to put up tubes at more than one level and discard those which give unsuitable values of response.

It is hardly necessary to draw the graph in order to compute the result of such an assay. The Std. line obviously passes through the "blank" and the "max. dose" points, so that if the mean response at these two levels are y_o and y_s respectively, the increase in response is $y_s - y_o$ for the dose of Std. Prep. used. For simplicity, take this dose, whatever it may be, as the arbitrary unit of Std. Prep. The Std. slope will then be simply $y_s - y_o$ ml. per unit of Std. Prep. Similarly, if the dose of Test Prep. giving the mean response y_t is taken as the arbitrary unit, the slope of the Test line is $y_t - y_o$ ml. per unit of Test Prep. The value of the Potency Ratio is thus given by

$$R = \frac{y_t - y_o}{y_s - y_o} \qquad (9)$$

A numerical example will make this clear. Suppose that the mean "blank" y_o is 1 ml.; that a dose of 0·2 μg. of riboflavine gives a mean response y_s of 9 ml.; and that a

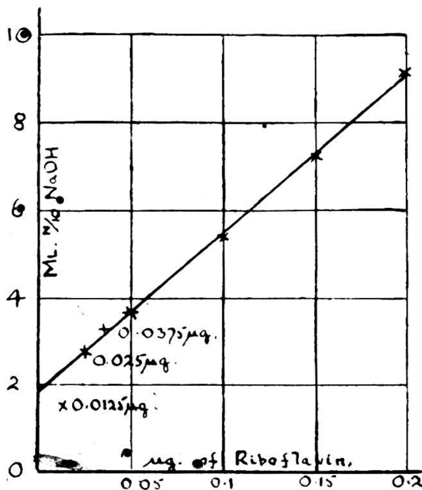

Fig. 5. A standard line in which the lower limit of linearity is 0·025 μg.

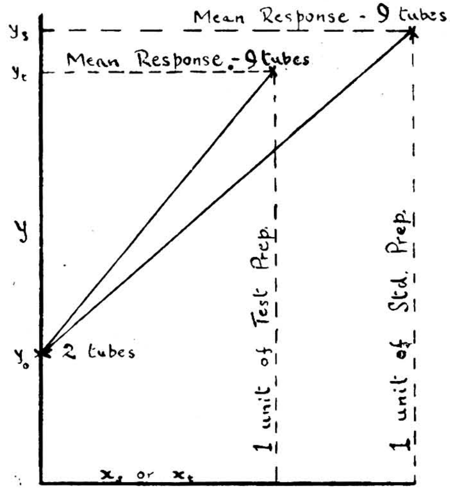

Fig. 6. The common zero 3-point design.

Note that $R = \dfrac{y_t - y_o}{y \quad - y_o}$.

quantity of Test Prep. representing 0·05 g. of a food gives a response y_t of 8 ml. Then our arbitrary units of Std. Prep. and Test Prep. are 0·2 μg. and 0·05 g. respectively. R is given by $(8 - 1)/(9 - 1) = 0{\cdot}875$. In other words the unit of Test Prep. contains 0·875 times as much riboflavine as the unit of Std. Prep., so that the food contains $0{\cdot}875 \times 0{\cdot}2/0{\cdot}05$ or 3·5 μg. per g.

It can be seen from equation (9) that inaccuracies in y_o do not affect the answer to anything like the same degree as inaccuracies in y_t or y_s, since they will alter the numerator and denominator of R in the same direction and to nearly the same extent. In our numerical example, if y_o had been wrongly estimated as 1·5 ml., R would have been estimated as 6·5/7·5 or 0·867, *i.e.*, less than 1% in error. For this reason, in an assay using 20 tubes in all, the best plan would be to use only 2 tubes as blanks and divide all the rest between the other two points, as shown in Fig. 6. This design will determine R with markedly more precision than an ordinary 4-point design with 5 tubes at each of 4 points and no common zero—an illustration of the fact that it is an advantage to arrange for the "blank" to be on the line and thus permit of a "common-zero" design being used.

Effect of Magnitude of Slope on Precision

There is another lesson to be learned from equation (9). The precision with which R, and hence the final result, is estimated can be increased in two ways only. Either the experimental error must be decreased—and this is a matter of practical refinements with which

I am not at present concerned—or the magnitude of both the numerator and the denominator must be increased. Now $y_s - y_0$ represents the maximum range of *titrations* over which the Std. line is truly linear. The actual size of the dose which produces the titration y_s is quite immaterial. If, then (Fig. 7), two workers A and B are using, for example, different basal media, or different strains of bacteria, such that A's Std. line is straight as far as 9 ml., while B gets a line which is straight up to 12 ml., then—other things being equal—B's results will be more accurate than A's. The doses A and B use to obtain their respective maximum titrations are quire immaterial. Similarly, if the upper limit of linearity is 9 ml. for both A and C, but C achieves this titration with a dose of 0·3 μg., whereas A needs only 0·15 μg. for the same titration, A has gained no real advantage. His Std. slope in ml. per microgram is indeed greater, but his useful range of titrations is no bigger, and this is all that matters. I mention this because some workers may have been thinking that to achieve an increase in the steepness of slope is an advantage *per se*. Actually it is valueless, unless the linear part of the curve is increased in terms of range of response.*

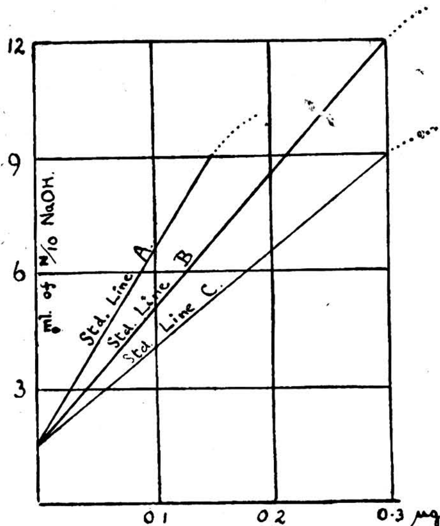

Fig. 7. The relation between slope and precision. Line A has the greatest slope; but B has the greatest precision.

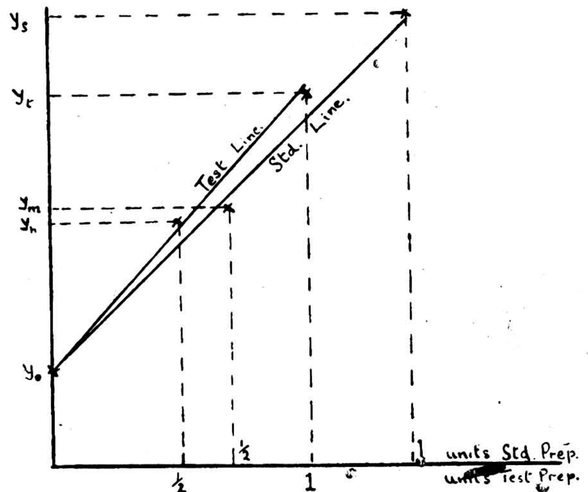

Fig. 8. The common-zero 5-point design.

The Common-zero 5-Point Design

I hope I may be forgiven for devoting so much attention to an assay design which, however attractive in its simplicity and inherent precision, would not be used in practice. It has served to illustrate without complications several of the fundamental principles underlying all statistically sound assay designs, and for that reason repays the time spent on it. But it has one great disadvantage—it *assumes* that both the Std. and Test regression curves are actually linear with a common zero, and therefore provides no means whatever of applying any test for validity. It is obviously essential that such a test should be possible; and this can be done if *there is at least one intermediate point on each line.* This arrangement leads to a "common-zero 5-point" design (Fig. 8); and as this is the design I would advocate for general use, I will now discuss it in detail.

It is possible to prove by fairly simple mathematical arguments that a design with one intermediate point exactly midway on each line is better than any with more than one intermediate point, in the sense that it gives more information for a given total number of observations. It is also easy to prove that the best method of distributing one's observations between the 3 points on any one line, in order to obtain the most sensitive test of linearity, is to have twice as many observations in the centre as at either end. This is most unfortunate,

* It may, however, be valuable for quite another reason, if obtained by improving the basal medium, for this may mean that less of one's assays will be invalid, as Price[8] suggests. But this is irrelevant to the present discussion.

for from the point of view of determining the *slope* of the line, which after all is our primary aim, the central observations contribute precisely nothing at all, so that half one's observations are wasted! Clearly a compromise is needed. It can be shown that the design which has ***an equal number of observations at each of the three levels*** is a quite satisfactory compromise; and this is a fortunate circumstance which mitigates the previous ill-fortune, for with so simple a design one's computations are correspondingly simplified.

The intermediate points are, as just stated, best located exactly halfway along the lines, *i.e.*, the dose should be half a unit of either the Std. Prep. or the Test Prep. Then, if the three points lie on a straight line, the mean response at the centre should be exactly the average of the other two. Let the mean responses at the "Half-Test" and "Mid-Standard" position be y_h and y_m respectively.

Then, taking the Std. line first, it is easy to see (Fig. 9) that if we calculate the quantity—

$$L_s = y_o + y_s - 2y_m \quad \cdots \quad (10)$$

—this should be zero. If in any given assay it differs appreciably from zero, we must compare it with its standard error, which is readily calculable—I will deal with this shortly—to see

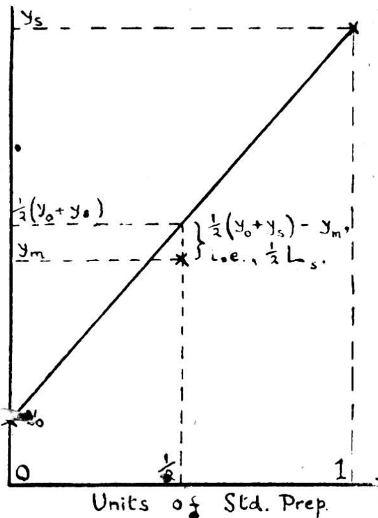

Fig. 9 A test for linearity.

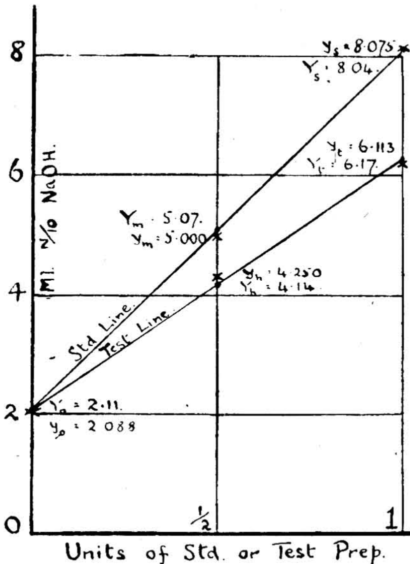

Fig. 10. Graph of the assay detailed in Table 1. Each y is an experimental mean response, while each Y is the corresponding point on the appropriate line.

whether the difference is significant. If not, there is no need to worry if it is, then that particular Std. curve is definitely not a straight line. The linearity of the Test line is examined in a manner exactly analogous to that used for the Std. line, by forming the quantity L_t defined by the equation

$$L_t = y_o + y_t - 2y_h \quad \cdots \quad (11)$$

—and examining the extent to which it departs from zero. Since we again need equal observations at the three levels, the design as a whole is fully symmetrical, with equal numbers of observations at each of the 5 points—or 4 tubes at each point for a 20-tube assay, which is a reasonable number to adopt.

I would again emphasise the advantages of inflating one's blank, so to speak, in order to bring it on to both lines. Dawson,[9] for instance, has used a "6-point" assay design with 3 points on each line, the "blank" not being used. Such a design is much superior to the usual multi-point technique. It lends itself to fairly simple statistical computation, and provides a check of the linearity of both the Std. and Test lines. Nevertheless, the "common-zero" 5 point design is superior, for it gives as much information as the 6-point design, with more accuracy (because a point is saved and the useful range of response is increased slightly)

and with less experimental labour (because there are only two dose-levels of each Prep. instead of three).

It is next necessary to discuss the method of computing the best result from such an assay. If the mean responses are plotted against the doses as usual, one soon learns to fit reasonably accurate lines to the data by eye, and to differentiate by inspection between the assay that is obviously valid and the one that is not; and the slopes of the lines can then be estimated from the graph. Nevertheless, it is only logical, having adopted a sound statistical design, to adopt an equally sound method of computing one's results. Not only can one thus obtain the very best mean estimate of the result; one can also obtain, if one wishes, a measure of its precision in the form of its standard error, together with a test of validity of the assay that is simple, sensitive, and sound. All this for half an hour's arithmetical work of an easy character. Let me, therefore, without more ado, summarise the necessary steps in the computation. Table I presents the essential data of a completed common-zero 5-point assay in which 20 tubes in all have been employed.

TABLE I

COMMON-ZERO 5-POINT 20-TUBE ASSAY

1 unit Std. Prep. = 0·20 μg. of riboflavin
1 unit Test Prep. = 0·05 g. of malt

Group	Individual responses (ml. $N/10$)	Mean response
Zero-dose	1·90, 2·25, 2·00, 2·20	y_o = 2·088
½ unit Std. Prep.	4·85, 5·00, 5·25, 4·90	y_m = 5·000
1 unit Std. Prep.	8·35, 8·20, 7·95, 7·80	y_s = 8·075
½ unit Test Prep.	4·00, 4·40, 4·50, 4·10	y_h = 4·250
1 unit Test Prep.	6·05, 6·20, 6·10, 6·10	y_t = 6·113

L_s = 2·088 + 8·075 − (2 × 5·000) = + 0·163
L_t = 2·088 + 6·113 − (2 × 4·250) = − 0·299
a = 2·088 + (0·136/7) = 2·107
b_s = 5·987 − (1·957/35) = 5·931
b_t = 4·025 + (1·277/35) = 4·061
R = 4·061/5·931 = 0·6847
Mean result = 0·6847 × 0·2/0·05 = 2·74 μg. per g.

The arithmetical work to be done is as follows.

(1) Compute the two quantities L_s and L_t from equations (10) and (11).

(2) The equations of the two regression lines are now obtained by calculating the three parameters a, b_s and b_t. These are given by

$$a = y_o - (L_s + L_t)/7 \quad \ldots \quad (12)$$
$$b_s = (y_s - y_o + (6L_t - L_s)/35 \quad \ldots \quad (13)$$
$$b_t = (y_t - y_o) + (6L_s - L_t)/35 \quad \ldots \quad (14)$$

The terms involving L_s and L_t are corrections for the fact that, unless L_s and L_t are both zero, the 5 points do not lie exactly on two straight lines, and therefore the best lines that can be drawn pass close to, but not through, the 5 points. If L_s and L_t are both zero, a becomes equal to the mean blank y_o, while the slopes reduce to $(y_s - y_o)$ and $(y_t - y_o)$ respectively, as would be expected.

The mean estimate of the Potency Ratio R is now given as usual by b_t/b_s, which in this instance = 0·6847, and the final answer in μg. per gm., or any other standard form can then be readily obtained. In the example given, the unit of Std. Prep. was 0·20 μg. and the unit of Test Prep. 0·05 g., so that the latter contained 2·74 μg. per g. Fig. 10 shows a graph of the assay; accompanying each mean response, the calculated value of Y, the corresponding point on the appropriate line, is shown for comparison.

Frequently no further information is required by the analyst, particularly if (as in this instance) it is obvious on mere inspection that the assay is valid, since the deviations of the five mean responses from the calculated "best" lines can only just be seen by eye. The result, as calculated by the simple process I have described, is the best mean estimate obtainable. Sometimes, however, the analyst requires the standard error of his result for some

purpose, or the validity of the assay may appear doubtful and need to be checked quantitatively. In such cases the further computations given below, and summarised in Table II, are required.

TABLE II

CALCULATION OF STANDARD ERRORS, ETC.

Group	Deviations of individual responses from group-mean			
Zero-dose	−0·188,	+0·162,	−0·088,	+0·112
½ unit Std. Prep. ..	−0·15,	0·00,	+0·25,	−0·10
1 unit Std. Prep. ..	+0·275,	+0·125,	−0·125,	−0·275
½ unit Test Prep. ..	−0·25,	+0·15,	+0·25,	−0·15
1 unit Test Prep. ..	−0·063,	+0·087,	−0·013,	−0·013

(Sum of squares of deviations)/15 = 0·54125/15 = 0·03608.

$s = \sqrt{0{\cdot}03608} = 0{\cdot}190$

$s_L = 1{\cdot}225s = 0{\cdot}233$

$s_R = \frac{0{\cdot}239 \times 0{\cdot}190}{5{\cdot}931}\sqrt{5{\cdot}5915} = 0{\cdot}0181$

Fiducial limits of R (P = 0·05) $\left.\right\}$ = 0·6847 ± (2·131 × 0·0181) = 0·646 to 0·723

Fiducial limits of result = (0·646 to 0·723) × 0·2/0·05 = 2·58 to 2·89 μg. per g.

(3). Write down the twenty (or as many as there are tubes) quantities given by subtracting from each individual response the mean response at the same dose-level. As a check, each group of 4 deviations should total zero.* Square them all (a book of tables such as that of Fisher and Yates[10] is invaluable here); divide by 15 (or if more than 20 tubes are used, by 5 less than the total number of observations); and take the square root of the answer. Algebraically, and much more concisely, compute

$$s = \sqrt{\frac{\Sigma(y - y_p)^2}{N - 5}} \quad \ldots \quad (15)$$

where y_p represents the appropriate group mean to be subtracted from any given observation y, and N is the total number of observations. In our numerical example, s is found to be 0·190. The quantity s is the standard error of a single observation.

(4) The linearity of the lines, and hence the validity of the assay, may now be checked. The standard error of either L_s or L_t is given by

$$s_L = \sqrt{\frac{30}{N}} \times s \quad \ldots \quad (16)$$

so that for a 20-tube assay, $s_L = 1{\cdot}225s$. If L_s and L_t are each less than twice s_L, the assay is valid. If either or both is over 2·5 times s_L it most certainly is not. If the ratio of either L to its standard error is between 2 and 2·5, the usual "t-test" will have to be applied; we are on the border line between validity and the reverse. Space does not permit further details of the use of the t-test, which has been explained very lucidly recently by Evans,[11] except to mention that the t-table should be entered with 15 (or $N - 5$) degrees of freedom. In the numerical example the assay is clearly valid.

I must here insert, however, a word of warning. While the result of any statistically invalid assay must be for that reason in error, it does not follow that the result of a statistically valid assay is not in error. If, for example, in a riboflavine assay the Test Preparation should happen to contain not only riboflavine, but also some other growth-stimulating factor, and if this other factor stimulated growth proportionally to the dosage at all dosage-levels, no statistical test and no method of calculating the result could possibly detect anything suspicious in the result obtained. The combined riboflavine and other factor would be estimated as riboflavine. All through this paper, therefore, the use of the words "valid" or "invalid" must be given the restricted statistical interpretation that I have previously explained.

* Or nearly so—there may be a small residue caused by approximating when calculating the mean responses.

(5) Finally, if the assay is valid, the standard error of the Potency-Ratio R can be calculated from the equation

$$s_R = \frac{s}{b_s}\sqrt{\frac{8}{7N}\,(8R^2 - 9R + 8)} \quad \ldots \quad \ldots \quad \ldots \quad (17)$$

which simplifies when $N = 20$ to

$$s_R = \frac{0{\cdot}239s}{b_s}\sqrt{8R^2 - 9R + 8} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (18)$$

It is now possible to state the answer in the form of its "fiducial limits," that is to say, two figures, one higher and one lower than the mean result, such that the true answer is unlikely to lie outside the range covered by these limits. More accurately, the odds are 20 to 1 against the possibility that the observed mean estimate of the result could have arisen from a true value lying outside these limits. They are given, not exactly, but usually quite accurately enough for all practical purposes, by the expression $R \pm t.s_R$, where t has its usual statistical meaning and for the 20-tube assay we are discussing is 2·131.

I believe that the design discussed above is a practical one and involves less experimental labour than the designs commonly employed to-day. The calculations at the end may perhaps be a little tedious, but are definitely less than those which every macro-biologist makes as a matter of course after every assay he performs; and in nine cases out of ten the full computations will not be needed. But I would strongly urge all micro-biologists to adopt either this design or some other which is equally sound statistically. The great advantage of using a sound design is that if at some future time one wishes to compare an old assay with a later one, an assay in one's own laboratory with one performed in another's, or even an experimental with an expected figure, the computations are made sufficiently easy for the analyst to be able to tackle them himself. If, however, the work is handed over to a statistician, the experimenter will have earned his praise and respect for presenting the data in a form from which he can readily extract the last ounce of information instead of having to spend vexatious hours making the best of a bad job.

I hope it will not be thought presumptious if I conclude with an exhortation to all analysts, no matter what their speciality may be. It is not making the best use of a statistician, nor is it fair to him, to call him in at the end of an experiment to extract therefrom information the analyst cannot obtain for himself; indeed, if the experiment was not properly designed the statistician will probably fail also. He should be consulted when the experiment is being designed. The result will be peace of mind for both analyst and statistician; a greater return of information per unit of experimental labour; and the satisfaction that comes from knowing that, whatever the results may be, no critic can infuriate the analyst by proclaiming pontifically "this assay is statistically unsound."

Summary

(1) An examination has been made of the theory underlying the design and computation of those analytical methods which are based on the existence of a linear relationship between the "dose" of the factor being estimated and the "response," or magnitude of the effect measured. In estimations of the strength or "potency" of a Test Preparation relative to a Standard Preparation, the following general principles can be laid down:

(*a*) The estimate of the Potency Ratio R is best obtained from the ratio of the slopes of the Test and Std. lines.

(*b*) The validity of such assays, *i.e.*, the extent to which the Test Prep. behaves purely as a dilution of the Std. Prep., can and should be examined; the best test is based on the fact that if the Std. observations are well fitted by a straight line, so should the Test observations be also.

(*c*) A given total number of observations is used most efficiently if the experimental design is symmetrical as regards the number of dose-levels, and number of observations at each level, along the Test and Standard lines.

(*d*) The method of distributing a given number of observations along a line which permits its slope to be estimated with maximum precision is quite different from that which provides the most sensitive test of its linearity. A satisfactory compromise is obtained by having one-third of one's observations at each of three points, *viz.*, the upper and lower dose-limits of linearity and halfway between them.

(*e*) A Std. line in which the lower dose-limit of linearity is zero leads to more precise

and simpler experimental designs than if the response to zero-dose is off the line. It is both possible and desirable to modify the latter case so as to convert it into the former.

(*f*) Other things being equal, that Std. line for which the distance, measured in units of *response*, between the upper and lower limits of linearity is a maximum, leads to maximum precision in the results. The distance in units of dose is immaterial.

(2) In the light of these considerations, an experimental design is worked out which should be suitable for routine analytical work, particularly in the field of micro-biological assays of vitamins and other specific nutrients. The method of extracting from it all the available information in a statistically sound form is given in detail.

It is a pleasure to record my indebtedness to Mr. D. J. Finney, M.A., for his most helpful advice and criticism and also for checking the accuracy of certain of the equations.

Appendix

A. The equations (12) to (14), by means of which the parameters a, b_s and b_t are computed, were obtained by applying the standard method of calculating the parameters of multiple regression equations (see Fisher, Sect. 29).[6] Using all symbols as in the main body of this paper, the Std. and Test regression equations may be combined into one in the form

$$y = a + b_s.x_s + b_t.x_t \qquad \text{(i)}$$

For the "zero-dose" group of observations, $x_s = x_t = 0$; for the "1 unit Std." group, $x_s = 1$, $x_t = 0$; for the "$\frac{1}{2}$ unit Std." group, $x_s = \frac{1}{2}$, $x_t = 0$; while for the "$\frac{1}{2}$ unit Test" and "1 unit Test" groups, $x_s = 0$, and $x_t = \frac{1}{2}$ and 1 respectively. The general equations given by Fisher will be found to reduce to

$$10a + 3b_s + 3b_t = 2(y_o + y_m + y_s + y_h + y_t) \qquad \text{(ii)}$$

$$6a + 5b_s = 2y_m + 4y_s \qquad \text{(iii)}$$

$$6a + 5b_t = 2y_h + 4y_t \qquad \text{(iv)}$$

These equations when solved lead to equations (12) to (14). Provided there is an equal number of observations in each of the 5 groups the results are independent of N, the total number of observations.

B. The standard method of attacking the computation of the results would be through an analysis of variance. This will be found to take the following form:

Fraction	Degrees of freedom	Sums of squares
Parameters ..	3	$4a\Sigma y_p + 2b_s(y_m + 2y_s) + 2b_t(y_h + 2y_t)$
Non-linearity	2	$4\Sigma(y_p - Y_p)^2$
Residual	15	$\Sigma(y - y_p)^2$
Total ..	20	$\Sigma(y^2)$

Following Fisher, Sect. 44, y_p represents any one mean response in each of the 5 groups and Y_p the corresponding value of the response as calculated from the appropriate regression line, *i.e.*, $Y_o = a$, $Y_m = a + \frac{1}{2}b_s$, etc. The error variance can be computed from the residual sum of squares in the manner set out above (p. 11), while non-linearity is assessed in terms of the squares of the 5 deviations of the mean responses from the lines, and its significance tested by means of a z-test as usual. However, the tests of non-linearity based on the quantities L_s and L_t are probably simpler to understand and use; if for no other reason, t-tests are easier to use than z-tests. Moreover, the two lines are tested *separately* for non-linearity if the L tests are used, whereas if the non-linearity fraction of variance is significant, a further partitioning would need to be made if information were sought as to, *e.g.*, the non-linearity of the Std. line considered separately. The quantities L_s and L_t are not quite independent, since y_o is common to both, but I believe the methods set out in the main text are the simplest, most practical, and most suitable for general use by practising analysts.

C. As regards equations (17) and (18) giving the standard error of the Potency Ratio R, these are derivable by fairly simple means, either by expressing R wholly in terms of the 5 group-mean responses, the standard error of each of these being $s \times \sqrt{(5/N)}$, or from the inverse matrix of the coefficients of equations (ii) to (iv) (see Fisher, Sect. 29,[6] and Finney[7]). It is hoped to publish elsewhere, in collaboration with D. J. Finney, a paper dealing, *inter alia*, with the underlying statistical theory of these and other cognate matters.

References

1. Bliss, C. I., and Marks, H. P., *Quart. J. Pharm. Pharmacol.*, 1939, **12**, 182.
2. Irwin, J. O., *J. Hygiene*, 1943, **43**, 121.

3. Gridgeman, N. T., "*The Estimation of Vitamin A*," Lever Bros. and Unilever, Ltd., 1944.
4. Wood, E. C., *Nature*, 1945, **155**, 632.
5. Barton-Wright, E. C., ANALYST, 1945, **70**, 283.
6. Fisher, R. A., "*Statistical Methods for Research Workers*," Oliver & Boyd, Edinburgh, 1944, Sect. 26.
7. Finney, D. J., *Quart. J. Pharm. Pharmacol.*, 1945, **18**, 77.
8. Price, S. A., *Nature*, 1945, **156**, 171.
9. Dawson, E. R., private communication.
10. Fisher, R. A., and Yates, F., "*Statistical Tables*," Oliver & Boyd, Edinburgh, 1943.
11. Evans, U. R., *Chem. and Ind.*, 1945, 106.

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DISCUSSION

The PRESIDENT congratulated Mr. Wood on his interesting and important paper and remarked on the difficulty of discussing adequately at short notice a paper of this character, which really required careful and prolonged study. In purely chemical work analysts were accustomed to eliminate any effects due to differences in composition of standard and test solutions by adding to the sample under examination small amounts of the constituent to be determined and ascertaining whether the technique employed gave an accurate determination of the amounts thus added. This procedure appeared to be trustworthy if the reaction curve was a straight line, but not necessarily if the relationship of response to concentration was more complex.

Mr. D. J. FINNEY said that before a particular dose-response relationship can be used as the basis of an assay technique, something must be known of the form of the relationship. Mr. Wood had been chiefly concerned with responses which are linearly related to the dose, at least over a fairly wide range of doses; another simple case is that of a linear relationship between response and the logarithm of the dose. There is no theoretical reason why more complex relationships should not also be used for assay purposes, providing that the same mathematical function defines the relation both for the standard and for the test preparation—indeed, certain sigmoid response curves are frequently used in biological assays. But research into the form of a response curve must precede its use in routine assays, in order to discover whether the curve is linear, if so, over what range; and if not linear, what its mathematical properties are, and whether any simple transformation of the measurements of dose or response will enable the curve to be converted into a straight line.

This research necessitates a detailed study of the curve for the standard preparation, based on observations at many different dose levels. He suspected that a partial realisation of the conflict between the needs of research on the response curve and the needs of routine assaying was responsible for the adoption of the single-curve arrangement of assays. In general, he thought that the conflict is best resolved by separating the two lines of work. Nevertheless, at least until the response curves are better understood than they are to-day, when several different test preparations are being assayed at one time, it may be worth making more observations on the standard than on any of the others, using additional dose levels to confirm that the behaviour of the standard is as would be expected from previous knowledge. For example, in an assay of four test preparations against a standard, the total number of tubes might be divided into thirteen and equal numbers assigned to the blank, each of four doses of the standard, and each of two doses of the four test preparations. This suggestion was not intended as a criticism of Mr. Wood's conclusions, for he (Mr. Finney) recognised that it involved some loss of precision on individual assays; in some circumstances, however, the increased assurance of the validity of the assay if his suggestion were adopted might more than compensate for the inevitable loss of precision in the estimates of potency.

Dr. J. H. HAMENCE pointed out that the statement by Mr. Wood—that the accuracy of the blank determination was of small importance—surely applied only when the standard and the test solution contained similar amounts of vitamin. He enquired whether or not it was possible to overcome the difficulty in the case of a determination found to be statistically incorrect due to the presence of interfering substances by making determinations on portions of the test solution to which known amounts of the vitamin had been added. Such a procedure had been found to give useful information in the past, but, so far as the speaker knew, the results had not been treated statistically.

Finally, it was interesting to note that the procedure for checking the efficacy of an analytical process by working with only half the usual quantity of the material, was apparently statistically correct.

Mr. WOOD, in replying to the discussion, said that "recovery" experiments, as referred to by the President and Dr. Hamence, would not detect the occurrence of all types of error. If, for example, the Test Prep. contained not only the "factor X" being assayed but also some other growth stimulant, the latter might well exert the same effect whether the Test Prep. was reinforced with additional "factor X" or not, in which case the recovery of added "factor X" would not be in any way abnormal and there would be no reason to suspect the presence of the interfering substance. Moreover, a cursory glance some time ago at the mathematics of recovery experiments indicated that the matter would repay further enquiry; it suggested that an apparent 100% recovery might in certain cases conceal relatively large errors. Mr. Finney's suggestion that extra dose-levels on the Std. line would be advantageous deserved consideration. In routine assays he himself had often put extra tubes at intermediate dose-levels as a matter of interest. But while he agreed wholeheartedly that there was a great need for further research, he emphasized that one should not attempt to combine research with one's routine assays. The object of a research experiment was quite different from that of an assay, ahd its design should be considered quite separately. The common-zero 5-point assay (or something closely akin to it) was the soundest design for routine analytical use, at least until advances in knowledge suggested that a modification would be desirable; but there was no reason why any analyst who wanted to should not make a few observations at intermediate dose-levels. For the purpose of working out the results, it was probably simplest to regard these intermediate observations merely as checks, and to ignore them in the computations. This entails a small loss of available information but avoids the necessity for other considerably more complicated calculations.

The Determination of Carotene and Vitamin A in Butter and Margarine

By T. W. GOODWIN and R. A. MORTON

(*Read at the Meeting of the Society on November 7th,* 1945)

BIOLOGICAL assays for vitamin A activity in butter reflect the joint contributions of preformed vitamin A and carotenoid pro-vitamins A. In order to assess the separate contributions it is necessary to use physico-chemical methods. Vitamin A has no effect on the colour of the butter, which is due to the provitamin carotene and the biologically inactive "xanthophylls." The yellow colour is best measured on the unsaponifiable fraction by determining the intensity of absorption near 450 mμ after removal of "xanthophyllic" pigments either by chromatography or phase separation (*vide infra*). On the other hand vitamin A is most easily measured (again on the unsaponifiable fraction) by determining the intensity of absorption near 620 mμ shown by the blue solution obtained with Carr - Price ($SbCl_3$) reagent. Direct absorption measurements at 325 mμ (after making allowance for carotenoid absorption) lead to the same result (see, for example,[1,2,3]). Reference will be made later to the special problems which arise in applying these methods to margarines.

PREPARATION OF UNSAPONIFIABLE FRACTION

The following adaptation of earlier procedures makes use of some suggestions made by Edisbury[4]:—20 g. of butter or margarine in a 250 ml. conical flask are treated with 8 ml. of 60% potassium hydroxide solution (wt./vol.) and 20 ml. of ethyl alcohol and the mixture is heated on a briskly boiling water-bath for 5 minutes after a homogeneous solution has been obtained. The soap solution is diluted with twice its volume of cold water and the whole transferred to a 500 ml. separating funnel. Ether (freshly redistilled from a little reduced iron) is used in three portions (150 ml. + 75 ml. + 75 ml.) to extract the carotenoids and vitamin A. The combined ether extracts are washed three times with tepid water to remove soaps and alkali. The solvent is then removed by distillation and the residue is blown dry, using nitrogen from a cylinder after twice moistening with a few drops of absolute alcohol.

DETERMINATION OF CAROTENE

The residue is dissolved in hexane (20 ml.) and extracted at least 7 times with 90% methyl alcohol saturated with hexane. The aqueous alcohol removes all the "xanthophylls," leaving α- and β-carotenes in the hexane. The carotene content is measured by determining $E^{1\%}_{1\,cm.}$ at 450 mμ, using a Hilger-Nutting Spectrophotometer. For pure β-carotene, $E^{1\%}_{1\,cm.}$ 450 mμ is taken to be 2500 in hexane.

A better estimation can be obtained with less trouble by a chromatographic procedure due to Mann.[5] The residue is dissolved in light petroleum (b.p. 40°–60° C.) and poured onto a small column (3 in. × $\frac{3}{4}$ in.) of defatted bone meal. Carotene (α- and β-) readily washes off the column with light petroleum and the eluate soon becomes colourless. Vitamin A, carotenols and artefacts are quickly eluted by small washings with acetone, and can be further separated, if necessary, by partition methods.[5]

DETERMINATION OF VITAMIN A

Vitamin A can be determined on the total unsaponifiable fraction or on the acetone eluate if the solvent is first removed. The vitamin-bearing residue is dissolved in 5 ml. of alcohol-free chloroform. The blue colour which appears when a suitable volume (0·4 ml.) is treated with the Carr - Price reagent (4 ml.), is measured, and the result is expressed as $E^{1\%}_{1\,cm.}$ 620 mμ.

Alternatively, the vitamin fraction is dissolved in purified *n*-hexane or cyclohexane and the intensity of absorption at 325 mμ is determined and expressed as $E^{1\%}_{1cm.}$. Whatever technique is used for this measurement a correction is needed, because the carotenoids exhibit some absorption near 325 mμ. (In the light of recent work by Zechmeister and others, the magnitude of the correction is somewhat uncertain, but Gillam's figure[1] of $E^{1\%}_{1\,cm.}$ 450 mμ divided by 6·5 works well in practice.)

EXAMPLES

(*a*) *Butter*—A sample of butter made available to the Vitamin A Sub-Committee (Accessory Food Factors Committee, Lister Institute and Medical Research Council) by Dr. S. K. Kon of the National Institute for Research in Dairying, gave the following results:

Vitamin A (by $SbCl_3$ colour test)	..	25·5 I.U./g.
,, (by ultra violet absorption)	..	26·4 ,,
Carotene	..	12·7 ,,
Total vitamin A activity	..	38·7 ,,

(Dr. A. E. Gillam, Dr. T. H. Mead and Mr. R. A. Finch obtained results in close agreement.) Biological assays carried out by several groups of workers associated with the Sub-Committee led to 35·3 I.U./g. (P = 0·99, 85 — 118%).

(*b*) During the past five years we have examined a fairly large number of samples of imported butter and butter fats and Fig. 1 shows the distribution of vitamin A and carotene in these samples.

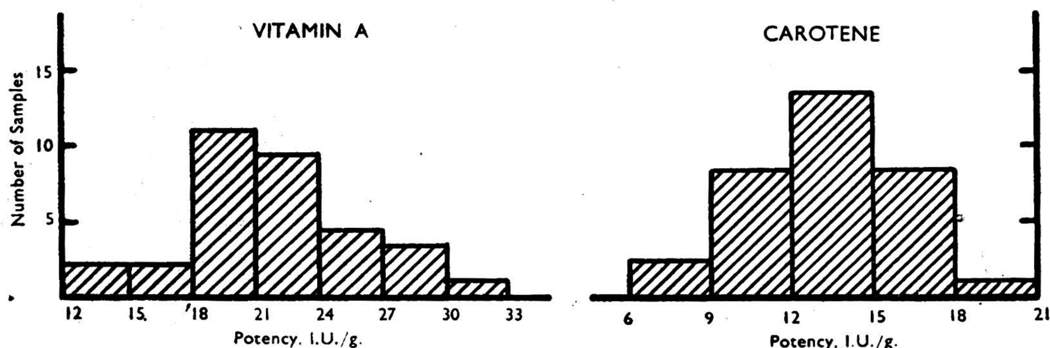

Fig. 1

MARGARINE ASSAYS

Difficulties are met in applying the above methods to margarines. The unsaponifiable fraction shows so much irrelevant absorption near 325 mμ that the ultraviolet test is useless for routine work. Moreover, constituents other than vitamin A react with the Carr - Price reagent to produce irrelevant absorption at 620 mμ in the colour test. Dyestuffs used to colour margarine may also appear in the unsaponifiable residue and produce colour (usually deep red) with $SbCl_3$; the absorption is, however, so far removed from 620 mμ that interference with spectroscopic measurement of the blue colour is surprisingly small. Nevertheless the gross absorption at 620 mμ for a margarine gives too high a result. Edisbury's "fading" technique[6] takes advantage of the transient nature of the vitamin A - $SbCl_3$ blue colour and his nett $E^{1\%}_{1 cm.}$ 620 mμ value is the difference between an initial "gross" $E^{1\%}_{1 cm.}$ reading and a reading made 20 minutes later. Unfortunately, it is known that the nett reading is too low, because (*a*) the vitamin A colour fails to fade away completely, and (*b*) the sterols present gradually give rise to a yellow colour with the antimony chloride reagent, resulting in a steady rise of irrelevant absorption at 620 mμ.

Empirically, the best compromise results from taking the mean of $E^{1\%}_{1 cm.}$ gross and $E^{1\%}_{1 cm.}$ nett. As will be shown below, there is strong justification for this, but for random samples of margarine it is perhaps unwise to claim greater precision than ±1 I.U./g. in results.

In order to assist our work, manufacturers of margarine have provided us with dyed unvitaminised fat blends as well as the corresponding margarines. The unsaponifiable fractions were prepared both from margarines and dyed fat blends, and the following data were collected:

(*a*) $E^{1\%}_{1 cm.}$ 620 mμ gross and nett on the margarine "unsaponifiable."

(*b*) $E^{1\%}_{1 cm.}$ 620 mμ initially, using a compensating cell containing the "fat blend unsaponifiable" plus $SbCl_3$ (*i.e.*, a complete control).

(*c*) $E^{1\%}_{1 cm.}$ 325 mμ was measured, again using the fat blend unsaponifiable fraction in the compensating cell. The 325 mμ maximum for vitamin A was clearly recorded.

No objection can be taken to methods (*b*) and (*c*), but they are applicable only if the dyed fat blend is available. Although not normally feasible, (*b*) and (*c*) here permit a check to be made on (*a*).

Example:

(i) 30 g. of margarine were saponified and the "unsaponifiable" was dissolved in 10 ml. of cyclohexane purified for spectroscopy ($\equiv$ 300% solution calculated on initial weight).

(ii) 24·5 g. of dyed fat blend were saponified, and the "unsaponifiable" was dissolved in 10 ml. of cyclohexane ($\equiv$ 300% for finished margarine).

(*a*) 0·4 ml. of solution + 4 ml. of $SbCl_3$ reagent gave

$$E^{1\%}_{1\,cm.}\ 620\ m\mu \begin{cases} 0{\cdot}0350 \text{ gross} \simeq 17{\cdot}85 \text{ I.U./g.} \\ 0{\cdot}0233 \text{ nett} \simeq 11{\cdot}9 \quad ,, \end{cases}$$

mean 14·9 ,,

(*b*) 0·5 ml. of (i) + 4 ml. of $SbCl_3$
0·5 ml. of (ii) in compensating cell + 4 ml. of $SbCl_3$ } $E^{1\%}_{1cm.}$ 620 mμ 0·030 $\simeq$ 15·3 I.U./g.

(*c*) 4 mm. thickness of 300% solution, E 325 mμ = 1·14.

$$E^{1\%}_{1\,cm.}\ \frac{1{\cdot}14 \times 10}{4 \times 300} = 0{\cdot}0095 = 15{\cdot}2 \text{ I.U./g.}$$

The agreement between the three methods justified procedure (*a*).

As a further test, a sample of margarine fortified with vitamin A and pure β-carotene was made specially for experimental purposes and tested against two margarines made with different fat blends. The results (for part of which the Vitamin A Sub-Committee is again responsible) are given in Table I.

TABLE I

Sample	Vitamin A I.U./g.	Carotene I.U./g.	Total activity (bio-assay)
(1) Intended to match butter			
Target figures	26·3	13·2	
Initial spectroscopic figures	24·4	12·7	35·3
Final ,, ,,	22·8	12·3	
(2) Margarine X			
Target	19	—	
Initial	17	—	15
Final	15·5	—	
(3) Margarine Y			
Target	17·8	—	
Initial	14	—	12
Final	12·5	—	

The samples were tested at the beginning and at the end of the rather long period necessary for the groups of biological tests. The evidence for an apparent initial loss of potency in manufacturing margarine is clear cut (*vide infra*).

As part of a programme for controlling National margarine for the Ministry of Food, the Oils and Fats Division arranged for us to have samples of finished margarine and of the concentrates used in known amounts, so that by applying standardised analytical procedures to both we could control the recovery of vitamin A more closely. The results are of interest from several points of view.

Three types of concentrate were used: C_1 of nominal potency 1456 I.U./g., C_2 4,368 I.U./g. and C_3 100,000 I.U./g. Each was examined in four ways:—(*a*) by the $SbCl_3$ colour test on the sample as supplied, (*b*) by the ultra-violet absorption at 325 mμ shown by the sample, (*c*) by the colour test carried out on the unsaponifiable fraction, and (*d*) by the ultra violet absorption at 325 mμ shown by the same fraction. The results of (*a*) and (*c*) agreed closely, but (*b*) gave readings which were obviously too high, since irrelevant absorption was practically eliminated in (*d*).

In calculating the potencies in Table II the conventional conversion factors

$$E^{1\%}_{1\,cm.}\begin{cases}620\ m\mu\ 1 = \ \ 510\ \text{I.U./g.}\\ 325\ m\mu\ 1 = 1600\ \ \ \ ,,\end{cases}$$

have been used. Both factors are probably a little low. From the present point of view, it is important to note that for nearly all the concentrate samples the saponification and extraction procedure results in a quantitative recovery of vitamin A in the unsaponifiable fraction.

TABLE II

	Estimated vitamin A potency by method—			
	(a)	(b)	(c)	(d)
C_1 concentrates (nominally 1456 I.U./g.)				
Factory M_1	1215	ca. 1660	1200	1250
,, M_2	1210	1660	1244	1328
,, M_3	1200	1660	1208	—
,, M_4	1352	—	1427	1408
,, M_5	1290	ca. 1660	1309	1360
,, M_6	—	—	1203	1332
C_2 concentrates (nominally 4368 I.U./g.)				
Factory M_7 C_2	3716	4000	3826	4190
,, M_8 C_2	3958	4800	3782	4190
,, M_9 C_2	3704	4480	3802	4190
,, M_{10} C_2'	4210	4950	4231	4450
,, M_{11} C_2'	4338	—	4344	4410
,, M_{13} C_2''	4900	5120	4880	4800
C_3 concentrate (nominally 100,000 I.U./g.)				
Factory M_{12}	93,000	96,000	90,300	86,500

As already mentioned, the antimony chloride colour test applied to margarine unsaponifiable fractions usually gives a deep red colour (due to dye-reagent interaction) superimposed upon the blue colour. $E^{1\%}_{1\,cm.}$ 620 mμ gross includes a contribution due to dye and $E^{1\%}_{1\,cm.}$ 620 mμ nett is too low because of the development of some irrelevant absorption. As has been shown, the mean of the gross and nett figures possesses experimental justification. When the weight of concentrate incorporated in a given weight of margarine is known, the expected potency may be calculated from the estimated potency of the concentrate.

TABLE III

Factory		Nominal potency I	Expected potency II	Estimated potency E gross	E nett	Observed I.U./g. III	Recovery %
M_1	..	22·6	18·8	17·4	14·1	16	86
M_2	..	21·45	17·95	15·3	12·8	14	78
M_4	..	21·5	20·15	17·9	15·3	16·5	82
M_5	..	21·5	18·8	17·3	14·6	16	85
M_7	..	22·7	19·73	16·8	14·6	15·5	79
M_8	..	21·65	18·82	16·1	13·6	15	80
M_9	..	21·95	19·1	16·4	13·4	15	79
M_{10}	..	22·65	21·9	17·5	14·4	16	76
M_{11}	..	21·92	21·45	19·4	16·3	18	84
M_{12}	..	21·8	19·25	16	13·6	15	78
M_{13}	..	21·7	24·3	22·4	18·8	20·5	85
*M_3	..	13·65	11·25	13·6	8·8	11	98
*M_6	..	13·4	10·9	12·7	8·4	10·5	96

* Margarines also fortified with Carotene (*vide infra*).

I Calculated from nominal potency and weight of concentrate used.
II Calculated from our assay of the concentrate and weight used.
III $\frac{1}{2}(E^{1\%}_{1\,cm.}$ gross $+ E^{1\%}_{1\,cm.}$ nett$) \times 510$.

Omitting M_3 and M_6 we have for 11 factories:

Average nominal potency 21·9 I.U./g.
,, expected ,, 20 ,,
,, observed ,, 16·1 ,,
,, "recovery" 80·5%.

For M_3 and M_6 the recovery is definitely higher than for the other 11 margarines, which varies within experimental error around 81 ± 5%.

The *absolute* potency of the margarine is a separate problem. The concentrates distributed to margarine manufacturers were prepared from oils or concentrates standardised by the ultra-violet absorption $E^{1\%}_{1\,cm.}$ 325 mμ multiplied by 1600. For very rich vitamin A preparations, it was found that $E^{1\%}_{1\,cm.}$ 620 mμ multiplied by 510 gave the same result. Since the time when these figures first came into use, experience has shown that for many types of concentrates the colour test appears to be somewhat inhibited, and for the present series a conversion factor of 540 would be more appropriate. Applying this new factor, the average *absolute* potency would be raised from 16 to 17 I.U./g. (still retaining 1600 as the ultra violet factor), but the average recovery would remain at 80·5%. Hence the average potency for the 11 fresh margarines works out to 28·4 × 17 = 483 I.U./oz.

The M_3 and M_6 margarines contain within experimental error the 11 I.U./g. of preformed vitamin A expected of them. They are also fortified with a carotene concentrate with a nominal activity of 350 I.U./g. According to our tests the carotene preparations contained

	C_5 (M_3)	C_5' (M_6)
Total carotenoids in "non-sap." extract	709	704 μg./g.
$\alpha + \beta$ Carotene portion	512	644 ,,

Margarines ..	M_3		M_6	
	expected	found	expected	found
Total carotenoids	11·6	12·2	11·5	12·7 μg./g.
α, β-carotene ..	8·4	9	10·6	11·5 ,,

5·4 μg. of β-carotene (= 9 I.U.) would raise the total activity from 11 to 20 I.U./g. so that there is evidently an appreciable margin of safety over the target of at least 20 I.U./g. Further chromatographic separation might show how wide is the margin, since α- and β-carotenes are not differentiated here.

In order to test the performance of a photoelectric instrument,[7] a concentrate (nominally 1500 I.U./g.) and 5 margarines (nominally 0, 10, 15, 20 and 25 I.U./g.) have been studied.

The concentrate (tested without previous saponification) gave

$E^{1\%}_{1\,cm.}$ 620 mμ 2·6 gross, 2·14 nett.

$E^{1\%}_{1\,cm.}$ 325 mμ 1·04.

Probably this concentrate was similar to C_1 but not enough was available for a full test. The preliminary test leads as in Table IV to lower expected values than the nominal ones. All the margarines were tested in the ways already described.

TABLE IV

Nominal potency I.U./g. y	Expected potency I.U./g. y'	$E^{1\%}_{1\,cm.}$ 620 mμ gross x	nett x'	$\frac{x+x'}{2}$	$\frac{625(x+x')}{2}$	$\frac{540(x+x')}{2}$
0	0	+0·0076	−0·0002	0·0037	2·4	2·0
10	8·8	0·0218	+0·0109	0·01635	10·2	8·8
15	13·2	0·0296	0·0178	0·0237	14·8	12·8
20	17·6	0·0383	0·0271	0·0327	19·4	17·65
25	22	0·0459	0·0343	0·0401	25·06	21·65

If the gross and nett values are plotted against nominal potencies two parallel straight lines are obtained:

$$\text{Gross} \quad x - 0{\cdot}006 = 0{\cdot}0016y$$
$$\text{Nett} \quad x' + 0{\cdot}006 = 0{\cdot}0016y$$
$$(x + x')/2 = 0{\cdot}0016y$$

implying a conversion factor of 1·0/0·0016 or 625. Similarly $(x + x')/2 = 0{\cdot}00181y'$, implying a conversion factor of 550. On similar concentrates $E^{1\%}_{1\,cm.}$ 620 mμ = 2·5 and $E^{1\%}_{1\,cm.}$ 325 mμ = $0{\cdot}83_7$; accepting the potency as $0{\cdot}83_7 \times 1600 = 1340$, a conversion factor 1340/2·5 = 536 is reached for the colour test.

The significant thing here is that the methods described lead to practically complete recovery of vitamin added to margarine. The calibration is thus satisfactory.

Conclusion

National margarine now bears on the wrapper a statement that 450–550 I.U./oz. is the vitamin A potency. A conservative interpretation of the tests on margarines containing no added carotene leads to an average potency of 483 I.U./oz. on the fresh products. Those containing carotene are at least as good; the complicated position concerning preparations of mixed carotenoids and their stability is responsible for the note of caution here.

The main problem is always, however, the retention of the vitamin A activity over the commercial life of the product. Separate and somewhat lengthy investigations made during the war show that the nature and quality of the fat blend is the controlling factor. A small but variable initial loss of potency is very frequent. This is followed by a period of little change which can be as long as three months, after which the rate of deterioration increases. Under normal peace-time conditions fat blend ingredients could be selected, processed and used without delay so as to minimise the initial loss and lengthen the period of induction.

A margarine fortified to 20 I.U./g. with preformed vitamin A and coloured with a carotenoid preparation equivalent to 10 I.U./g. would eliminate the need for added dyestuff and preserve the 2 : 1 ratio of preformed and provitamin A activity characteristic of butter.

This paper represents a selection from a considerable mass of data accumulated during the war, and we wish to express our thanks to the Ministry of Food (Scientific Adviser's and Oils and Fats Divisions) for permission to publish. We are also indebted to many other workers concerned with margarine or vitamin technology as well as those mentioned in the text for the exchange of experience and the provision of facilities, and to the Vitamin A Sub-Committee (Secretary, Miss E. M. Hume) for permission to use the results of biological assays.

References

1. Gillam, A. E., Heilbron, I. M., Morton, R. A., Bishop, G. and Drummond, J. C., *Biochem. J.* 1933, **27**, 878.
2. Gillam, A. E., *ibid.*, 1934, **28**, 79.
3. Morton, R. A., Lord, J. W. and Goodwin, T. W., *J.S.C.I.*, 1941, **60**, 310.
4. Edisbury, J. R., private communication.
5. Mann, T. B., ANALYST, 1943, **68**, 233.
6. Edisbury, J. R., *ibid.*, 1940, **65**, 484.
7. Bowen, J. L., Gridgeman, N. T., and Longman, G. F., ANALYST, this vol. p. 20.

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(*For discussion see end of next paper.*)

A Photoelectric Photometer for the Estimation of Vitamin A in Margarine

BY J. L. BOWEN, N. T. GRIDGEMAN AND G. F. LONGMAN

(*Read at the Meeting of the Society on November 7th,* 1945)

INTRODUCTION—Although statutory minima for the vitamin-A content of margarines have been in force in this country for some years, a wholly satisfactory method of test is still wanting. The bio-assay is unsuitable for routine estimation of vitamin A, not only because of the well-known difficulties in obtaining inter-laboratory agreement, but because of its cost and the length of time involved in making the test. It is therefore necessary to make use of the light-absorption characteristics of vitamin A itself or of its chloroform - antimony trichloride reaction product, and the literature furnishes a choice of methods,[1,2,3,4] each of which, however, is open to one objection or another. The present paper offers yet another method—one, it is hoped, that approaches a little nearer the ideals of simplicity, inexpensiveness and reproducibility.

Normal trading in vitamin-A oils and concentrates is conducted on the basis of the "E325" test, *i.e.*, on the determination of the extinction coefficient, referred to a 1% solution, at 325 mμ. Unfortunately this test cannot be applied to margarine whose normal content

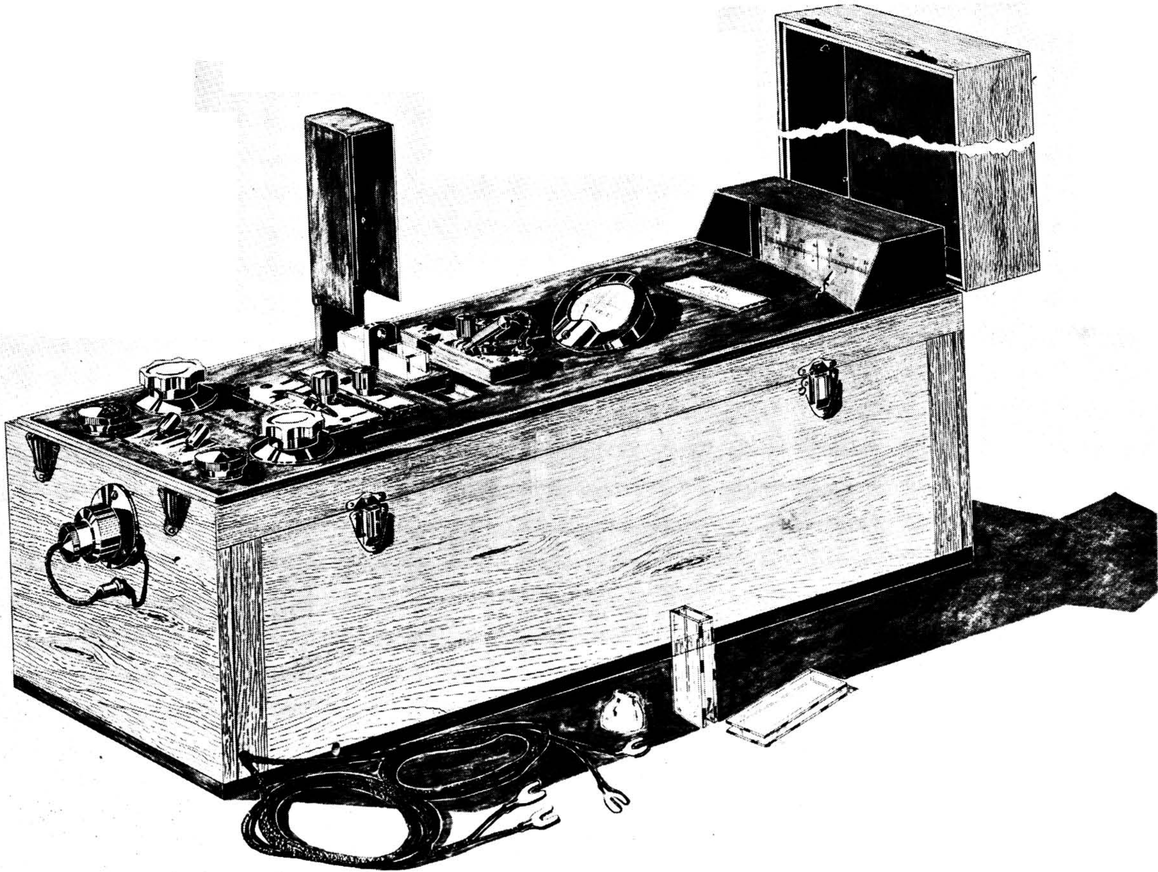

Fig. 1. Photoelectric Photometer.

of vitamin A is below the critical level of estimation by this means, and recourse has to be made to the "spectroscopic blue" test, *i.e.*, to the determination of the extinction coefficient, again referred to a 1% solution, at 620 mμ, of the antimony trichloride - vitamin A reaction mixture in chloroform solution. This can be done on a visual spectrophotometer, which, however, is an expensive instrument that does not usually form part of the equipment of the ordinary routine laboratory. It appeared desirable therefore to evolve a much less costly piece of apparatus that would be simple to use and capable of furnishing results of a high degree of reproducibility. The method about to be described, which in essence is the evaluation by means of a photoelectric photometer of the "E620" antimony trichloride test on the unsaponifiable matter, was primarily devised for factory control purposes, *i.e.*, to enable the works laboratory to check the vitamin-A content of the manufactured margarine, and it is from this point of view that the bulk of this paper is written. It is believed, however, that the technique is adaptable to the purpose of the outside analyst who has to examine margarine, and this application of the method is dealt with separately. It is also hoped that the instrument described will be of general use in other fields of absorption photometry.

The distinction between factory control work and outside analysis lies in the fact that the absorption at 620 mμ in a margarine test *via* the unsaponifiable matter is not wholly due to vitamin A; a portion is contributed by other ingredients, and the making of the necessary correction is simplified if the analyst has access to the raw materials.

The Instrument

The instrument (see Fig. 1) derives from several predecessors, see, for instance, Evelyn,[5,6] Yudkin[7] and Hoch.[8] The principle common to all is the measurement, by means of a photoelectric cell and a galvanometer, of the absorption of filtered light by the test solution—a mixture of antimony trichloride and the unsaponifiable portion of the margarine in chloroform. The total cost of this apparatus* will be of the order of £50, *i.e.*, probably less than a quarter of the cost of a visual spectrophotometer.

In this instrument the light source is an adjustable, horizontally mounted 6-volt 6-watt automobile lamp run from an accumulator. A rheostat and voltmeter enable the voltage to be kept constant. To minimise reflection from the glass of the bulb the emergent light has first to pass through a circular hole 4·5 mm. in diameter, *i.e.*, rather larger than the filament. The light beam then enters a simple biconvex lens whose focus is at the lamp filament, *i.e.*, about 55 mm. away, and immediately beyond the lens is an iris diaphragm. The beam, now slightly convergent and of adjustable cross-section, traverses the filter and the reaction cell in that order. The filter is a 620 mμ Wratten 26, sandwiched between 1 mm. Chance glasses OB.2 and ON.13. (When circumstances permit, this filter could advantageously be replaced by an all-glass one, made from a specially prepared melt exhibiting absorption characteristics the exact inverse of those shown by the antimony trichloride - vitamin A complex.) After leaving the reaction cell (described below) the light impinges on a photoelectric cell whose output (of the order of 2 microamps.) is taken to a light-spot galvanometer fitted at the far end of the instrument. A shutter protects the cell when this is not in use. The galvanometer scale is double: one reads normally from 100 to zero and registers percentage transmission; the other reads logarithmically, in the same direction, from zero to infinity, and registers optical density.

The components are housed in a wooden case with a cast-aluminium base and a top—which serves as an instrument panel—of Tufnol. The reaction cell carriage is also of Tufnol, a material whose particular advantage in this connection is its resistance to stains of the antimony trichloride solution. This carriage is lidded and holds two optical cells side by side and its lateral movement enables either cell to be brought into the light path. The cell holders accommodate either ½ cm. or 1 cm. cells, but all the work here reported was done with the former, which are about 6 cm. high by 3 cm. wide and in use require not less than 3 ml. of solution. A special funnel is provided to facilitate rapid and splashless filling of the "reaction" cell, in the top of which it sits stably and does not need to be removed immediately after use, as the hinged lid of the carriage fits comfortably over it. The carriage lid keeps out extraneous light, and the instrument as a whole is designed to minimise the entry of stray light.

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* Manufactured by Unicam Instruments Ltd., Arbury Road, Cambridge.

Other Apparatus and Reagents

Apparatus

250 ml. round-bottomed narrow-necked flasks fitted with 30 in. ground stoppered air condensers.
1000 ml. glass-stoppered separating funnels.
300 ml. flat-bottomed narrow-necked flasks.
Distillation apparatus for ether recovery. This consists of a 300 ml. flat-bottomed wide-necked flask fitted with a 500 ml. separating funnel. The flask is connected through an Evans double-surface condenser to a 2000 ml. conical flask which is fitted with a vertical double-surface condenser to prevent escape of distilled ether.

6 × 1 cm. test tubes marked at the 3 ml. level.
Burettes, 10 ml. stoppered measuring cylinders and fine pipettes—including at least one ½ ml. pipette, graduated in hundredths of a ml. and fitted with a capillary tip 5 cm. long.

Reagents

50% aqueous potassium hydroxide solution.
Absolute alcohol.
Ether (B.P.) distilled fresh from powdered caustic soda as required.
Hydroquinone.
Chloroform (B.P.). Before use wash with water, dry with K_2CO_3, filter through a fast paper and add 1% of absolute alcohol.
Vitamin-A reagent. This is a 21–23% solution of antimony trichloride in chloroform purified as above. If obtained ready made it must be repurified as described by Edisbury.[1]
Acetic anhydride (pure).

Preparation of Unsaponifiable Matter from Margarine

It must be emphasised that the reliability of the technique as a whole depends first and foremost upon the care taken to ensure that all the vitamin A in the sample of margarine is mobilized in the extracted unsaponifiable matter. No detail is therefore spared in the following description of the process of extraction. It is an improved version of that described earlier from this laboratory (Edisbury[1]), and inter-laboratory trials have led us to believe that it is also an improvement on other methods to be found in the literature already quoted—and some of those methods have theoretically unnecessary or undesirable features, such as preliminary removal of non-fatty constituents, the addition of a slightly acidic antioxidant, the use of light petroleum or of a large excess of caustic soda (see the evidence of Benham[9] on these last two points) and the creation of conditions that increase the ever-present danger of emulsification.

Weigh 15 g. taken from the inside of the sample into a 250 ml. flask fitted with a ground glass stoppered air condenser, add 10–20 mg. of hydroquinone, 12 ml. of 50% aqueous potassium hydroxide solution and 35 ml. of absolute alcohol. Boil gently for 15 minutes on a steam bath, cool, wash the condenser and stopper with distilled water, and pour the contents of the flask into a litre separating funnel, using a total of 150 ml. of distilled water for the transfer. Rinse the flask and air condenser three times with small quantities of ether, transfer the washings to the separating funnel, and make up the volume of ether in the funnel to 150 ml. Invert the funnel and swirl the contents with a single sharp movement of the wrist, which should produce an intimate mixing of the two phases, and repeat several times. Avoid vigorous shaking. If the phases do not separate within a period of two minutes the test should be rejected. Separate the layers and re-extract the aqueous-alcoholic phase twice with 60 ml. quantities of ether. Wash the combined ether extracts four times with 50 ml. quantities of distilled water at about 30° C., using the same swirling motion as described above to mix the two phases.

After removal of final wash water, decant the ether phase into the separating funnel of the distillation apparatus, run the ether solution into the flask at distillation rate, *i.e.*, so that the ether is removed at the same rate as the fresh solution enters. When about 1 ml. of solution remains in the funnel rinse with about 5 ml. of absolute alcohol, and run it swiftly into the distillation flask. Remove the flask immediately from the apparatus and cork it. The unsaponifiable matter is now in alcoholic solution and may be kept in the dark for two or three hours, if necessary, before being tested.

Method of Test when Control Samples of Vitamin Concentrate and Fat Blend are Available

In this section we assume that the analyst has been supplied with samples of vitamin-A concentrate (together with information regarding its potency based on $E^{1\%}_{1\,cm.}$ 325 mμ) and

with the fat blend used in the manufacture of the margarine he is to test, together with quantitative details. If the finished margarine contains carotene, the appropriate quantity of the carotene concentrate must be mixed in with the control fat blend. Any dye that has been used may also be mixed into the fat blend, although it is our experience that none of the margarine dyes known to be in use at the present time contributes any significant absorption in the region of 620 mμ. The water-soluble ingredients of the margarine are not required, because they are all removed during saponification.

Prepare the unsaponifiable fractions from a 15 g portion of the margarine and from a 15–20 g. portion of the corresponding fat blend (plus carotene, if present in the margarine) as described above.

The instrument, in a shaded and vibration-free position, is prepared for use as follows: Switch on the current, adjust the voltage to 6 and allow 10 minutes' steadying time. With the photocell screened, set the galvanometer spot exactly at "maximum absorption." Place a reaction cell filled with chloroform in the light path, open the screen, and adjust the iris diaphragm until the galvanometer spot registers maximum transmission. The instrument is now ready.

Remove the alcohol from the solution of unsaponifiable matter by heating on a steam bath in a stream of hydrogen. Still maintaining the gas flow, cool the flask and add about 1·5 ml. of chloroform. Pour the solution into a small test tube having a mark at 3 ml., rinse the flask with three 0·5 ml. lots of chloroform, and make up the solution to the 3 ml. mark. With a 0·5 ml. pipette, graduated in hundredths of a ml. and fitted with a capillary tip 5 cm. long, transfer an aliquot of this solution (usually 0·25 to 0·35 ml.) to a clean reaction cell. (After a few tests this cell will become slightly frosted with antimony oxychloride; immediately clean the cell with 4 *N* hydrochloric acid, rinse with distilled water, and dry with acetone). Place the cell in the vacant holder in the carriage and move it across into the light path. Fit the funnel into the cell mouth. Allow time for the galvanometer to re-settle. Put 3·5 ml. of antimony trichloride solution and two drops of acetic anhydride into a test tube, then pour this solution into the funnel with a rapid smooth motion, and quickly and carefully (to prevent vibration) close the lid of the carriage. Note the point of minimum transmission on the galvanometer. If this reading is less than 35 or more than 50 on the transmission scale (*i.e.*, outside the limits 0·456–0·301 on the log, or optical density scale) the test must be repeated with an appropriately greater or smaller aliquot of the solution of unsaponifiable matter so that the reading does fall within these limits.

Repeat the test twice on each sample, checking the zero and maximum transmission points on the galvanometer each time. The three readings for each sample should be very close (see Table I). In the case of the fat blend, and in contrast to the vitaminised margarine, it will be observed that the galvanometer spot remains steady at its deflection point and does not almost immediately begin to move back. This is because the absorption is not transient as is that of the vitamin-A reaction mixture.

Finally, test the vitamin concentrate directly, *i.e.*, weigh out 1–2 g. and dilute with chloroform to such an extent that an aliquot of less than 0·5 ml. gives a test reading within the limits prescribed above.

Interpretation of Readings—The calculation of results proceeds as follows:

Let D = reading on the optical density scale of the galvanometer.
C = percentage concentration (of margarine, or fat blend, or vitamin concentrate) in the chloroform test solution.
V = volume of test solution put in the ($\frac{1}{2}$ cm.) reaction cell.
R = volume of reagent plus acetic anhydride added to the test solution in the cell.
P = given potency of vitamin concentrate in I.U./g.

$$\text{The "effective" } E^{1\%}_{1\,\text{cm.}}\ 620\ \text{m}\mu = \frac{2D(V + R)}{CV}.$$

The term "effective" is used because the light transmission actually recorded includes rather more than that at 620 mμ—a certain quantity on either side, depending upon the quality of the filter. It should be noted that the use of a filter instrument of this type prohibits the direct translation of absorption data to I.U./g. by means of standard conversion factors.

If we abbreviate "effective" $E^{1\%}_{1\,\text{cm.}}$ 620 mμ as E, the required result can be written as

$$\text{Potency of margarine in I.U./g.} = P \times \frac{E(\text{marg.}) - E\ (\text{fat blend})}{E(\text{conc.})}.$$

Example:

		An experimental margarine		
		Marg.	Fat blend	Conc.
Weight of sample taken		15·04 g.	20·26 g.	1·261 g.
Diluted to		3 ml.	3 ml.	25 ml.
Therefore, concentration	= C =	501·3%	675·4%	5·044%
Volume of test solution in cell	= V =	0·3 ml.	0·9 ml.	0·4 ml.
Volume of reagent and anhydride in cell	= R =	3·56 ml.	3·56 ml.	3·56 ml.
Galvanometer reading (optical density)	= D =	0·400	0·385	0·455
Therefore "E" = 2D(V + R)/CV ..	=	0·02053	0·00565	1·786

The given potency of the concentrate was 1560 I.U./g.* The potency of the margarine is therefore calculated as

$$1560 \times \frac{0{\cdot}02053 - 0{\cdot}00565}{1{\cdot}786} = 13{\cdot}0 \text{ I.U./g.}$$

The convenience of the optical density scale will be apparent in these calculations; the density, which is the log of the ratio of the intensities of the incident and transmitted light, is directly proportional to concentration and to cell thickness, and density readings are therefore simpler to handle than the transmission percentages given on the ordinary galvanometer scale. There are nevertheless two limitations to the use of either scale. First, the error of reading has a minimum effect on the error of estimation when the transmission is 36·8%, corresponding to an optical density of 0·4343; this can easily be derived from first principles—see, for instance, Twyman and Lothian.[10] On either side of this position the influence of the reading error increases. Second, it is a characteristic of the reaction mixture that its optical density is not strictly proportional to the concentration *in the cell*, so that unless cell concentration is always confined within certain fairly narrow limits results will not be comparable. These two considerations have led us to define the working limits given above, *viz.*, 35% to 50% transmission, 0·456 to 0·301 optical density.

Accuracy Attainable—In order to assess the reliability of the method, several series of margarine samples, some from experimental batches and some from ordinary production, were tested. Three preparations of unsaponifiable matter were made from each sample and three or more photometer tests were carried out on each preparation. A typical set of results on one sample is shown in Table I.

TABLE I

REPLICATION OF ESTIMATES OF I.U./G. IN A SAMPLE OF MARGARINE

Each result is calculated from a single reading

Unsap. prep. No.	1	2	3
1st test	21·4	22·3	21·8
2nd ,, ...	21·4	22·0	21·4
3rd ,, ...	21·8	22·3	21·9
4th ,, ...	21·4	22·7	22·0
Means	21·5	22·3	21·8

A typical set of results on one series of samples is given in Table II. These samples were from batches of margarine made in the laboratory under carefully controlled conditions. The figures tabulated are the means of three photometer tests on each preparation of unsaponifiable matter.

From all these experiments it has been possible to estimate the accuracy of individual photometer tests, one test being defined for this purpose as the whole process from the taking of an aliquot of the solution of the unsaponifiable matter to the recording of the galvanometer reading. At the same time an indirect estimate of the accuracy of the method of extraction of the unsaponifiable matter was made. Statistical analysis revealed that the error of one test, defined as above, is represented by ±2·3% for P = 0·95, while that of preparation of unsaponifiable matter is ±4·5% for P = 0·95. It follows that the error of one reading on one preparation of unsaponifiable matter is ±5·05% (the square root of the sum of the squares of the two component errors) for P = 0·95. This means that only once in 20 such estimates

* The limiting factor in this method of arriving at the potency of the margarine is clearly the reliability of the ascribed potency of the concentrate. If an ultra-violet spectrophotometer is available, concentrates can of course be checked for their $E^{1\%}_{1\,cm.}$ 325 mμ values, on which unitages are based. If not, it is advisable to calibrate the galvanometer scale with concentrates of warranted potency, as described in the latter part of this paper.

should we be more than 5% away from truth. Normally of course more than one reading is taken and, preferably, more than one preparation of unsaponifiable matter is tested.

TABLE II

REPLICATION OF ESTIMATES OF I.U./G. IN FOUR SAMPLES OF LABORATORY-MADE MARGARINE

Each estimate is derived from the mean of three photometer tests

Marg. No.	Vitamin A added, I.U./g.	Vitamin A found, I.U./g.			
		Unsap. 1	Unsap. 2	Unsap. 3	Mean
I	10	9·9	9·2	8·9	9·3
II	15	14·8	14·7	14·1	14·5
III	20	19·0	19·2	18·8	19·0
IV	25	25·9	25·6	25·3	25·6

The influence of the two kinds of replication on the error of the mean result is shown in Table III.

TABLE III

INFLUENCE OF THE NUMBER OF PREPARATIONS OF UNSAPONIFIABLE MATTER, AND THE NUMBER OF TESTS ON EACH, ON THE P, 0·95 PERCENTAGE ERROR OF THE MEAN RESULT

No. of unsaps.:	1	2	3	5	10	∞
No. of tests on each unsap.						
1	5·05	3·57	2·92	2·26	1·60	0
2	4·78	3·38	2·76	2·14	1·51	0
3	4·69	3·32	2·71	2·10	1·48	0
10	4·56	3·22	2·63	2·04	1·44	0
∞	4·50	3·18	2·60	2·01	1·42	0

It is suggested that 3 tests on each of 2 preparations of unsaponifiable matter, giving an error of about $\pm 3{\cdot}3\%$ for $P = 0{\cdot}95$ should be made standard practice. The accuracy of the final result is also dependent on the error of the observations on the fat blend and the concentrate, both of which are less susceptible to error than is the actual margarine test, and the former of which makes only a small contribution to the final error. If the margarine test is replicated as suggested above, if three readings are taken on one preparation of fat blend unsaponifiable matter, and if three readings are taken on the concentrate, the error of the final result should be of the order of $\pm 5\%$, with $\pm 6\%$ the maximum range.

METHOD OF TEST WHEN MARGARINE ALONE IS AVAILABLE

If $E^{1\%}_{1\,cm.}$ 620 mμ is estimated from the antimony trichloride reaction with the unsaponifiable fraction of a margarine and no further information is available, the question arises, how much of the absorption can be attributed to vitamin A? Experience has shown that constituents other than vitamin A may contribute anything up to, in exceptional cases, 50% of the total E value. Fortunately the absorption due to the vitamin-A reaction product has a peculiar characteristic that can be used to distinguish it quantitatively from the irrelevant, non-vitamin-A absorption. The characteristic is that the absorption is transient; it declines, rapidly at first and more slowly later, eventually reaching a minimum that is almost invariably 10% of its initial, maximum, value. The fading period is about 20–30 minutes. The difference between the "gross" initial absorption and the minimum absorption is known as the "nett" absorption; thus for pure vitamin A and high-potency concentrates the nett value can be taken as 90% of the gross value. But for low-potency materials in which there is a significant non-vitamin-A contribution to the absorption at 620 mμ the value is a little less than 90%, for two reasons: (*a*) the recorded gross value is not the simple sum of the values for the vitamin-A and non-vitamin-A fractions; there is a slight inhibition of total colour development when the two fractions conjoin, and (*b*) the non-vitamin-A contribution increases slightly during the fading period. The accompanying diagram (Fig. 2) illustrates these points.

The above applies to the absorption at 620 mμ only, *i.e.*, as estimated on a spectrophotometer. When a light-filter instrument is employed the recorded absorption embraces a small region of the spectrum maximised at 620 mμ. The correction factors will therefore not necessarily be the same, and each instrument must be calibrated. For this purpose three or four vitamin-A concentrates of the type used in margarine are required. The potency of each, in I.U./g., based on $E^{1\%}_{1\,cm.}$ 325 mμ and a conversion factor of 1600, must be known.

Because the "fading" test necessitates the taking of galvanometer readings well outside the recommended limits, it is advisable to use the concentrates to calibrate the whole scale—or at least between the 30% and 90% transmission points. (Incidentally, as a calibration curve has to be used for the interpretation of readings in this type of test the straight transmission scale only need be used.) At the same time the nett : gross ratio (0·9 : 1·0 for pure vitamin A and E 620 mμ only) for the instrument can be ascertained.

A weighed amount of each concentrate should be diluted in two stages to the strength required to produce a galvanometer reading of about 30, and the exact reading noted. Leave the cell in position,—preferably with a small glass cover to prevent evaporation losses—for

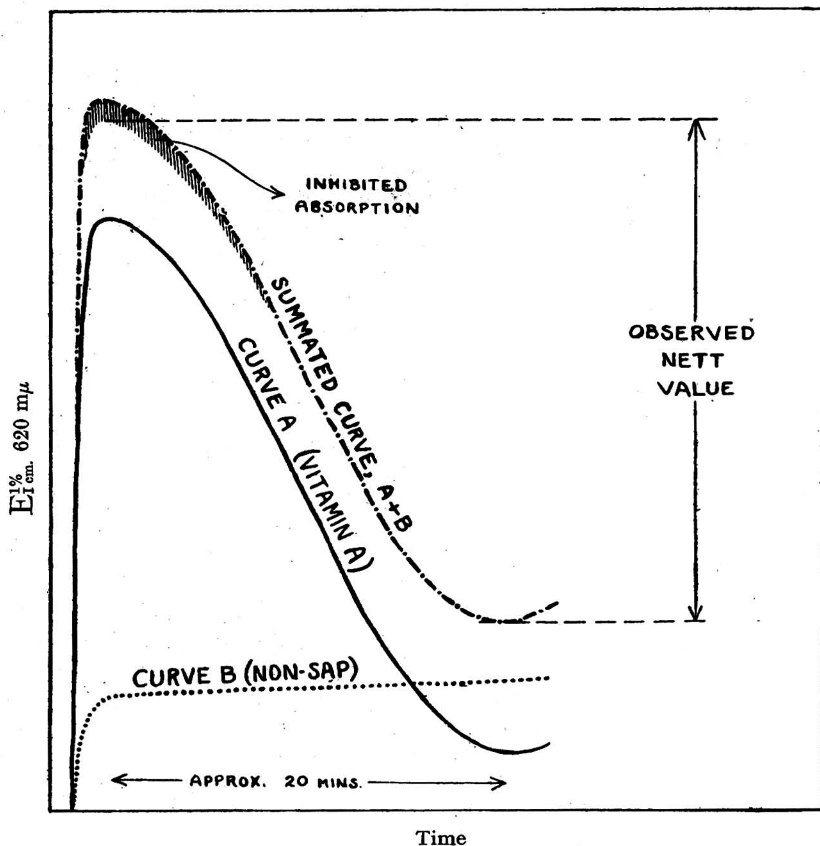

Fig. 2. Typical course of absorption of $SbCl_3$ reaction on the unsap. of an A-vitaminised margarine.

about half-an-hour, during which time the transmissibility will rise to about 80, *i.e.*, the colour and absorption of the solution fade to a minimum at this point. Then make up a series of weaker solutions (from the original first-stage dilutions)—say 80%, 60%, 40% and 10% of the strengths that gave readings of about 30. Only the initial, gross, readings need be recorded for these. From all the gross readings a mean calibration curve may now be constructed relating gross readings to I.U./ml. of cell solution. A curve such as that in Fig. 3 will be obtained.

This curve may now be used to check the value of the nett figures, obtained by fading tests on the concentrates, in terms of the gross figures; as already indicated they should be approximately 90%. Let us suppose that a mean value, for all the concentrates tested, of 89% is obtained. This value can then be applied to the "fading" test on a margarine as shown in the following example:

The unsaponifiable matter from 15 g. of margarine was dissolved to 3 ml. in chloroform. A reaction cell containing 0·2 ml. of this solution plus 3·5 ml. of reagent

plus 0·06 ml. (2 drops) of acetic anhydride, gave a galvanometer reading of 42. On fading, the furthest point on the scale that the light spot reached was 76·5. From the graph (Fig. 3) these values were read off:

Transmission	Equivalent I.U. per ml. in cell
42	6·56
76·5	1·70
	4·86

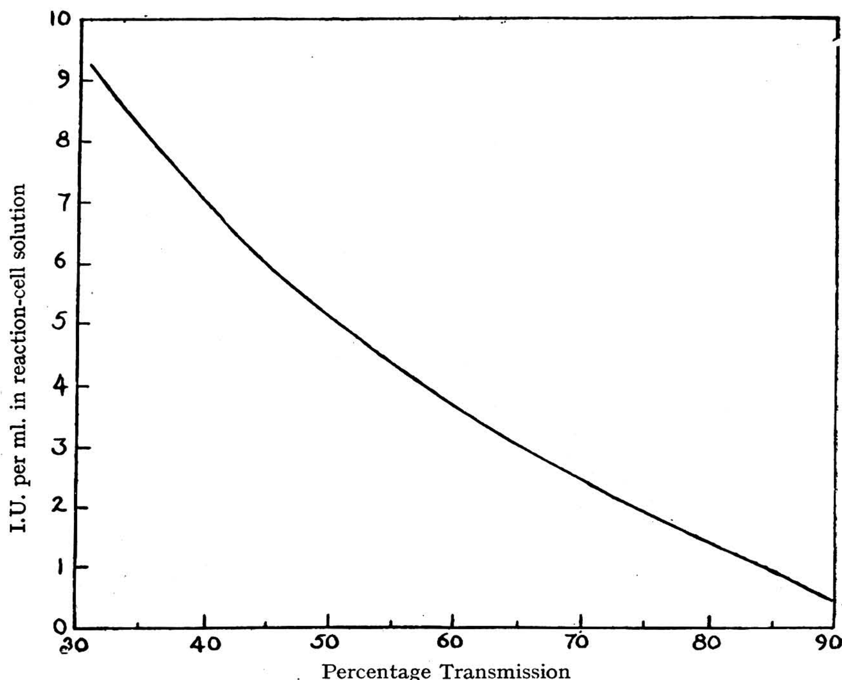

Fig. 3. Relation between galvanometer readings (% light transmission) and strength of Vitamin A in reaction cell.

This difference, 4·86 I.U. per ml. of cell solution is clearly the "nett" which, as already indicated, corresponds to a true, vitamin-A-only, gross value of 4·86/0·89 I.U. per ml. in the cell. Now the cell contained altogether 3·76 ml., of which 0·2 ml. was an aliquot of the 3 ml. solution of the unsaponifiable matter from 15 g. of margarine. Therefore the required potency of the margarine is

$$\frac{4{\cdot}86 \times 3{\cdot}76 \times 3}{0{\cdot}89 \times 0{\cdot}2 \times 15} = 20{\cdot}5 \text{ I.U./g.}$$

It should be noted that we have made no allowance for the slight over-rating of the nett/gross factor (here 0·89) due to inhibition, etc., the reason being that we cannot be confident of its precise value in any particular margarine. By ignoring it, however, we are unlikely to underestimate by more than 5%, a figure that is within the experimental error of the method.

Summary

Vitamin-A potency is normally expressed on the basis of $E^{1\%}_{1\,cm.}$ 325 mμ, but this value cannot be successfully determined on margarine. The best alternative is to measure the absorption at 620 mμ of the antimony trichloride - vitamin A complex, and an improved type of photoelectric photometer has been desigued for this purpose. The margarine is tested *via* the unsaponifiable matter and the instrument is calibrated with margarine-type vitamin-A concentrates.

The principal case considered is that in which control samples of the particular concentrate and the unvitaminised fat blend are available. The other case is when the margarine sample alone is available, and is met by the "fading technique," *i.e.*, advantage is taken of the fact that of the gross registered absorption at 620 mμ, the fraction due to vitamin A declines by about 90% over a period of approximately 20–30 minutes.

The authors wish to express thanks to Dr. J. R. Edisbury, Mr. R. J. Taylor and Mr. A. L. Wilkie, of these laboratories, for their help; to Professor R. A. Morton and Mr. T. W. Goodwin, of the University of Liverpool, for much useful discussion; and to the directors of Lever Brothers & Unilever Limited for permission to publish this paper.

References

1. Edisbury, J. R., Analyst, 1940, **65**, 484.
2. Oser, B. L., Melnick, D., and Pader, M., *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 724.
3. Neal, R. H., and Luckman, F. H., *Ibid.*, 1944, **16**, 358.
4. Wilkie, J. B., and De Witt, J. B., *J.A.O.A.C.*, 1945, **28**, 174.
5. Evelyn, K. A., *J. Biol. Chem.*, 1936, **115**, 63.
6. Dann, W. J., and Evelyn, K. A., *Biochem. J.*, 1938, **32**, 1008.
7. Yudkin, S., *Ibid.*, 1941, **35**, 551.
8. Hoch, H., *Ibid.*, 1943, **37**, 425.
9. Benham, G. H., *Canad. J. Res.*, 1944, **22**, 21.
10. Twyman, F., and Lothian, G. F., *Proc. Phys. Soc.* (Lond.), 1933, **46**, 643.

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Discussion on the Preceding Two Papers

Mr. J. I. M. Jones said that the rationale of the conversion factor merited careful consideration. The margarine manufacturer based his calculation of the amount of vitamin concentrate to be added to his margarine on a potency in international units given by the supplier of the vitamin concentrate. This potency was determined from $E^{1\%}_{1\,cm.}$ 328 mμ on the whole concentrate. When the margarine was analysed it was subjected to saponification and the Carr–Price reaction was used for estimating the vitamin A from $E^{1\%}_{1\,cm.}$ 617 mμ. It was therefore necessary to relate the result obtained by the latter process to that obtained by the former. This had been done by testing the vitamin mixture as added to the fat blend by both methods. The factor necessary to convert the $E^{1\%}_{1\,cm.}$ 617mμ values to yield the same value in international units as those given by $E^{1\%}_{1\,cm.}$ 328mμ $\times$ 1600 was then found to vary between 560 and 590 on different concentrates and to average about 565, and gave values of approximately 18 I.U. of vitamin A per gram of fresh margarine in samples examined in his laboratory. A considerable amount of work had been done on loss in the analytical procedure, which it was hoped to publish in due course.

Mr. Jones enquired what steps had been taken in the instrument shown to ensure stability of the light source and absence of drift in the galvanometer.

Dr. H. Wilkinson said he would like to agree with the remarks made by Mr. Jones concerning conversion factors. At Port Sunlight they had found, after examining a large number of margarine concentrates, that the average E-620/E-325 ratio was 2·85. Professor Morton had used the ratio 3·1 which obtains for pure vitamin A in calculating his 510 conversion factor for the gross and nett E-620 value. If one took the ratio 2·85 and used the conversion factor 1600 for the E-325 value, which is standard commercial practice, then one obtained a conversion factor 570 for the gross E-620, which was very close to the 565 quoted by Mr. Jones. They had found in their laboratories that for the nett E-620 value determined on the unsaponifiable matter of margarine a factor of 680 gave the best results. This value was attained by consideration of the fact that pure vitamin A gives a nett figure only 89% of the gross E-620 value, and also by making an allowance of 7½% for inhibition and contribution; the 7½% value was at present *sub judice* as they have not sufficient results available at the present time to consider it absolute. More work was at the present moment in progress.

One other point he would like to mention concerned the method of chromatographic separation of carotene and vitamin A on de-fatted bone meal. When Mr. Mann published his method they used it on the unsaponifiable matter from margarine and found that the dye associated itself with carotene, and they were unable to obtain quantitative elution of the vitamin A. It should be noted that these results refer only to tests with the unsaponifiable matter. He wondered whether Professor Morton had any details which he could give on the matter, although he (Dr. Wilkinson) felt sure that Professor Morton would not recommend the chromatographic separation as regular routine but would prefer to determine the vitamin A on a separate portion of the unsaponifiable matter.

Lastly, he would like to take this opportunity of publicly thanking Professor Morton and Mr. Goodwin, on behalf of his colleagues and himself, for the help they had given them in designing the instrument which his colleague had just described.

Dr. H. E. Cox asked whether much destruction of vitamin A could occur during the spectrographic observations owing to the presence of ultra-violet light.

Mr. J. V. Smart enquired if the authors had had any experience of a new reagent for vitamin A, glycerol-1·3-dichlorohydrin (*cf.* this Vol., p. 28), which was said to give a purple colour much more stable than the colour given by antimony trichloride.

Mr. J. H. High asked whether the Spekker absorptiometer could be used. Rapidity of setting and reading, elimination of trouble from fluctuations in the light source, and ability to allow for "drift" (Analyst, 1943, **68**, 78) were its chief advantages. Its chief disadvantage was that of cost.

Mr. R. A. C. Isbell said there was no fundamental reason why the Spekker absorptiometer should not be used for vitamin A or carotene determinations with suitable filters. Hilgers did not specifically recommend it for this purpose as it seemed unnecessarily expensive for an instrument for a limited use. They had therefore developed a simplified version of the "Spekker," which is a single beam type similar in some respects to the instrument which had been described. The light source is a 6 volt, 18 watt headlamp energised from a high capacity accumulator, or a stabilising transformer. By carefully selecting filters it has been found possible to make a combination with sufficient transmission to enable a Unipivot type needle galvanometer to be used. Instead of an iris diaphragm they preferred to use a potentiometer form of control for setting the full scale deflection of the galvanometer, as this has been found to give smoother variation. With regard to the drift of the photocell mentioned by an earlier speaker, it is possible to select photocells with minimum fatigue. The type of photocell used may have either positive or negative drift, and it follows that cells can be found in which this effect is very slight. They had for some years selected such cells for special purposes.

Mr. K. A. Williams remarked that the instrument demonstrated by Mr. Gridgeman and that mentioned by Mr. Isbell were almost exactly the same as the one described to the Society ten years before by E. R. Bolton and himself (Analyst, 1935, **60**, 447). He had used that instrument very successfully for blue value determinations for some years, and could confirm that it possessed great advantages over the visual method, not only in speed of operation, but also by making it possible to work near to the head of the absorption band of the test solution. In the early days of the use of photoelectric cells in this way some doubt had arisen as to the validity of results owing to the limitations imposed on selecting the operating wavelength through the use of dyed gelatin filters. He would be interested to hear whether it had proved possible for the authors to narrow the spectral range of their filter more than had earlier been possible.

He noted that the vitamin A content of a margarine showed an initial falling off which might well be attributed to effects of the manufacturing process; this was followed by a comparatively long period in which there was little change, and this period seemed to correspond to the known long period in which edible fats remained sweet; it was probably the result of the action of the natural or added inhibitors present. Finally there appeared a period in which the vitamin content dropped quickly, and he thought that this would be found to correspond to the final deterioration of the fat and onset of rancidity. The industry could congratulate itself that, during the difficult time of the war, fats had proved as well refined as in peace time, and to have very similar keeping properties Where they had shown themselves to keep a little less well, it was probably due to the great changes that had taken place in transport and storage; this was perhaps the price that had to be paid for progress in such matters.

Mr. J. G. Lunt noted that Mr. Goodwin had suggested the possibility of selecting the constituent oils so as to achieve good keeping quality, and would like to ask on what basis such selection should be made. His firm were continually selecting new oils and having trouble with their suppliers on that account.

Mr. A. E. Parkes enquired if any special precautions had to be observed in the saponification process, and why acetic anhydride was used with the antimony trichloride reagent.

Professor Morton, replying to Dr. Cox, said that the risk of destroying vitamin A photochemically during the spectrographic examination was not serious. If the potency is approximately determined first (*e.g.*, by means of the colour test), the optimum concentration and cell thickness for the ultra-violet test can be foreseen sufficiently closely to limit the period of exposure to ultra-violet light to a short time. The proposed new test referred to by Mr. Smart had not been tried yet at the Liverpool laboratory.

The question of light filters arose frequently in discussions concerning photoelectric colorimetry. The interference filters associated with Geffcken transmitted very narrow strips of the spectrum and it was hoped that before long such filters would become more easily available.

Mr. Goodwin, in reply to Mr. Parkes, said that acetic anhydride (2 or 3 drops in 4 ml.) is used in the test solutions to remove any water which might make the solutions turbid. Even a slight cloudiness introduces errors.

The Composition of Commercial Methylene Blue

By C. M. MARTIN, J. W. G. NEUHAUS and F. H. REUTER

In the course of an investigation involving the use of methylene blue, differences in the appearance of different samples of this compound were observed. Eight samples in hand (Table I and II) could readily be divided into two groups, one of golden-green crystals and the other of reddish-brown crystals; another sample (No. 9) was different from the others in appearance and purplish in colour.

Except for the Merck Index[3] there appears to be no published information as to the identity and composition of commercial grades, nor do the labels of the respective samples disclose the composition. Accordingly it was decided to examine these samples to determine whether the appearance of the crystals indicated any fundamental difference in composition, and also what degree of uniformity is to be expected among commercial methylene blues.

The samples concerned were analysed for methylene blue by the method of Ferrey,[2] matter volatile at 110° C., ash (by incineration at 550° C.) and zinc. The results of these

determinations are given in Tables I and II, with similar data for a specimen of pure methylene blue prepared by the authors by the usual chemical method.

TABLE I

GOLDEN-GREEN CRYSTALS(*a*)

Sample No.	Designated on label	$C_{16}H_{18}N_3SCl$ %	Volatile at 110° %	Ash(*b*) %	Total %
1	B.D.H. Standard stain	69·6	17·7	9·1(*c*)	96·4
2	Methylene blue I.C.I. (commercial)*	74·6	17·1	4·6(*d*)	96·3
3	Methylene blue, chlorzinkfrei, chemischrein, Merck	77·7	18·8	0·7	97·2
4	Methylene blue B extra, Merck	77·9	18·6	1·2	97·7
5	Methylene blue, G. T. Gurr	78·8	16·4	2·0	97·2
10	Sample 1 after recrystallisation(*e*)	87·5	9·5	0·0	97·0
11	Pure methylene blue prepared by the authors(*e*)	85·7	11·7	0·0	97·4
	Calculated from the trihydrate $C_{16}H_{18}N_3SCl,3H_2O$	85·5	14·5	—	100·0

(*a*) All samples showed freedom from zinc.
(*b*) Ignited at 550° C. to constant weight in electric muffle furnace.
(*c*) Ash coloured red, due to Fe_2O_3.
(*d*) Ash coloured grey.
(*e*) Recrystallised from 6 parts of ethanol (5% methanol).

* Obtained from H. B. Selby & Co. Pty. Ltd., Sydney.

TABLE II

REDDISH-BROWN CRYSTALS(*a*)

Sample No.	Designated on label	$C_{16}H_{18}N_3SCl$ %	Volatile at 110° %	Ash(*b*) %	$ZnCl_2$(*c*) %	Total %
6	Aniline blue, methylene blue B, Merck	57·8	4·0	5·6	14·2	80·0
7	Methylene blue B B, Merck	56·9	3·8	7·2	14·7	75·4
8	Methylene blue B B, Merck	56·6	4·3	5·8	14·7	75·6
9(*a*)	Methylenblau, Grubler	65·9	9·4	10·2	12·9	88·2
12	A commercial zinc chloride double salt recrystallised(*d*) by the authors ..	77·9	2·3	7·9	20·4	100·6
	Calculated for $(C_{16}H_{18}N_3SCl)_2,ZnCl_2$ ..	82·45	—	—	17·55	100·0
	Calculated for $(C_{16}H_{18}N_3SCl)_2ZnCl_2,H_2O$	80·55	2·27	—	17·18	100·0

(*a*) Sample No. 9 was purplish in appearance.
(*b*) Ash estimations at 550° C. were of no significance in this instance as ZnO was tenaciously retained; however, they served to show that the deficiency cannot be accounted for in terms of mineral impurities.
(*c*) Zinc determined after wet oxidation with sulphuric and nitric acids by the A.O.A.C. method.[4]
(*d*) 200 ml. of 10 *N* HCl were added to the solution of 25 g. of zinc chloride double salt in one litre of hot water; yield on cooling 16·8 g. (67% recovery) of material after drying to constant weight at 65° C.

From these two tables it is evident that the commercial samples tested varied considerably in composition, and that the samples showing reddish brown or purplish colour contained zinc, while the golden green specimens were zinc-free. For the latter group it was demonstrated that the pure ash-free hydrochloride could readily be prepared by recrystallisation from ethanol (5% methanol). The varying amounts of water found in the commercial samples confirm the observation of Wales and Nelson[5] that methylene blue does not form a definite hydrate, that is, the water present is not bound as water of crystallisation.

No attempt was made to account for the discrepancy occurring in the totals of percentages of the components of the methylene blue samples in Table II or to identify the missing portion. As will be seen in Table III, the zinc chloride contents of the samples are not in accordance with the commonly stated ratio of 2 mols. of $C_{16}H_{18}N_3SCl$ to 1 mol. of zinc chloride. No explanation is offered for this discrepancy, which was met both in the commercial samples of the zinc chloride double salt and in the one purified by the authors.

Originally it was intended to employ the method of Bolliger[1] for the methylene blue estimations, but when in 1944 Ferrey[2] published his procedure the Bolliger method, although eminently satisfactory for very dilute solutions, was abandoned on account of its tediousness and the difficult end point experienced with the more concentrated solutions encountered in the present work. That the Bolliger and Ferrey methods yield comparable results is demonstrated in Table IV

As a further check on the Ferrey Method a comparison was made with the methylene blue content calculated from the nitrogen determined by the Kjeldahl method. This was done on sample No. 12, and gave 78·2% of $C_{16}H_{18}N_3SCl$ as compared with 77·9% by Ferrey's method.

TABLE III

Sample No.	$C_{16}H_{18}N_3SCl$ %	$ZnCl_2$ Found %	$ZnCl_2$ Calculated for $(C_{16}H_{18}N_3SCl)_2.ZnCl_2$ %
6	57·8	14·2	12·30
7	56·9	14·7	12·05
8	56·6	14·7	12·12
9	65·9	12·9	14·03
12	77·9	20·4	16·60

TABLE IV

COMPARISON OF THE BOLLIGER METHOD WITH THE FERREY METHOD

Sample No.	Bolliger method %	Ferrey method %
1	71·66	69·56
2	58·25	57·75
11	87·17	85·71

SUMMARY—Differences in appearance of samples of commercial methylene blue have been shown to be due to the presence or absence of $ZnCl_2$. Commercial zinc-free methylene blue is conveniently purified by recrystallisation from denatured ethanol. The view that methylene blue does not form a definite hydrate has been confirmed. Commercial zinc chloride double salts do not appear to conform to the ratio of 2 mols. of methylene blue to 1 mol. of zinc chloride. The method for estimating methylene blue recently introduced by Ferrey has been found to be very satisfactory.

REFERENCES

1. Bolliger, A., *J. Royal Soc. N.S.W.*, 1933, **67**, 240; ANALYST, 1939, **64**, 416.
2. Ferrey, J. W., ANALYST, 1944, **69**, 54; *Quart. J. Pharm.*, 1943, **16**, 208–212.
3. Merck's Index, 1930, pp. 337–38.
4. "*Methods of Analysis*," A.O.A.C., 5th Ed., 1939, p. 104.
5. Wales, H., and Nelson, O. A., *J. Amer. Chem. Soc.*, 1923, **45**, 1659.

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The Separation and Determination of Lead as Iodate and its Application to Glass Analysis

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

INTRODUCTION—The analysis of lead nitrate or acetate solutions by precipitation of the iodate has previously been investigated.[1,2,3] The methods have usually consisted in addition of an excess of potassium iodate and estimation of the excess in the supernatant liquid. The accuracy obtained was not good, and the use of precipitation from neutral or slightly acid solution was of no great value for separation. Amperometric,[4] conductometric and potentiometric[5] titrations under similar conditions have also been published. Attempts to filter and wash lead iodate precipitates were apparently subject to solubility errors.[2]

The present investigation was undertaken in order to provide a rapid routine method for the analysis of glasses containing approximately SiO_2 50%, PbO 30%, Al_2O_3 1%, CaO 2%, K_2O 14%, Na_2O 2%, and traces of the oxides of iron, manganese and antimony. The chief objection to the standard methods for the analysis of such glasses is the time involved in the separation of lead as sulphide and its subsequent determination as lead sulphate. By precipitating and determining lead as the iodate, the time of analysis has been very considerably reduced.

The following methods were the result of the present investigation and they were subsequently adapted to glasses containing small percentages of barium oxide.

PROCEDURE—(*a*) *Main Analysis*—Weigh 1·0–1·1 g. of the crushed sample into a platinum crucible. Add 3 ml. of water, 12 ml. of hydrofluoric acid (40%) and 5 ml. of perchloric acid (70%), and evaporate to moist dryness in a radiator. Extract with a hot mixture of 2·0 ml. of nitric acid (sp.gr. 1·42) and 20 ml. of water. Filter through a small Whatman 540 paper into a 400 ml. beaker. Wash the precipitate of antimony oxide with 50 ml. of hot 4% v/v nitric acid. Dilute the filtrate to 125 ml., heat to boiling and add slowly, with constant stirring, 25 ml. of 6% iodic acid solution. Stir for 2 minutes and keep at 60–70° C. for 30 minutes. Cool to room temperature, filter the lead iodate through a weighed sintered glass crucible (porosity 3) and wash with 75 ml. of 0·2% iodic acid in 1% v/v nitric acid. Finally wash three times with 2 ml. portions of cold water and then twice with acetone, rejecting the acetone washings. Suck dry, and weigh as $Pb(IO_3)_2$ after drying for one hour at 140° C.

To the filtrate add 5 ml. of nitric acid (sp.gr. 1·42), 0·2–0·4 ml. of hydrochloric acid and 4 ml. of 40% formaldehyde. Boil vigorously until all the iodine is volatised; add a further 5 ml. of nitric acid and boil until the formaldehyde is destroyed. Dilute to 250 ml. in a standard flask.

Use a 150 ml. aliquot for the separation of aluminium with ammonia and weigh as Al_2O_3. In the filtrate precipitate calcium with ammonium oxalate from acid solution[6] and neutralise to methyl red. Filter and wash the precipitate with 0·1% ammonium oxalate solution and finally with water. Estimate the calcium volumetrically after solution in hot 7% perchloric acid.

Determine sodium on a 20 ml. aliquot, using 20 ml. of zinc uranyl acetate reagent.[7,8]

Determine potassium on a 50 ml. aliquot, by evaporating to fumes with 1 ml. of perchloric acid (70%) and extracting once with ethyl acetate.[9]

In presence of barium follow the above procedure for the precipitation of lead iodate and digest for 30 mins. as before, but filter hot and wash with the same wash liquor at 60–70° C. Evaporate the filtrate twice with hydrochloric acid to remove iodic and nitric acids before proceeding with the remainder of the analysis. Estimate the barium as sulphate on a suitable aliquot.

(*b*) *Determination of Silica*—This is made on a 0·5 g. sample by fusion with sodium carbonate and dehydration with perchloric acid.[8] Add 3% hydrogen peroxide just prior to the filtration in order to dissolve any precipitated manganese dioxide.

(*c*) *Volumetric Estimation of Lead Iodate*—Separate the lead iodate as above, omitting the acetone washing. Dissolve the precipitate in hot 10% sodium hydroxide solution, washing out the filter crucible with hot 2% sodium hydroxide solution. Cool the solution and add 3–4 g. of potassium iodide. Dilute to 150 ml., acidify with a marked excess of hydrochloric acid (sp.gr. 1·18) and titrate with a standard solution of sodium thiosulphate. If necessary, use an aliquot of the sodium hydroxide solution.

RESULTS—The results obtained on the determination of lead as iodate from pure solutions of lead nitrate and from solutions containing calcium and barium nitrates are set out in Table I. The conditions used are those described in the procedure given above.

TABLE I

Excess iodic acid g./100 ml.	Other elements present g.	Lead iodate expected g.	Lead iodate obtained g.	Lead in filtrate mg.
0·5	—	0·1976	0·1976	—
0·75	—	0·4939	0·4939	—
1·0	—	0·1976	0·1975	—
0·5	1·6 g. $Ca(NO_3)_2$	0·1976	0·1972	0·2
1·0	1·6 g. $Ca(NO_3)_2$	0·1976	0·1985	—
2·0	1·6 g. $Ca(NO_3)_2$	0·1976	0·1991	—
0·5	3·3 g. $Ca(NO_3)_2$	0·1976	0·1971	0·4
0·5	4·9 g. $Ca(NO_3)_2$	0·1976	0·1968	0·7
0·75	0·05 g. BaO	0·7744	0·7745*	<0·1

* Average of ten results. Average deviation ±0·0005 g. In the separation of lead from barium three of the filtrates were analysed for barium; the weights of barium sulphate obtained were 0·0817, 0·0816 and 0·0818 g.; calculated 0·0817 g.

Some of the results obtained on lead glass samples are set out in Table II. The first column shows the results obtained by separating the lead as sulphide and estimating it as sulphate, whilst the second column shows those obtained by the procedure described.

TABLE II

PbO %		
As sulphate	As iodate	Remarks
28·1	28·10	—
27·9	28·10	
32·8	32·64	—
	32·57	
32·9	32·97	—
28·50*	28·50	The results by the iodate method are the extremes of 7 determinations
	28·58	
28·50*	28·50	The equivalent of 2% of barium oxide was added to the glass.
	28·33	
	28·51	
	28·41	

* Results obtained by independent referee analyst

DISCUSSION—The apparent stability of cold supersaturated lead iodate solutions previously reported[4] has been confirmed, and incomplete precipitation of lead iodate from cold solutions, after twenty hours' standing, has been found. However, by the slow addition of iodic acid to a lead solution and digestion of the hot solution, quantitative precipitation of lead iodate in easily filterable form is obtained. The conditions finally selected, employing 3 ml. of nitric acid (sp.gr. 1·42) and an excess of 0·75 g. of iodic acid per 100 ml. of solution represent the maximum acidity in which lead can be quantitatively precipitated with a reasonable excess of iodic acid.

The effect of introducing large amounts of other ions into the solution is shown by the traces of lead found in the filtrates from the lead - calcium separations. Presumably, activity effects have increased the solubility of lead iodate.

The determination of lead as iodate is subject to interference from titanium, zirconium, thorium, silver, mercury and bismuth. Ferric iron precipitates with the lead, small amounts colouring the precipitate yellow and larger amounts affecting the nature of the precipitate. However, the traces of iron in lead glasses are too insignificant to interfere with the lead determination.

The results cited have shown that calcium and barium do not interfere with the lead determination, provided that they are present in the amounts normally encountered in glass analysis. Subsidiary experiments have shown that moderate amounts of aluminium, manganese, zinc, cadmium, strontium, magnesium, sodium and potassium do not interfere. The purity of the precipitate of lead iodate obtained in actual glass analyses has been confirmed by spectrographic examination.

In the volumetric estimation of the lead, preliminary solution of the lead iodate in sodium hydroxide was adopted, as a protective layer of lead iodide formed when an attempt was made to dissolve the precipitate directly in a solution containing hydrochloric acid and potassium iodide. Many other methods of titrating iodates are available, and could no doubt be used.

Several methods were investigated for the removal of iodic acid. Reduction with formaldehyde in presence of a small amount of hydrochloric acid was found to be very rapid, and had the advantage of not introducing any interfering compounds. The best alternative method of eliminating iodic acid is by evaporation with hydrochloric acid.

The remaining methods of analysis are well known, the conditions outlined being suitable for glasses having the composition quoted.

SUMMARY—A simple rapid scheme for the analysis of lead glasses is given, employing the separation and weighing of the lead as iodate. The results indicate that the iodate method is a valuable alternative to the sulphide - sulphate method for determining lead.

We are indebted to Mr. A. E. Ross for carrying out the experimental work on lead - barium separations. We wish to thank Mr. J. A. M. van Moll and the directors of Philips Lamps Ltd. for permission to publish this work.

REFERENCES

1. Moser, L., *Chem. Zeit.*, 1906, **30**, 9.
2. Banerjee, P. C., *J. Indian Chem. Soc.*, 1942, **19**, 87.
3. Cameron, C. A., ANALYST, 1878, **3**, 338.
4. Kolthoff, I. M., and Yu-Djai Pan, *J. Amer. Chem. Soc.*, 1940, **62**, 3335.
5. Dragulescu, C., and Latui, E., *Z. anal. Chem.*, 1943, **126**, 63.
6. Kolthoff, I. M., and Sandell, E. B., "*Textbook of Inorganic Chemistry*," 2nd Ed. (1943).
7. Barber, H. H., and Kolthoff, I. M., *J. Amer. Chem. Soc.*, 1928, **50**, 1625.
8. A.S.T.M. Tentative Methods for Soda-Lime Glass, *J. Soc. Glass. Tech.*, Feb. 1942.
9. Willard, H. H., Liggett, L. M., and Diehl, H., *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 234.

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A New Method for the Absorptiometric Determination of Chromium in Low Alloy Steels by Oxidation with Potassium Bromate

BY M. Z. DE LIPPA

INTRODUCTION

Methods at present available for the rapid determination of chromium by absorptiometric (or colorimetric) analysis are carried out by oxidation of chromium to dichromate and involve one or other of the following oxidising agents: (i) ammonium persulphate,[1] (ii) hot perchloric acid,[2] (iii) potassium permanganate.[3] The last of these reagents is used for the primary oxidation of chromium and followed by the addition of diphenylcarbazide; a violet coloured complex is formed, the intensity of which is a measure of the chromium present.

Each of the above mentioned methods, while capable of giving good results, possesses certain disadvantages which render the determination somewhat lengthy and complicated. The following comments are offered on them.

Ammonium persulphate necessitates the use of silver nitrate as a catalyst, which is objectionable, since it is practically impossible to avoid traces of chloride in the reagents and even the laboratory atmosphere and consequent slight opacity of the solution gives rise to erroneous results. Moreover, ammonium persulphate oxidises manganese in addition to chromium and therefore requires either subsequent reduction with sodium nitrite or the use of a manganese correction graph. The use of sodium nitrite is inconvenient, since it often causes nitrogen bubbles which adhere to the cell walls and, once formed, are difficult to remove. In an endeavour to avoid the use of silver nitrate, the author developed a method by which chromium is oxidised by ammonium persulphate alone, at low acid concentration, but this involves much longer boiling and manganese is also partly oxidised. For this reason it was discarded in favour of the method to be described.

With perchloric acid oxidation, extreme care must be taken to keep a constant acid concentration during all determinations, since this factor greatly influences the colour intensity. The time of boiling must be very strictly controlled, too long boiling leading to loss of acid and high results. Even the type of vessel used for boiling (open beaker or conical flask) slightly influences the results. In addition, the dichromate colour in perchloric acid appears to be more sensitive to temperature changes than that in sulphuric or sulphuric-phosphoric acid, as the values in Table I indicate. All readings were taken with the same solution in one cell.

TABLE I

Temp. °C.	10·5	12·5	14·0	15·5	16·5	18·0	19·0	20·0	22·0	25·0	30·0
Readings × 1000	410	410	410	410	410	407	405	403	400	392	380
Cr% (actual 0·82)	0·820	0·820	0·820	0·820	0·820	0·827	0·830	0·835	0·840	0·850	0·870

It can be seen from the Table that constant readings can be obtained only when the temperature of the solution is below 17° C.

The potassium permanganate - diphenylcarbazide method has the disadvantage of requiring protracted boiling and the separation of iron by sodium hydroxide which, in turn, involves filtration.

Experimental Work

Potassium bromate has been suggested[4] as an oxidising agent for the volumetric determination of chromium, and consequently work was undertaken to discover whether it could also be applied in an absorptiometric method. The main obvious advantages of such a method would be:

(1) avoidance of the use of silver nitrate (as in the persulphate method);
(2) selective oxidation of Cr^{III} to Cr^{VI} without oxidation of the manganese present to permanganate.

The experimental work was carried out on (*a*) pure salts, (*b*) a solution of "spectrographically pure" iron with additions of chromium, and (*c*) solutions of steel with different chromium contents. The Spekker photoelectric absorptiometer was employed, with a mercury vapour lamp as source of illumination. As a result, the following conclusions were reached:

(i) Chromic salts are readily oxidised to dichromate by boiling with a slight excess of potassium bromate in a solution acidified with sulphuric and phosphoric acids, the speed of oxidation being increased by the presence of small amounts of manganese.

(ii) Manganese is oxidised only to the quadrivalent state, not to permanganic acid.[4]

(iii) To open the analysis, it was decided to take an aliquot portion (25 ml.) of a solution of 1 g. of steel in 40 ml. of "Spekker acid" (see list of reagents) oxidised with HNO_3 and diluted to 100 ml. This enables the method to enter the composite one for Mn, Ni, Cr, Mo, V from one weighing.[5]

(iv) After the oxidation of chromium, an excess of bromine remaining in the solution must be removed, as this would impart a yellow colour, thus interfering with the determination. This is conveniently carried out by further boiling with ammonium sulphate solution. The present work also confirmed the statement by Kolthoff and Sandell (*loc. cit.*) that even protracted boiling with ammonium sulphate alone does not remove the last traces of bromine and a small addition of hydrochloric acid becomes necessary for this purpose. This is not objectionable in a colorimetric determination, since the amount of hydrochloric acid added is small and any formation of *yellow* ferric chloride is suppressed by the presence of phosphoric acid.

Numerous experiments were carried out in order to establish the conditions for the complete removal of bromine from the solution. The final time of boiling and the minimum concentration of hydrochloric acid were investigated on solutions both with and without chromium. It was eventually found for the conditions prescribed in the method that a minimum of 3 minutes' boiling with 2 ml. of diluted hydrochloric acid (1+4) was necessary to obtain the final solution completely free from bromine.

(v) During the final boiling (with hydrochloric acid), it was found necessary to add from time to time a little water to replace loss on evaporation and maintain the total volume of the solution at 30 ml. approx. If evaporation goes too far, there is some danger of reducing chromium again. However, this reduction took place only when the rate of evaporation was high and the volume was reduced to 10–15 ml.

(vi) The quantity of potassium bromate added has no influence upon the resulting dichromate colour, providing all the excess of bromine is boiled off as described. 0·3 g. of potassium bromate (or 5 ml. of 6% aqueous solution) is sufficient to oxidise completely all chromium present, at least up to 3·5% (if an aliquot corresponding to 0·25 g. of steel is taken). In the experimental work, the amount of potassium bromate was increased from 0·3 to 0·5 g. (in steps of 0·05 g.) without any influence on the results.

(vii) The time of boiling with potassium bromate also has no influence upon the dichromate colour; 2 minutes are quite sufficient for a 3·5% chromium steel. In otherwise identical conditions, the time of boiling was extended to 3, 5, 8 minutes and consistent results were obtained in all cases.

(viii) The amount of ammonium sulphate necessary for the removal of bromine was found to be 0·5 g. which is conveniently added as 2·5 ml. of a 20% aqueous solution of the salt.

(ix) The stability of the dichromate colour developed by potassium bromate oxidation was examined and found very satisfactory for at least 3 hours.

(x) The effects of temperature variations on the colour readings are shown in the following table.

TABLE II

Temp. °C. ..	16	17	18	19	20	21	22	23	24	25	23	27	28
Readings × 1000 ..	655	655	655	655	650	650	650	645	645	642	640	640	640
Cr% (actual 0·855) ..	0·855	0·355	0·855	0·855	0·870	0·870	0·870	0·885	0·885	0·895	0·900	0·900	0·900

It can be seen that the readings should be taken at a temperature not above 19° C.; this indicates a slight advantage over the other method with respect to the permissible temperature range (*cf.* Table I).

(xi) Ammonium persulphate may, under certain conditions, oxidise chromium to perchromic acid,[1] this being one of the reasons for the addition of urea in the persulphate method (*ibid.*). The possibility of this taking place with potassium bromate oxidation was investigated, but the ether test revealed no perchromic acid present.

(xii) Of the elements occurring in steel which may cause interference, vanadium, molybdenum and titanium are the most important. An investigation of the effect of their presence gave the following results:

(*a*) Vanadium is oxidised by potassium bromate to the quinquevalent state, but not to pervanadates (as occurs with hydrogen peroxide) and therefore does not give rise to any coloration interfering with the determination of chromium.

(*b*) Molybdenum, as in the reaction with hydrogen peroxide, is oxidised to permolybdate, but only temporarily. The complex is almost immediately decomposed by boiling and therefore does not interfere with the determination.

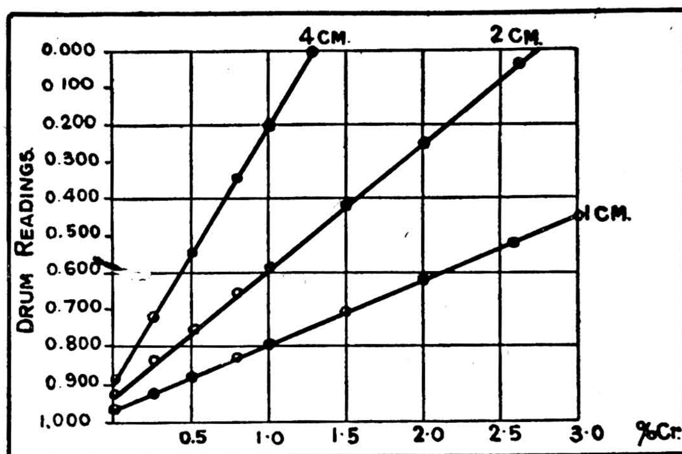
(*c*) Titanium is not oxidised to pertitanate (as it is with peroxide) and therefore does not interfere.

After the foregoing experimental work, the following procedure was eventually adopted.

THE METHOD

REAGENTS—(1) Spekker acid: 150 ml. of sulphuric acid (sp.gr. 1·84) + 150 ml. of phosphoric acid (sp.gr. 1·75) in 1000 ml. (2) Nitric acid: 1 vol. of conc. acid (sp.gr. 1·42) + 1 vol. of water. (3) Potassium bromate: 6% aqueous soln. (4) Ammonium sulphate: 20% aqueous soln. (5) Hydrochloric acid: 1 vol. of conc. acid (sp.gr. 1·19) + 4 vols. of water.

PROCEDURE—Treat 1·000 g. of drillings with 40 ml. of Spekker acid, heat until dissolved oxidise dropwise with diluted nitric acid (1 + 1), boil off nitrous fumes, cool and dilute to 100 ml. with water. Pipette 25 ml. of the solution into a conical beaker (150 ml.), bring



to boiling, add 5 ml. of 6% potassium bromate solution, boil 2–3 min., add 2·5 ml. of 20% ammonium sulphate solution, boil for 5 min., add 2 ml. of diluted hydrochloric acid (1 + 4) and boil for a further 3–4 min., replacing the evaporation losses by a few ml. of water. Cool to below 19° C., dilute to 50 ml. with water, mix, fill a 4, 2 or 1 cm. cell (depending upon the colour intensity) and measure the absorption with Ilford Spectrum Violet filters No. 601 and setting water to water 1.

The method can also be applied to steels of higher chromium contents by (1) using a smaller aliquot portion, (2) setting water to water 1·5 or even higher and (3) using a 0·5 cm. cell.

The calibration curve (page 36) was obtained by analysing several samples of steel initially containing 0·014% of chromium, to which a standardised solution of potassium dichromate was added to make up to the required chromium percentage. A sample of pure iron gave a small constant "blank," so the curve does not originate from zero for 0·0% chromium.

The time taken for the analysis of a single sample is 20 minutes. Batch analysis considerably shortens the average time. The accuracy of the method lies within ±1% of the chromium present.

In order to confirm the validity of the above calibration curves, 4 standard steels of known chromium contents were analysed several times, with the following results:

TABLE III

Designation of sample	Elements other than Cr present %	Certified Cr% or result of chemical anal. %	Cr found %	Deviation from standard
B.C.S. steel "B"	Mo=0·36; Ni=3·05	0·683	0·69	+0·007
,,	,,	,,	0·69	+0·007
,,	,,	,,	0·685	+0·002
,,	,,	,,	0·675	−0·008
B.C.S. steel "V"	V=0·29	0·861	0·850	−0·011
,,	,,	,,	0·860	−0·001
,,	,,	,,	0·858	−0·003
,,	,,	,,	0·850	−0·011
Am. St. 30C	V=0·235	0·977	0·970	−0·007
,,	,,	,,	0·970	−0·007
,,	,,	,,	0·970	−0·007
,,	,,	,,	0·970	−0·007
Steel No. 1	Mo=0·63; Ni=2·63	0·700	0·700	nil
,,	,,	,,	0·695	−0·005
,,	,,	,,	0·693	−0·007
Steel No. 2	Carbon steel	0·115	0·110	−0·005
,,	,,	,,	0·110	−0·005

SUMMARY—Methods available at present for the rapid absorptiometric determination of chromium in steel possess several disadvantages, depending upon the type of oxidising agent used. A new absorptiometric method has been developed which obviates these disadvantages and enables chromium to be very rapidly determined in low alloy steels with a reasonable degree of accuracy.

The author wishes to thank the Management of Colvilles Ltd., Clydebridge Steel Works, for permission to publish this work and also expresses his gratitude to T. F. Pearson, M.Sc., Chief Chemist, for his encouragement and constructive criticism.

REFERENCES

1. Vaughan, E. J., "*The Use of the Spekker Photo-electric Absorptiometer in Metallurgical Analysis.*" Monograph published by the Institute of Chemistry, 1941, p. 15.
2. Singer, L., and Chambers, W. A., *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 507.
3. Evans, B. S., ANALYST, 1921, **46**, 38.
4. Kolthoff, I. M., and Sandell, E. B., *Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 140.
5. Vaughan, E. J., "*Further Advances in the Use of the Spekker Photo-electric Absorptiometer in Metallurgical Analysis.*" Monograph published by the Institute of Chemistry, 1942, p. 8.

RESEARCH LABORATORY
COLVILLES LTD.
CLYDEBRIDGE STEEL WORKS

September, 1945

Notes

A WORKS TEST FOR EXCESS LIME IN WATER TREATMENT

WHERE excess lime treatment is used in water purification it is necessary to provide a simple test for use by the attendants controlling the treatment. At one of the Gold Coast water supplies where this process is used a test which could be applied by semi-skilled Africans was required. The colour changes given by silver nitrate and brilliant cresyl blue were not sufficiently pronounced for the purpose. The following test utilises thymol blue, the colour changes of which the African has no difficulty in distinguishing.

To 100 ml. of the excess lime treated water, which has been allowed to stand for calcium carbonate to precipitate and settle, are added 5 ml. of a hydrochloric - boric acid buffer solution. The *p*H value of the mixture is then taken by means of the Lovibond comparator and thymol blue in the usual way. The excess lime in parts per 100,000 corresponding to the various *p*H values can be read off from the following Table.

TABLE

*p*H value	8·1	8·2	8·3	8·4	8·5	8·6	8·7	8·8	8·9	9·0	9·1	9·2	9·3	9·4
Excess lime, parts per 100,000	0·25	1·10	1·70	2·00	2·25	2·40	2·50	2·65	2·80	3·00	3·20	3·40	3·60	4·00

The graph of this relationship is the usual *p*H curve.

The buffer solution referred to is made up of 0·75 g. of boric acid, 20 ml. of *N* hydrochloric acid and distilled water to make 1 litre.

The method makes no pretension to great accuracy, but is sufficiently accurate for works use. The following results on two batches of six samples each show the sort of agreement reached:

EXCESS LIME—PARTS PER 100,000

Sample	By works test	By analysis
1	2·50	2·52
2	2·80	2·48
3	3·00	3·08
4	2·25	2·48
5	3·00	2·80
6	2·65	2·52
Average	2·70	2·65
1	2·25	2·30
2	2·80	2·80
3	2·65	2·46
4	3·00	2·91
5	3·20	2·94
6	2·25	2·36
Average	2·69	2·63

A typical mineral analysis of the water being treated is:

	Parts per 100,000
Calcium, Ca	2·78
Magnesium, Mg	1·16
Sodium, Na	3·00
Oxides of iron and aluminium, R_2O_3	0·14
Carbonates, CO_3	7·03
Sulphates, SO_4	1·66
Chlorides, Cl	3·35
Nitrates, NO_3	0·29
Silica, SiO_2	2·76
	22·17
Organic matter, combined water, etc.	0·59
Total solids in solution	22·76
Suspended matter	3·16
Total solids	25·92

This water is usually highly coloured, and in the treatment an excess of 2 to 3 parts of lime per 100,000 is aimed at; to achieve this it is necessary to add from 10 to 12 parts of lime per 100,000.

If this method were applied to waters having a different composition and total solids content the relationship between the *p*H values found and the excess lime content would also be different, and a curve would have to be drawn for each water. The test has been in use for a number of years with satisfactory results. For the original idea behind this test I am indebted to my predecessor, Mr. H. E. Course, A.R.I.C.

Thanks are due to the Director of Public Works, Gold Coast, for permission to publish this note.

PUBLIC WORKS DEPARTMENT
KUMASI, GOLD COAST

J. S. DUNN
October, 1945

TEST OF THE LOCAL THICKNESS OF ZINC PLATING

A SOLUTION containing 200 ml. of concentrated hydrochloric acid and 0·8 g. of antimony trichloride per litre, which is approximately a third of the strength of the stripping solution employed in Specification D.T.D. 903 A for Zinc Plating, attacks zinc rapidly with vigorous effervescence, even when it is passivated, and the action rapidly falls off as the solution becomes exhausted. The zinc in a confined space on a piece of plating may thus be titrated by adding the solution, drop by drop, until an addition produces no further effervescence.

Recommended Procedure—A small flat surface of the zinc plated metal is necessary for the test. A glass cylinder, conveniently prepared by cutting about half an inch from the top of a test-tube and grinding the cut surface perfectly flat, about 0·7 inch in diameter, is placed in a shallow dish of molten beeswax so as to coat the lower half. The cylinder whilst still warm is placed on the chosen flat surface. If the surface is perfectly flat a watertight joint is obtained; if it is not perfectly flat contact can be effected by gently warming the metal until the wax again melts and seals any gaps. In this event the area attacked by the reagent is slightly reduced and may be measured after the test. If no particles of dirt or fibre are in the wax the area is still perfectly circular. The reagent is then added from a small burette (of 10 ml. capacity or less) in drops, or fractions of drops, until an addition produces no renewed effervescence. The volume of solution added is normally quite small but is accurately measured by the burette, and gentle rocking of the test ensures perfect mixing of the liquid. In the majority of tests the progress of the stripping can be observed when the metal surface is exposed by tipping the test piece.

Calculation—The exact acidity of the reagent solution is determined by titration, but is approximately 2 *N*, which is sufficiently accurate for routine tests. Hence 1 ml. ≡ 0·0654 g. of zinc. The thickness of the zinc plating is then calculated as in Specification D.T.D. 903 A, *viz.*,

$$\text{thickness of plating in inches} = \frac{\text{weight of plating in grams}}{113 \times \text{area of plating in sq. inches}}$$

Examples—The thickness of miscellaneous samples of zinc plating was determined by the above method and the results compared with those calculated from the loss in weight during test.

Sample	Diameter, inches	Area, sq.in.	Titration, ml. (2 *N* soln.)	Calculated wt. of zinc, grams	Thickness, inches	Thickness by loss in wt., inches
1	0·7	0·384	0·20	0·0131	0·00030	0·00030
2	0·7	0·384	0·30	0·0196	0·00045	0·00045
3	0·7	0·384	0·32	0·0209	0·00048	0·00045
4	0·65	0·332	0·22	0·0144	0·00038	0·00039
5	0·60	0·285	0·13	0·0085	0·00026	0·00031

Summary—Local thickness of zinc plating on flat surfaces is determined by measuring the volume of a standard stripping solution of hydrochloric acid (containing antimony to inhibit action on the base metal) required to dissolve the zinc on a definite area. This area is isolated by cementing a glass cylinder on the surface to be tested. The end-point is judged by the cessation of effervescence.

174, EASTCOTE ROAD
RUISLIP, MIDDLESEX

H. W. PARKER
September, 1945

Order in Council

STATUTORY RULES AND ORDERS*

1945 No. 1454. Order in Council amending Regulation 3 of the Defence (Sale of Food) Regulations, 1943. Price 1d.

This Order adds to Regulation 3, *which enables samples to be taken for the purpose of enforcing the Regulations, the following paragraph*:

"(5) Where a sample has been taken under this Regulation, any proceedings in respect of the article sampled may be taken before a court having jurisdiction in the place where the sample was taken or, if the prosecutor so elects and the article sampled was sold and actually delivered to the purchaser, the proceedings may be taken before a court having jurisdiction at the place of delivery:

Provided that this Regulation shall not prejudice the taking of proceedings before any other court having jurisdiction in respect of such an offence."

The provision is similar to Section 80 (2) *of the Food and Drugs Act*, 1938.

Ministry of Food

STATUTORY RULES AND ORDERS*

1945 No. 1383. Order, dated October 31, 1945, amending The Egg Products (Control and Maximum Prices) Order, 1943. Price 1d.

This Order amends The Egg Products (Control and Maximum Prices) Order, 1943, by bringing "sugar - dried egg" within the definition of "egg products." This product is distributed for manufacturing purposes only and its maximum price is accordingly fixed by an amendment

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

to the Third Schedule. An additional Article provides that the certificate of a public analyst or the Government Chemist shall be conclusive evidence of the facts stated therein, unless challenged.

"Sugar - dried egg" means the product obtained by the spray-drying of whole egg pulp and added sucrose.

1945 **No. 1444. Order, dated November 14, 1945, amending the Fish (Maximum Prices) (No. 2) Order, 1944.** Price 2d.

This Order prescribes new maximum prices for fish during the coming winter months. Bass and mullet are excluded from the definition of "fish" and are thus freed from price control. An amendment to the definition of "first hand sale" places imports of fresh fish from foreign countries in the same position, as regards first hand sale, as fish from Eire and the Isle of Man. No maximum first hand price is prescribed for wet salted and dried salted fish. The minimum size for mackerel and horse mackerel is reduced from 9 in. to 8 in.

— **No. 1475. Order, dated November 21, 1945, amending the Feeding Stuffs (Regulation of Manufacture) Order, 1944.** Price 2d.

This Order, which came into force on 3rd December, 1945, alters the composition of all National Compounds, other than Baby Chick Food, by deleting the minimum requirement of maize, increases the maximum fibre content of certain compounds and reduces the animal protein content of National Poultry Foods Nos. 1 and 1A. It also makes obligatory the inclusion of cod liver oil in National Pig Food No. 1 and the inclusion of decorticated groundnut cake in National Poultry Foods Nos. 1 and 1A.

— **No. 1550. Order, dated December 10, 1945, amending the Labelling of Food (No. 2) Order, 1944.** Price 1d.

Hitherto the labelling provisions of the Labelling of Food (No. 2) Order, 1944, have not applied to intoxicating liquor. This amending Order brings them into operation as regards "sweets," including British wines, and spirituous liquors containing not more than 40% proof spirit, subject to certain modifications necessitated by the nature of these products. An additional Article imposes restriction on the labelling of such products if the fruit basis of the product is not exclusively grapes, or products derived from grapes. The Order will come into force on April 1, 1946, except that as regards the sale or delivery of intoxicating liquor by the packer or labeller otherwise than by retail the Order will come into force on February 1, 1946.

The following definitions are added to those in par. (1), *Article I, of the principal Order.*

"Intoxicating liquor" means spirits, wine, beer, porter, cider, perry and sweets and any fermented, distilled or spirituous liquor which cannot be lawfully sold without an excise licence.

"Spirits" means spirits of any description, and includes all liquors mixed with spirits and all mixtures, compounds or preparations made with spirits, other than fortified wines or fortified sweets.

An amendment to List B of Schedule I of the principal Order restricts the intoxicating liquors exempted from the operation of the Order to "spirits containing more than 40% of proof spirit, wine imported into the United Kingdom, beer, porter and any product sold under the description cider or perry."

Other intoxicating liquors are subject to labelling requirements which prescribe a statement, in specified lettering and wording, of the kinds of fruits or fruit products used in their production and of their minimum alcohol content.

A new Article, 4A, *states that* no person shall sell or have in his possession for sale intoxicating liquor produced in the United Kingdom described in a label, whether attached to or printed on the container or not,

(i) by any name or words calculated to indicate either directly, or by ambiguity, omission or inference, that the liquor is or resembles imported wine or is a substitute for or has the flavour of imported wine, unless such liquor, in so far as it is derived from fruit, is derived exclusively from grapes; or

(ii) (in the case of liquor derived wholly or partly from fruit other than grapes) by the use of the word "wine," unless that word is immediately preceded in identical lettering by a word or words accurately specifying the description of fruit or fruit product used.

CHOCOLATE FLAVOURED FLOUR MIXTURES

In Circular FSL/18/45 (FSL/18S/45, Scotland), dated November 27, 1945, the Food Standards and Labelling Division of the Ministry of Food notifies local authorities that the Ministry has had under review the composition and descriptions of

Chocolate Sponge Mixtures
Chocolate Cake Mixtures } and variations thereof,
Chocolate Pudding Mixtures

the manufacture of which is regulated by Ministry of Food Licences issued under the Manufactured and Pre-packed Foods (Control) Order, 1942. After taking account of the views expressed by a number of Food and Drugs Authorities and of the manufacturers concerned, the Ministry has decided only to license the manufacture of mixtures which contain a minimum of 10% of cocoa. Action will be taken to amend existing licences accordingly. This step is designed to secure that the finished product (*i.e.*, after preparation by recipe) shall contain approximately 4% of fat-free cocoa, which in the opinion of the Ministry should justify the proposed description. While every effort will be made to bring the revised formulae into effect as early as possible, some period of time must elapse before the full effects of this revision are reflected in retailers' shops.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

The Diagnostic Value of Rothera's Test on Milk. A. B. Paterson (*Veterinary J.*, 1945, **101**, 199–204)—It is shown that the application of Rothera's test (*J. Physiol.*, 1908, **32**, 491) to milk samples is a valuable aid to diagnosis of the condition in cows described by Stimson (*Vet. Rec.*, 1928, **8**, 880) as "bovine hyperglycaemic ketosis." *Method*—Transfer 10 ml. of milk to a test tube containing 5 g. of powdered ammonium sulphate, shake to ensure complete solution, add 0·1 ml. of a freshly prepared 5% aqueous soln. of sodium nitroprusside followed by 2 ml. of conc. ammonia soln. and again shake. After 5 min. note the colour; this varies from a faint pink for small amounts of ketones to an intense purple for larger quantities. A modification for field use employs an intimate mixture of 95 parts of crystalline ammonium sulphate and 5 parts of coarsely powdered sodium nitroprusside as reagent, together with test tubes, 5/8 in. in diameter, marked at 1·7 in., 3·0 in. and 3·5 in. from the bottom. The reagent is added to the first mark, the milk under examination to the second and, after shaking to dissolve, conc. ammonia soln. up to the third mark. The colour is noted after shaking and allowing to stand for 5 min. It is stated that the test is inapplicable to bovine urine by reason of its sensitivity, since healthy stall-fed animals may give a positive reaction. As applied to milk it is less sensitive and a positive test is not given until the level of blood ketones has risen to several times its normal value. The level of ketones in milk is shown to be about half that of the corresponding blood serum, while β-hydroxytartaric acid, which may constitute about 70% of the ketones in blood and which does not react in Rothera's test, only occurs in milk to the extent of 30% of the total ketone bodies. The test must be conducted on fresh milk, as souring causes the sensitivity to decrease, probably owing to conversion of aceto-acetic acid into acetone. No interference is found when sulphonamides, strychnine, sodium salicylate, iodine, formalin, camphor, turpentine or linseed oil is used medicinally. It is concluded that when Rothera's test on milk is used as an aid to diagnosis, the findings may be grouped as follows: (1) test negative: ketosis definitely absent, even when the test on urine is positive; (2) test weakly positive: ketonaemia definitely present and condition of ketosis confirmed if clinical symptoms such as fall in milk yield are shown; (3) test strongly positive: ketonaemia pronounced, although the severity of clinical symptoms may vary considerably, as they are not directly proportional to the intensity of the reaction.

J. A.

Determination of Tin in Canned Foods. D. Dickinson (*Ann. Rep. Fruit and Vegetable Preservation Research Station*, Campden, 1944, 46–52)—The method is based upon that of Stone (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 791; ANALYST, 1942, **67**, 142) It is rapid and sufficiently accurate for routine work. Weigh accurately about 10 g. of the sample containing 0·1–5 mg. of tin and evaporate almost to dryness in a porcelain crucible. Ignite the residue in a muffle furnace at about 600° C. until it is free from carbon and scrape any ash remaining on the sides of the crucible to the bottom by means of a stainless steel spatula. Cover the ash with 1 g. of a fusion mixture containing 25 parts of potassium cyanide and 75 parts of anhydrous sodium carbonate and hold the crucible in the flame of a Bunsen or Meker burner until the mass melts and can be stirred by gentle rotation of the crucible. Place the cold crucible in a lipped beaker containing 10 ml. of water, cover with a watch glass and pipette 10 ml. of dil. hydrochloric acid into the crucible by inserting the tip of the pipette through the lip of the beaker. Gently boil in a fume cupboard for 30 min., cool to room temp., remove the ppt. of Prussian blue by filtration, wash the beaker and crucible with water and make the combined filtrate and washings up to 50 or 100 ml. according to the amount of tin present. To an aliquot containing 0·02–0·1 mg. of tin, but measuring not more than 11 ml., add 1 ml. of dil. hydrochloric acid and enough water to make 12 ml. Add 0·5 ml. of dithiol reagent (*infra*), mix, and after 5 min. compare the colour with that of standard solns. containing known amounts of tin or evaluate the colour in a tintomer; 0·1, 0·2, . . . 1·0 mg. of tin ≡ 0·7, 1·3, 1·9, 2·5, 3·1, 3·6, 4·2, 4·7, 5·1, 5·5 red units. Expts. with known solns. indicated a recovery of tin of from 85% to 109%. To prepare the dithiol reagent dissolve 0·1 g. of dithiol (1-methyl-3, 4-dimercaptobenzene) in 2·5 ml. of 5 *N* sodium hydroxide and 0·5 ml. of thioglycollic acid and dilute to 50 ml. with water.

Residual iron not removed in the form of Prussian blue does not interfere with the estimation, Copper when present in relatively high concn. may be removed by the following procedure. Treat the filtered soln. of the fused mass in hydrochloric acid in a separating funnel with 5 ml. of a 1% soln. of sodium diethyldithiocarbamate and 15 ml. of carbon tetrachloride. Shake the stoppered funnel and allow the liquids to separate. The lower layer of carbon tetrachloride contains the copper and is rejected. Wash the aq. layer with 10 ml. of carbon tetrachloride, transfer it into a standard flask together with rinsings of the funnel, and after adjustment of the volume determine the tin colorimetrically as previously described. Interference by copper does not become serious until its concn. is at least one-tenth that of the tin. When the concn. of copper is 50% or more of that of the tin, further extraction with sodium diethyldithiocarbamate and carbon tetrachloride may be necessary, but such repeated extraction tends to give low results. In practice only products containing a high proportion of tomato purée or jams are likely to contain sufficient copper to justify its extraction. Other metals likely to occur in canned foods cause no interference with the method.

A. O. J.

Colorimetric Method for the Estimation of Cinchona Alkaloids [in Blood]. P. B. Marshall and E. W. Rogers (*Biochem. J.*, 1945, **39**, 258–260)—It was observed that quinine forms with bromothymol blue a yellow colour extractable by shaking with chloroform and that the intensity of the colour is proportional to the concentration of quinine. The reaction was also given by the other three main alkaloids of cinchona bark, quinidine, cinchonine and cinchonidine, by certain derivatives of these alkaloids and by some other nitrogen-containing compounds. The method was adapted to the estimation of total cinchona alkaloids

in the blood of experimental animals, using benzene in place of chloroform.

Lake 1 ml. of oxalated blood in 29 ml. of water, add 5 ml. of *N* sodium hydroxide and heat for 30 mins. on a steam-bath. Cool, transfer to a separating funnel and add 2 ml. of acetone and 50 ml. of ether. Shake, leave for a few mins. and shake again. When both layers are clear, discard the aqueous layer. Wash the ether layer with 50 ml. of 0·5 *N* potassium hydroxide and then with 50 ml. of water. Add 2·5 ml. of 0·05 *N* hydrochloric acid, shake, allow to separate and run the acid layer into a 25-ml. beaker. Extract the ether with a second 2·5-ml. portion of the acid and combine the acid extracts. Warm to remove dissolved ether, add 1 ml. of 0·04% (w/v) bromothymol blue solution and neutralise to about *p*H 7·0 (green colour) with sodium hydroxide soln. Transfer the soln. to a narrow bore tube, add 1 ml. of Sorensen's buffer of *p*H 7·0, mix and leave for 60 mins. Add exactly 1 ml. of benzene, close the tube with a tightly fitting cork covered with cellophane and shake for 40 mins. Centrifuge at moderate speed for 10 mins. and transfer the benzene layer to a 0·5-ml. cell. Evaluate the colour in a Spekker absorptiometer, using Ilford spectrum filter No. 601 (violet). Prepare standard curves by adding known amounts of alkaloid to volumes of blood equal to those used in the estimation. The procedure described above is also applicable to tissues, which should be digested with 2% sodium hydroxide soln. before extraction. The recoveries of amounts of cinchonidine, ranging from 1·5 to 13 μg., added to 1-ml. quantities of blood, were theoretical. The method also gave satisfactory agreement with results obtained by the method of Kyker, Webb and Andrews (*J. Biol. Chem.*, 1941, **139**, 551).

F. A. R.

A Chemical Evaluation of Digitalis. F. K. Bell and J. C. Krantz, Jr. (*J. Pharmacol. Exp. Therap.*, 1945, **83**, 213)—The colorimetric method of Knudson and Dresbach (*J. Pharmacol. Exp. Therap.*, 1923, **20**, 205), based on the colour reaction of Baljet (*Schweiz. Apoth. Zeit.*, 1918, **56**, 71 and 89), has been modified by measuring photoelectrically the colour produced. Jacobs (*Physiol. Rev.*, 1933, **13**, 222) has shown that the activity of the cardiac glycosides is dependent upon the presence in the unsaturated lactone group of an active hydrogen atom and that the Baljet colour reaction is due to this portion of the molecule, whence the test is a true measure of the pharmacological potency. *Method*—To 5 ml. of the sample in the form of a tincture add about 15 ml. of water and 2 ml. of a freshly prepared 12·5% soln. of lead acetate, mix, dilute to 25 ml. and filter. To 12·5 ml. of the filtrate add 2 ml. of a 4·7% soln. of sodium phosphate, $Na_2HPO_4.7H_2O$, mix, dilute to 25 ml. and filter. Transfer 12·5 ml. of the filtrate to a suitable container, add 12·5 ml. of a reagent freshly prepared by mixing 95 ml. of a 1% soln. of picric acid and 5 ml. of a 10% soln. of sodium hydroxide, transfer to a 23-ml. absorption tube and measure the log. transmission by means of a Fisher Electrophotometer with a green filter No. 525 exactly 20 min. after adding the reagent, using 12·5 ml. of the reagent diluted with 12·5 ml. of water as a blank and reading to the nearest 0·5 or whole division on the scale. Treat 5 ml. of the U.S.P. Reference Standard tincture and also 5 ml. of a mixture of the U.S.P. Reference Standard tincture with an equal vol. of 71% alcohol in precisely the same manner. The percentage potency of the unknown tincture in terms of the standard is given by

$$\text{Potency} = 50\left(\frac{R_x - R_{100}}{R_{100} - R_{50}}\right) + 100$$

where R_{100}, R_{50} and R_x are respectively the instrument readings (log. transmission) corresponding to the U.S.P. Reference Standard tincture, the half-strength U.S.P. Reference Standard tincture and the unknown tincture. Substituting the average (median) values for R_{100} and R_{50} found by the statistical analysis of 50 typical pairs of readings in this equation, it becomes

$$\text{Potency} = 50\left(\frac{R_x - 42{\cdot}5}{16{\cdot}5}\right) + 100.$$

Data are presented which indicate that U.S.P. Reference Standard Ouabain is unsuitable as a standard since the development of the colour follows a different course from that of tincture of digitalis. The Knudson and Dresbach procedure has been criticised on the ground that reducing sugars are also determined, but it is shown that under the specified conditions of the assay interference from this source can be disregarded. Agreement between the proposed procedure and the U.S.P. cat method is shown to be satisfactory, and it is claimed that assays can be performed with a percentage standard error of 1·2 compared with that of 5·7 permitted by the U.S.P.

J. A..

Identification of Sulphapyrazine through Ultra-violet Fluorescence of 2-Aminopyrazine H. W. Raybin (*J. Amer. Pharm. Assoc.*, **1945**, **34**, 196)—Aqueous solns. of 2-aminopyrazine, the pyrolysis product of sulphapyrazine, have been found to give an intense violet-blue fluorescence, most vivid in the 365 mμ region. This fluorescence is characteristic for 2-amino-pyrazine, and, therefore, for sulphapyrazine. The test is performed as follows. After determining the melting-point of the sample of sulphapyrazine, allow the capillary melting-point tube to remain in the bath at the melting-point temperature (254° C.) for 3 min. to obtain sufficient 2-aminopyrazine for the test. Remove the tube, cut off the lower end containing the charred residue and grind the upper portion with 5 ml. of water in a mortar. Observe the filtered soln. in ultra-violet light in a dark room; a blue fluorescence indicates the presence of 2-aminopyrazine, thus identifying the sample as sulphapyrazine. Of the sulpha drugs in the U.S.P. XII and New and Non-official Remedies (1944), only sulphadiazine, sulphamerazine (sulphamethyldiazine) and sulphapyrazine give crystalline identification products suitable for identification purposes. The melting points of sulphadiazine (250/4° C.) and sulphapyrazine (252/6° C.) and their respective pyrolysis products, 2-aminopyrimidine (126/7° C.) and 2-aminopyrazine (120/2° C.) are too close for positive differentiation. Sulphamerazine melts at 235/6° C. and its pyrolysis product, 2-amino-4-methylpyrimidine, at 159/61° C. Sulphadiazine may be identified by the colour reaction of 2-aminopyridine with resorcinol (Raybin, *J. Amer. Pharm. Assoc.*, 1944, **33**, 158).

J. A.

Biochemical

Estimation of Histidine in Protein Hydrolysates with *Leuconostoc Mesenteroides* P-60. M. S. Dunn, N. N. Camien, S. Shankman and L. B. Rockland (*J. Biol. Chem.*, **1945**, **159**, 653–662)—It was found that histidine could be

estimated satisfactorily, using the basal Medium D and the experimental conditions previously employed for the assay of lysine (Dunn, *et al.*, *id.*, 1944, **156**, 703; this vol., p. 182). The standard curves were most satisfactory when the microörganisms were grown for 5 days. Hydrolysates of casein and silk fibroin were found to contain 3·1 and 0·34% of histidine respectively. Mixtures of amino acids containing known amounts of histidine gave results equal to 97·5% of the theoretical. F. A. R.

Polarographic Studies of Proteins and their Degradation Products. 1. The Protein Index. O. H. Müller and J. S. Davis (*J. Biol. Chem.*, 1945, **159**, 667–679)—Proteins and polypeptides containing cystine or cysteine when dissolved in a suitable buffered cobalt solution produce a catalytic reduction during electrolysis at the dropping mercury electrode. It has been claimed that the sera of normal individuals and persons suffering from cancer can be differentiated because the height of the polarographic waves are different, but the test is now known not to be specific for cancer. Nevertheless, the polarographic protein test has proved of value for the study of disease in general.

In order to compare normal and abnormal serum proteins, the use of the "protein index" is now suggested. The wave heights of a sample of plasma are measured before and after precipitation of protein and the ratio multiplied by an arbitrary factor is termed the "protein index." To 0·5 ml. of oxalated plasma add 0·5 ml. of water and 0·25 ml. of *N* potassium hydroxide. Mix and leave for 30 mins. at room temperature. Add 0·05 ml. of the alkaline digest to 10·0 ml. of a buffered cobalt soln. ($1{\cdot}6 \times 10^{-3}$ *M* cobaltous chloride, 0·1 *N* ammonium chloride and 0·1 *N* ammonium hydroxide) and polarograph immediately in an open beaker starting at −0·8 volt *versus* the saturated calomel electrode. To another 0·5 ml. of oxalated plasma add 1·0 ml. of water and 0·1 ml. of *N* potassium hydroxide. Leave for 30 mins. at room temperature, add 1·0 ml. of 20% sulphosalicylic acid soln. and shake vigorously. Exactly 10 mins. after the addition of the precipitant, filter through a Whatman No. 5 filter paper, and add 0·5 ml. of the filtrate to 5·0 ml. of a soln. consisting of 0·001 *N* hexammonium cobaltic chloride, 0·1 *N* ammonium chloride and 0·8 *N* ammonium hydroxide. Polarograph immediately, starting at −0·8 volt. Divide the wave height (expressed in microamperes) obtained in the second test by that obtained in the first test and multiply by 15 to obtain the "protein index." F. A. R.

Modified Method of Extracting Cholesterol. L. F. Potter (*Science*, 1945, **102**, 333)—Most of the published methods of extracting cholesterol from liver are tedious and many do not give reproducible results. The following method was found to be satisfactory. Grind the liver with anhydrous sodium sulphate and one 10-ml. and two 5-ml. portions of acetone - alcohol mixture (3 : 1). Transfer the mixture to a centrifuge tube, add 15 ml. of anhydrous ether and shake for 10 mins. Centrifuge and evaporate the supernatant liquor in nitrogen under reduced pressure. Estimate the cholesterol in the residue by the Liebermann - Burchard reaction with the Evelyn photoelectric colorimeter. F. A. R.

Estimation of the Cystine and Methionine Content of Plant and Animal Material by a Differential Oxidation Procedure. R. J. Evans (*Arch. Biochem.*, 1945, **7**, 439–445)—The differential oxidation procedure described in this paper is a modification of the method of Blumenthal and Clarke (*J. Biol. Chem.*, 1935, **110**, 343) and involves the estimation of sulphur by three different methods on each protein concentrate studied. Total sulphur is determined by the Parr bomb method or by the nitric and perchloric acid digestion method of Evans and St. John (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 1630), inorganic sulphate by precipitation as barium sulphate after hydrolysis of the sample with dilute hydrochloric acid, and inorganic + cystine sulphur by digestion with conc. nitric acid as follows:—Weigh a 2·00 g. sample of the protein concentrate into a 500 ml. Kjeldahl flask, add 35 ml. of conc. nitric acid and heat on the steam-bath for 24 hrs. Rinse the soln. into a 250-ml. beaker containing about 0·5 g. of potassium nitrate and evaporate to dryness on the steam bath. Add 10 ml. of conc. hydrochloric acid, again evaporate to dryness, and dissolve the residue in water containing 1·0 ml. of conc. hydrochloric acid. Filter, precipitate, and weigh as barium sulphate. Cystine = 3·747 × (sulphate S after nitric acid oxidation — inorganic • S). Methionine = 4·651 × (total S — sulphate S after nitric acid oxidation).

The factors 3·747 and 4·651 were calculated from the theoretical sulphur contents of cystine and methionine. By the nitric acid oxidation alone, the recovery of the two amino acids added to dextrose, soya bean oil and herring fish meal were 92·5, 99·3 and 98·5% of the added cystine, and 0·0, 0·9 and 2·8% of the added methionine. It appears that considerable organic matter is necessary for cystine sulphur to be completely oxidised to sulphate. The results obtained for methionine were in close agreement with those obtained by McCarthy and Sullivan's method (*J. Biol. Chem.*, 1941, **141**, 871). The cystine and methionine contents of a number of plant and animal proteins are recorded. F. A. R.

Spectrophotometric Study of a New Colorimetric Reaction of Vitamin A. A. E. Sobel and H. Werbin (*J. Biol. Chem.*, 1945, **159**, 681–691)—Vitamin A reacts with glycerol 1 : 3-dichlorohydrin to give a blue colour which changes rapidly to a violet colour similar to that of a dilute solution of potassium permanganate. The colour is more stable than that obtained with antimony trichloride and its intensity can be measured any time from 2 to 10 mins. after addition of the reagent. The reagent is also more stable than antimony trichloride soln., is not affected by traces of moisture and its intensity can be measured at any time from light absorption of the coloured soln. The new method suffers from the disadvantage that the extinction coefficient ($E^{1\%}_{1\,cm.}$ λ 550 mμ) of the violet colour is about 1/4 that of the yellow colour produced with antimony trichloride ($E^{1\%}_{1\,cm.}$ λ 615 mμ). Beer's law is obeyed up to concentrations of 25 I.U. of vitamin A in 5·0 ml. of soln. The immediate blue colour has maximum absorption at 625 mμ and obeys Beer's law up to 33 I.U. in 5·0 ml. of soln. $E^{1\%}_{1\,cm.}$ at 550 mμ and 625 mμ are 1010 and 1385 respectively. The results agreed well with those obtained by the antimony trichloride method. Interference from vitamin D and related sterols was negligible but carotene interfered to the same extent as in the antimony trichloride method, giving a green colour the absorption spectrum of which showed two maxima, one at 475 mμ and the other at 625 mμ, with a minimum at 550 mμ. At the

point of maximum absorption for vitamin A, therefore, the absorption for carotene was minimal. The colour due to 1 μg. of carotene at 550 mμ was equivalent to about 0·49 I.U. of vitamin A when measured 6 mins. after the addition of the reagent. When the immediate blue colour was used for measuring vitamin A concentrations, 1 μg. of carotene was equivalent to only 0·17 I.U. of vitamin A. Thus, the presence of carotene can be corrected for either by measuring the absorption of the soln. at 550 mμ after 6 mins., or at 625 mμ after 5 secs. and subtracting the absorption due to carotene.

Pipette 1·0 ml. of a chloroform soln. of the vitamin A preparation into a 10-ml. glass-stoppered graduated cylinder and add 4 ml. of glycerol 1 : 3-dichlorohydrin from a bulb pipette. Mix and leave the soln. in a water-bath at 25° C. for 2 mins., transfer to the cuvette of a Coleman spectrophotometer and evaluate the absorption using filter PC–4. When the immediate blue colour is to be evaluated, pipette the chloroform soln. of vitamin A directly into the cuvette, add 4 ml. of the reagent from a fast delivery pipette and measure the colour within 5 secs.

F. A. R.

Studies on Vitamin B_c Produced by Micro-organisms. P. R. Burkholder, I. McVeigh and K. Wilson (*Arch. Biochem.*, 1945, **7**, 287–303)—Vitamin B_c is a factor which cures anaemia in chicks and cytopenia in monkeys and stimulates the growth of *Lactobacillus helveticus*. It occurs in yeast, chiefly in the form of a simple non-protein conjugate. A method of assaying yeast and animal tissues using *L. helveticus* has been described. Prepare the inoculum from a 24-hr. culture grown in 10 ml. of yeast - glucose broth, centrifuge and resuspend the organisms in 10 ml. of steirle physiological saline, centrifuge again, resuspend in saline and transfer 1 ml. of the suspension aseptically into 20 ml. of sterile saline. Inoculate each assay tube with 1 small drop of the inoculum by means of a fine-tipped sterile pipette. The double-strength basal medium has the following composition: casein amino acids, 10 g.; glucose, 20 g.; sodium acetate, 12 g.; asparagine, 0·5 g.; tryptophan, 0·2 g.; cystine, 0·2 g.; salt soln. A,* 10 ml.; salt soln. B,*. 10 ml.; glutamine, 10 mg.; guanine, 10 mg.; adenine, 10 mg.; xanthine, 10 mg.; uracil, 10 mg.; aneurine, 200 μg.; biotin, 1 μg.; riboflavin, 400 μg.; calcium pantothenate, 400 μg.; nicotinic acid, 400 μg.; pyridoxine, 1200 μg.; *p*-aminobenzoic acid, 400 μg.; water to make 1000 ml. of soln. Run the assays in triplicate and put 5 ml. of the double-strength basal medium into each test-tube. Add graded amounts of the soln. to be tested and of standard solns. of crystalline vitamin B_c, make up to 10 ml., autoclave, inoculate and incubate for 72 hr. at 37° C. Vitamin B_c gives a typical dosage - response curve with maximum acid production after 72 hr., but in absence of glutamine maximum acid production is only obtained after 120 hr. Titrate the tubes with 0·1 *N* sodium hydroxide and calculate the results from the standard curve.

Fresh chicken pancreas is used to liberate conjugated vitamin B_c. Grind the pancreas, weighing about 4 g., in a glass mortar or in a Waring blendor and suspend in 0·2 *M* phosphate buffer at *p*H 5·0.

* Salt soln. A consists of K_2HPO_4, 10 g.; KH_2PO_4, 10 g.; water to make 100 ml. Salt soln. B of $MgSO_4.7H_2O$, 4 g.; NaCl, 0·2 g.; $FeSO_4.7H_2O$, 0·2 g.; $MnSO_4.4H_2O$, 0·2 g.; water to make 100 ml.

Alternatively prepare dried pancreas by grinding the tissue in acetone, filtering through cheese cloth and drying the residue; the product retains its activity for several months when stored at 0° C. The pancreas preparation contains 0·0016 μg. of vitamin B_c per mg. of dried solids, so that except with materials containing very small amounts of vitamin B_c the error introduced by addition of the pancreas is negligible. Mix 25 mg. of dried yeast or its equivalent with 20 ml. of phosphate buffer, *p*H 5·0, and add 5 or 10 mg. of dried pancreas powder (fresh pancreas contains about 44% of dry solids) in 1 ml. of phosphate buffer. Add a little benzene and incubate at 37° C. for 18 hr. Stop the enzyme activity by steaming the sample at 100° C. for 10 mins., adjust the *p*H to 6·8, dilute to 100 ml. and filter through Super-cel on a Buchner funnel. This removes fatty substances as well as suspended solids. Assay an appropriate volume of the filtrate as described above.

F. A. R.

Microbiological Assay of Vitamin B_c Conjugate. O. D. Bird, B. Bressler, R. A. Brown, C. J. Compbell and A. D. Emmett (*J. Biol. Chem.*, 1945, **159**, 631–636)—It has long been known that part of the vitamin B_c present in substances such as yeast is in a combined form, incapable of stimulating the growth of *Lactobacillus helveticus*. Enzymatic procedures are now described for releasing the vitamin B_c to enable it to be estimated microbiologically. The digestion method of Cheldelen *et al.*, using takediastase and papain proved ineffective, but takadiatase or clarase in high dilution was partially effective. The enzymes of certain animal organs were still more effective and the most convenient preparation was desiccated hog's kidney. This was highly active, had a low vitamin B_c content and was extremely stable.

Grind a fresh hog kidney and immerse in 5 vols. of acetone, filter, re-wash with acetone, air-dry and reduce to a fine powder. Transfer the sample to be tested, containing 25–50 μg. of vitamin B_c, to a small beaker, add a few ml. of water and 0·3 g. of the powdered, dry kidney. Adjust to *p*H 4·3–4·5, and transfer to a test tube or conical flask, keeping the vol. between 10 and 20 ml. Add a few drops of toluene and incubate for 48 hours at 37° C. Heat for a short while in an autoclave, adjust to *p*H 7·0 and dilute to 50 ml. Centrifuge and use an aliquot portion of the supernatant liquid for microbiological assay by a modification of the method of Mitchell and Snell (*Univ. Texas Pub.*, 1941, No. 4137, 36), using crystalline vitamin B_c as standard. The following alterations in the original procedure are recommended. Increase the pyridoxine hydrochloride content from 200 to 1,000 μg. per litre. Prepare two stock cultures each month; maintain one undisturbed and subculture the other weekly in solid medium. Twenty-four hours before assay make a transfer from the subculture to a tube containing 5 ml. of the riboflavin medium of Snell and Strong (*Ind. Eng. Chem. Anal. Ed.*, 1939, **11**, 346), 5 ml. of water and 1 μg. of riboflavin. Dilute 1 ml. of this culture aseptically with sterile saline to 10 ml., and use one drop of the resultant suspension as the inoculum for each tube. Grow the cultures in Evelyn colorimeter tubes and measure the turbidities after 40 hours' incubation at 37° C.

Instead of the kidney enzyme, an extract of almonds can be used. Grind unheated almonds in a mortar with 0·1 *M* phosphate buffer of *p*H 7·0, using in all 10 ml. per g. of almond and filter the emulsion through muslin. Incubate a sample containing 25–50 μg. of the vitamin with 10 ml. of

the filtrate for 48 hours at 37° C. and proceed as described on p. 44.

Either method gave results in excellent agreement with the results obtained by the chick assay method of Campbell, Brown and Emmett (*J. Biol. Chem.*, 1944, **152**, 483). F. A. R.

Organic

Determination of the Methyl Ester Content of Pectin. C. H. Hills, C. L. Ogg and R. Speiser (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 507–510)—The viscosity of pectinate solns., the setting time of pectin jellies, the formation and stability of calcium pectinate gels and the solubility of pectin are greatly influenced by the degree of esterification, and accurate methods for determination of the methyl ester content of pectin are required. The Zeisel method and the saponification method are the chief methods now used for this purpose. The saponification method is empirical but is considered accurate because it gives results in agreement with those of the Zeisel method, which is the reference method. In the authors' hands both methods gave erratic results. The principal cause of erratic Zeisel values was discovered by Jansen *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 523) and independently by the authors. It was found that a portion of the ethanol commonly used in the preparation of pectin is so strongly adsorbed that it cannot be removed by drying *in vacuo* at 80° C. Ethyl iodide formed from the adsorbed ethanol is measured as methoxyl in the usual Zeisel procedure, and, since the amount of adsorbed ethanol may be 4% of the pectin, the error from this source is considerable. Jansen *et al.* (*loc. cit.*) detected the presence of bound ethanol in pectin by the observation that the Zeisel value was lowered when the pectin was treated with water vapour. Ethanol was considered to be completely removed when treatment of the sample with water vapour no longer affected the Zeisel value and the Zeisel value corresponded with the saponification value. This proof does not preclude the possibility that a portion of the ethanol is not removed or that the saponification values are inaccurate. The present work shows that the results of the saponification method depend upon the conditions of saponification and the type of pectin being analysed and, therefore, this method cannot be considered a reference method. More direct proof of the presence of adsorbed ethanol or of its complete removal is afforded by separation and measurement of ethyl iodide in the Zeisel distillate. The Zeisel method as modified by Clark (*J. Assoc. Off. Agr. Chem.*, 1932, **15**, 136; Analyst, 1932, **57**, 402) was used except that an aq. suspension of red phosphorus was used in the washer. Attempts to remove adsorbed ethanol by drying *in vacuo* for 2 hr. at 80° C. were unsuccessful. The ethanol can, however, be removed by the following method. Place the sample in the inner chamber of a Conway diffusion cell with water in the outer ring. Place the cell under a bell jar, evacuate and seal off the bell jar and allow the apparatus to stand overnight. Finally dry the sample, in which the ethanol has now been replaced by water, for 2 hr. *in vacuo* at 80° C. Many experimentally and commercially prepared pectins are pptd. by acetone, ethanol or *iso*propanol. These alcohols and, under certain conditions, the acetone react with hydriodic acid to form volatile iodides and determination of the methoxyl values of pectins by the Zeisel method should therefore include a preliminary treatment with water vapour, unless the sample is definitely known to be free from adsorbed precipitant. A study of the usual procedure for saponification as described by Myers and Baker (*Del. Agr. Expt. Sta., Bull.*, **187**, 1934) showed that no single set of conditions can be used for the accurate determination of methyl ester in various types of pectin by saponification. Acid de-esterification progressively removes non-galacturonide compounds from the polygalacturonide portion of the pectin, whereas enzyme de-esterification does not appreciably affect that portion, and it is apparent that the methoxyl values of pectins differing in galacturonide content are not strictly comparable. Two saponification procedures were therefore developed, one for acid-demethylated pectins and another for all other types.

For all types except acid de-esterified types moisten 2 g. of pectin with alcohol in a 600-ml. beaker and dissolve in 400 ml. of water by mechanical stirring. Titrate the soln. to *p*H 7·5 by adding 0·5 *N* sodium hydroxide, preferably with the aid of a *p*H meter equipped with extension electrodes. Transfer the neutralised soln. into a 500-ml. Erlenmeyer flask, adjust the temp. to 25° ± 1° C., and add an amount, *x*, of 0·5 *N* sodium hydroxide which is 5 ± 0·5 ml. in excess of the amount equiv. to the methoxyl content of the sample. (It may be necessary to carry out a preliminary saponification to determine the approximate amount.) Allow the soln. to stand at room temp. for 30 ± 2 min., add 0·5 *N* sulphuric acid equiv. to the amount *x* of sodium hydroxide used in the saponification and titrate the soln. to *p*H 7·5 with 0·5 *N* sodium hydroxide. The amount of 0·5 *N* sodium hydroxide *y* added in the final titration subtracted from the amount *x* added for saponification should be 5 ± 0·5 ml. If not, the saponification should be repeated with a different amount of sodium hydroxide determined by a new preliminary estimate. The % of methoxyl is calculated from the amount of 0·5 *N* sodium hydroxide consumed in the saponification by multiplying by 1·55 and dividing by the wt. of pectin saponified. For acid de-esterified pectin the saponification is carried out in the same way except that 40 ml. of 0·5 *N* sodium hydroxide are used and the time of saponification is increased to 40 min.

A reliable and convenient method in which pectase is used for determination of pectin methyl ester has been developed. The enzyme may be prepared as follows. Grind firm ripe tomatoes to pulp in a food chopper, adjust the reaction to *p*H 7·5 by addition of 2 *N* sodium hydroxide and allow the mixture to stand for a few min. Drain the extract through cheese cloth and filter through coarse filter paper. Store the filtrate under xylene at room temp. for one day and then at about 0° C. until required. To 2 g. of pectin moistened with alcohol and dissolved in 400 ml. of water as before, add 10 ml. of 2 *N* sodium acetate and 10 ml. of 2% sodium oxalate soln. Neutralise to *p*H 7·5 with 0·5 *N* sodium hydroxide as before, add 20 ml. of tomato pectase extract (adjusted to *p*H 7·5), and allow to stand at room temp. for 2 hr. after which titrate the mixture to *p*H 7·5 with 0·5 *N* sodium hydroxide. The % of methoxyl in the sample is calculated by multiplying the amount of alkali used in the titration (ml.) by 1·55, dividing by the wt. of pectin hydrolysed and adding a correction of 0·75. If the sample is an acid de-esterified pectin with a methoxyl value less than 3% the multiplying factor is 1·94, and this includes a correction (25%) for unhydrolysed pectin. A. O. J.

Spectrophotometric Determination of α-Elaeostearic Acid in Freshly Extracted Tung Oil. Determination of Extinction Coefficients in Oil Solvents. R. T. O'Connor, D. C. Heinzelman, A. F. Freeman and F. C. Pack (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 467–470)—The excellent drying properties of tung oil are due to the presence of 70–80% of α-elaeostearic acid,* which is not found in appreciable amounts in other common commercial oils. An important characteristic of this acid is its absorption of ultraviolet radiation, a property due to the triene conjugation of the double bonds within the molecule. None of the other constituents of the oil shows any significant absorption of ultraviolet radiation above 220 mμ, and no corrections for overlapping absorption by diene- or tetraene-conjugated constituents are required. Also the high content of triene-conjugated material in the oil, together with the high value of the extinction coefficient of the acid, necessitates measurements of very dilute solns., and thus the effects of absorption by other constituents are negligible. Only the α-isomer of elaeostearic acid occurs naturally in the oil of the common species, the β-isomer apparently being formed after extraction of the oil by action of light or certain catalysts. The present method is intended for freshly extracted samples containing only the α-isomer, and in which polymerisation by heat and other agencies has been minimised. Determination of the wave length positions of max. absorption and the corresponding extinction coefficients were made with specially prepared samples, and these measurements confirmed the value of the extinction coefficient in 99% alcohol, *viz.*, 189, found by van der Hulst (*Rec. trav. chim.*, 1935, **54**, 629) and Miller and Kass (Amer. Chem. Soc. Meeting, St. Louis, 1941), and showed that there is an appreciable difference between the value of the extinction coefficient found in 99% alcohol soln. and in iso-octane (169·8) and cyclohexane (168·6). Further expts. with stored material supported the suggestion of Kass (*Protective and Decorative Coatings*, ed. by J. J. Matiello, Vol. IV, Chap. 12, New York, John Wiley & Sons, 1944) that the lower values found by Dingwall and Thomson (*J. Amer. Chem. Soc.*, 1934, **56**, 899) are due to deterioration of the oil during storage. Iso-octane and cyclohexane are recommended as solvents and the procedure is as follows.

Dissolve an accurately weighed sample of the oil in the solvent and dilute to a concn. of about 0·005 g. per litre. Read the optical density of the soln. directly in a Beckman spectrophotometer in a 1 cm. cell at 270 mμ with iso-octane as solvent and at 271 mμ with cyclohexane, and calculate the α-elaeostearic acid content from the equation:

$$\text{concn. \%} = \frac{\text{optical density}}{\alpha} \frac{100}{cl}$$

where α is the extinction coefficient, c the concn. of the soln. and *l* the internal length of the cell in cm. To establish the accuracy of the method, known mixtures of tung oil and mineral oil were examined. The results showed that admixture with more than 4% of mineral oil can be detected. Whilst expts. indicated the extreme instability of pure α-elaeostearic acid crystals, chemical and spectrophotometric evidence indicates that in tung oil the acid is considerably more stable. Although the freshly extracted oil need not be examined on the day of extraction the measurements should be made as soon as is practicable. Changes in the oil depend more upon conditions of storage than upon time of storage, exposure to light, heat or oxidation being the most important factors causing the changes.

A. O. J.

* Hilditch found 90·7% on the total fatty acids (*Industrial Fats and Waxes*, p. 165).—EDITOR.

Agricultural

Determination of 1-Trichloro-2,2-*bis* (*p*-Chlorophenyl) Ethane in Technical DDT. S. J. Cristol, R. A. Hayes and H. L. Haller (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 470–472)—Technical DDT consists essentially of a mixture of two isomeric compounds, *viz.*, 1-trichloro-2,2-*bis* (*p*-chlorophenyl)ethane (*p,p'*–DDT) and 1-trichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane (*o, p'*–DDT), with small amounts of by-products and impurities. The technical product is obtained by condensation of chloral with chlorobenzene in presence of sulphuric acid, and when pure chloral is used the product contains 70–80% of *p, p'*–DDT with 15–25% of the less insecticidal *o, p'*–DDT. When dichloroacetaldehyde is present in the chloral the product may contain dichlorodiphenyldichloroethane isomers (DDD). A method for determination of *p, p'*–DDT in presence of the other compounds is therefore required, and the following procedure was found satisfactory for products containing at least 40% of *p, p'*–DDT.

Prepare a saturated stock soln. of *p, p'*–DDT in 75% v/v aqueous ethanol and allow it to remain in a thermostatically controlled bath at 25° ± 0·5° C. or other convenient temp. Reflux 2 g. of the technical sample with 150 ml. of the stock soln. in a 250 or 300-ml. Erlenmeyer flask. After the sample has completely dissolved allow the stoppered flask to cool slowly in air to about 26° to 30° C. Crystals of *p, p'*–DDT will be deposited. If separation of oil occurs re-dissolve the oil by refluxing again and, if necessary, seed the cooling liquid with a crystal of *p, p'*–DDT. Shake the flask in the thermostat intermittently for 4 hr., collect the crystals by filtration through a tared Gooch crucible containing a disc of filter paper, using an additional 20 ml. of the sat. soln. to wash the crystals into the crucible and avoiding suction of air through the crystals during filtration. Dry the crystals at 78° to 80° C. until their wt. is constant and finally determine their m.p. The corrected m.p. should be above 106° C. Add an empirical correction of 1·4% to the amount found. To apply the method to material containing less than 40% of *p, p'*–DDT add a known amount of the pure substance sufficient to bring the new mixture within the range of the method. Treat 2 g. of the mixture as previously described, add 0·028 g. (=1·4%) to the wt. of the crystals, subtract the wt. of material added and divide by the amount of the original product taken. To apply the method to insecticidal dusts, extract a weighed amount of the dust containing 5 to 10 g. of material soluble in ether with ether in a Soxhlet extractor for 4 hr. Remove the solvent by evaporation on the water-bath and finally under reduced pressure and weigh the residue. Grind the residue in a mortar to ensure representative sampling and treat 2 g. as already described. A. O. J.

Gas Analysis

Quantitative Absorption of Oxygen by Chromous Salt. H. W. Stone and E. R. Skavinski (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 495–498)—The reagent is a 2 *M* soln. of crystals

of green chromic chloride in cold 2 *M* acetic acid; it is forced by pressure through 0·1% mercury - zinc amalgam into the absorption pipette, the exit end of the reductor being provided with a compact plug of glass wool to arrest fine particles of amalgam. The reagent has a capacity of 11 volumes of oxygen and absorbs it at a much faster rate than does alkaline pyrogallol; it is most useful for the separation of oxygen from acid gases. The authors used it for the precise determination of oxygen in air, employing the apparatus sketched and described; they found 20·947% as an average of 60 determinations with an average deviation of ±0·007%. Three passes of the air were required for complete absorption. The objection against the use of chromous salt in gas analysis—evolution of hydrogen in acid soln.—does not hold good in the case of acetic acid solns. with their relatively high *p*H. Some hydrogen is evolved on long standing, but the reagent is still safe if used within 10–12 hrs., and in the procedure described the chromous salt is prepared immediately before use. W. R. S.

Reviews

THE MEASUREMENT OF COLOUR. By W. D. WRIGHT, A.R.C.S., D.Sc. Pp. vii + 223. London: Adam Hilger, Ltd. Price 30s.

The colour of an object depends on both the nature and the relative proportions of the vibration frequencies which give rise to it, and, as is well known, it can be appreciated both qualitatively and quantitatively with varying degrees of efficiency by the normal eye, the photographic plate and various devices capable of transforming its energy into heat and electricity. A colour may be recorded by comparison with the standard shades of a colour atlas or other graded series of colours, by measurement of the relative energy radiated at the different wavelengths of the visible spectrum, by matching against mixtures of white, black and the spectral colour of a single visible wavelength, or by matching against a suitable mixture of three selected radiations.

The last-mentioned of these methods received international recognition and standardisation in 1931, when the Commission Internationale de l'Eclairage adopted a set of tables defining the characteristics of a standard observer and established a reference framework for specifying colours on the trichromatic system. This system has now become the fundamental one underlying all others, and forms the main subject of this book.

It is not simple for any one unfamiliar with the C.I.E. system to visualise colours defined in its terms. The standard observer and reference stimuli are mathematical abstractions, and their relation to spectral hues must be understood for such visualisation. The deliberate removal of the reference standards from a practical plane has probably resulted in a narrower use being made of the system than if other standards had been chosen; it is obviously desirable that its popularity should become greater for there is a remarkable unanimity amongst physicists regarding its merits.

There are very few books on the subject, and it may well be that the use of the C.I.E. system would be stimulated if it were expounded clearly and concisely in a readily available work. Such an exposition is admirably provided here. Starting with a general description of visible radiations, the author proceeds to deal fully with the trichromatic systems having RGB and XYZ reference stimuli and with the C.I.E. system of colour specification. He describes the colorimeters adapted to make measurements on these systems and the practical means for transforming their results into terms of different systems. Three appendixes contain details of the illuminants and distribution coefficients required for computations, and chapters are devoted to spectrophotometry in the measurement of colour, to photoelectric and other colorimeters, to the colour atlas as a substandard and to the practical applications of colorimetry.

The author must be congratulated on having written an extremely interesting and well balanced book. K. A. WILLIAMS

COLLECTED PAPERS ON METALLURGICAL ANALYSIS BY THE SPECTROGRAPH. Edited by D. M. SMITH. Pp. x + 162; 58 Figs. London: British Non-Ferrous Metals Research Association. 1945. Price 21s.

Twelve years ago the British Non-Ferrous Metals Research Association published a monograph by D. M. Smith on Metallurgical Analysis by the Spectrograph (reviewed in THE ANALYST for March, 1934), with the expressed intention of "encouraging the more

general adoption of spectrographic methods." The methods then described were those which had been developed in the Association's own laboratory, special attention being given to the quantitative analysis of zinc, tin, copper and lead. As a result of the Association's continued activity in the spectrographic field, interest has now developed to such an extent that it is most convenient for the Association's Spectrographic Sub-Committee to operate through a number of specialist panels. These panels consider reports, not only from the Association's own research staff, but also from the laboratories of member companies.

The present volume contains a number of papers based on these reports. These include two papers on photographic processing and plate calibration, four on aluminium and its alloys, three on lead and its alloys, one on zinc alloys, two on copper alloys and one on platinum. It is interesting to note the almost universal adoption of microphotometry and calibration curves, as compared with the somewhat cumbersome "analytical tables" of the previous book, in which the comment was made (p. 54), " it is open to question whether the extra time and manipulation required for microphotometric methods are justified by the results which may be obtained." Mr. Smith has now provided the answer to his own question. It is valuable to have contributions in the present volume dealing with photographic processing and calibration, since attention to this aspect of technique has contributed not a little to the increased accuracy of spectrographic methods.

The book is admittedly not a systematic treatise on spectrographic analysis, but is a valuable supplement of expert contributions, which should interest all users of the method, particularly those concerned with the metals mentioned. An adequate subject index is provided, and production and printing are excellent. B. S. COOPER

PHYSICAL METHODS GROUP

AN Ordinary Meeting of the Group will be held at the Chemical Society's Rooms, Burlington House, Piccadilly, London, W.1, at 6.30 p.m. on Tuesday, February 26th, on the subject of Chromatographic Analysis.

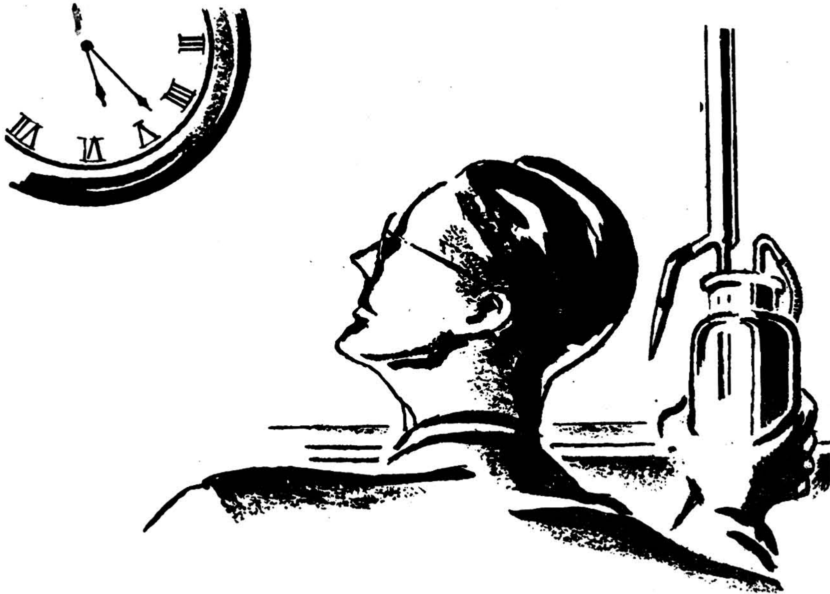
The following short papers will be read:

"The General Principles," by F. A. Robinson, M.Sc., LL.B., F.R.I.C.

"Partition Chromatography," by R. L. Synge, B.A., Ph.D.

"Chromatography in the Analysis of Fatty Oils," by K. A. Williams, B.Sc., F.R.I.C.

"Some Applications of Chromatographic Analysis in Industry," by F. R. Cropper, Ph.D., F.R.I.C.



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