THE ANALYST

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

Published for the Society by
W. HEFFER & SONS LTD., Cambridge, England

Volume 74 Price 3s. 6d.
No. 884, Pages 577—616 November, 1949

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held on Wednesday, October 5th, 1949, at 6.45 p.m., in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1. The President, Mr. George Taylor, O.B.E., F.R.I.C., was in the chair. The following papers were presented and discussed: "The Calculation of the Botanical Composition of Wheat Flours and Offals from the Chemical Analysis," by J. Straub, Chem.Ing.; "A Photo-electric Method of Determining the Colour of Flour as Affected by Grade, by Measurements of Reflecting Power," by D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C., and W. Martin, B.Sc.; "Experiments in the Photo-electric Recording of Flour Grade by Measurements of Reflecting Power," by D. W. Kent-Jones, B.Sc., Ph.D., F.R.I.C., A. J. Amos, B.Sc., Ph.D., F.R.I.C., and W. Martin, B.Sc.

NEW MEMBERS

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DEATHS

WE regret to have to record the deaths of

Sir Robert Howson Pickard (Honorary Member).
William Pearson Skertchly.

The Assay of Curare and Curarimimetic Substances

The following four papers were read at a meeting of the Biological Methods Group on Thursday, October 21st, 1948.

The Assay of *d*-Tubocurarine Chloride on the Isolated Rat-Diaphragm A Statistical Examination

BY G. A. MOGEY, J. W. TREVAN AND P. A. YOUNG

THE use of the Bülbring rat diaphragm preparation (Bülbring[1]) for assay of curarising drugs was described by one of us (J. W. T.) at the meeting of the Pharmacological Society in January, 1946, and Chou[2] has independently described a similar method. We have now examined the method statistically.

The preparation was essentially the same as that described by Bülbring except for the electrode for stimulating the nerve. This is represented in Fig. 1. It consists of a ¼-inch glass tube, A, with an oblique opening at the bottom over which is stretched a rubber membrane, B, with a hole in the middle made by boring slowly with a wet darning needle; pushing the needle straight through will split the rubber; silk thread is attached to the central

end of the phrenic nerve, C, which is gently drawn through the hole in the membrane. One silver - silver chloride electrode, E1, is placed inside the tube, which contains a little Ringer - Locke solution, and another, E2, in the bath which contains the rat diaphragm, D. The rubber membrane dips into the bath and a potential difference between the electrodes causes stimulation of the nerve where it passes through the membrane. The current should pass down the nerve to avoid anodal block. Slightly supramaximal stimuli, varying in voltage with the size of the hole in the membrane, are used; 1·5 volts is about the maximum necessary. The stimulus is a short pulse of direct current less than about 1 m.sec. in duration. We use 0·33 m.sec. Longer stimuli produce repetitive responses (Mogey and Trevan[3]) in the nerve, with tetanic responses of the muscle, varying in height from nerve volley to nerve volley, and often during a long assay diminishing in average height too much for accurate work. Ringer - Locke solution of the following composition, with oxygen bubbled through, is used:

NaCl	90 g.
KCl	4·8 "
$CaCl_2$	2·4 "
Glucose	10·0 "
$NaHCO_3$	5·0 "

Distilled water to 10 litres.

Tyrode solution gives rather smaller regressions of response on dose.

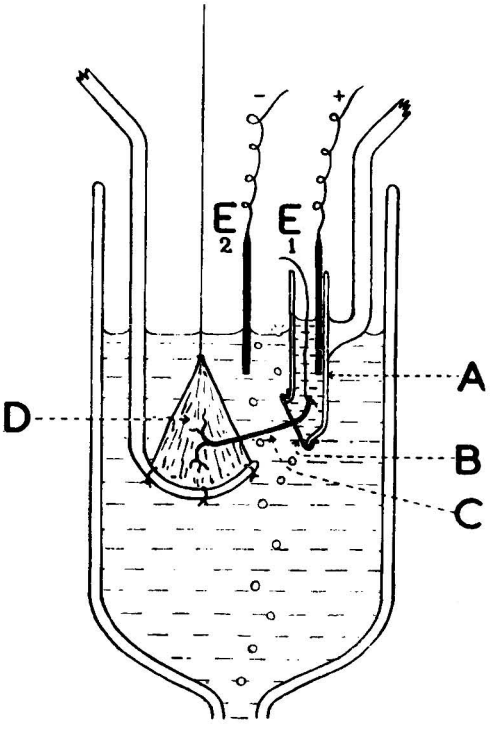

Fig. 1. Diagram of isolated organ bath showing: A, glass tube sealed at lower end with B, a thin rubber membrane through which C, the phrenic nerve passes. The diaphragm segment, D, is tied to a glass supporting rod below, the apex being connected to a writing lever by a thread. A potential applied between the silver - silver chloride electrodes E1 and E2 stimulates the nerve where it passes through B.

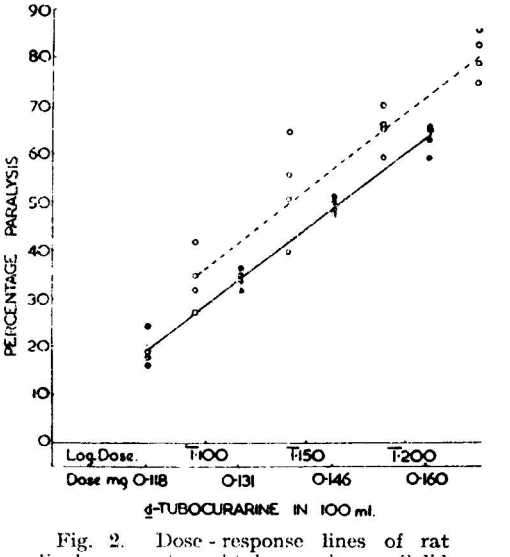

Fig. 2. Dose - response lines of rat diaphragm to *d*-tubocurarine. Solid circles: Experiment 1. Open circles: Experiment 2.

The accuracy of the method has been examined by applying the analysis of variance to a series of randomised doses. Four doses, each 0·9 of the next, were added to the bath, the Ringer - Locke solution being replaced between successive doses. The doses were given in an order determined from a randomly chosen 4 × 4 Latin square. The first two or three doses administered after the isolation of the diaphragm show a fairly rapidly increasing effect, which will be the subject of another communication. Three or four preliminary doses are therefore given before the actual assay is begun. These preliminary doses can be used to determine the approximate equivalent doses of the standard and test. The response after these preliminary doses sometimes still shows a significant alteration, but it is of smaller dimensions and rarely if ever of such extent as to invalidate the experiment by the occurrence of complete paralysis with any dose. This alteration in response can be eliminated from the calculation of the error by the variance analysis.

Fig. 2 shows two examples of the response to such a series of 4 × 4 doses of a solution of *d*-tubocurarine randomised as described, with a regression line fitted to each. Linearity of regression is found between 20 per cent. and nearly 100 per cent. paralysis in different experiments. Above about 85 per cent. the variance alters, so that for accurate error estimation, doses should be so adjusted that the paralysis is between 20 and 80 per cent. Table I shows the arrangement of the injections and the recorded responses measured on a smoked drum for experiments where A, B, C and D represent the doses in increasing magnitude from A to D, A being 0·9 of B and so on. Underneath each letter corresponding to a dose is the percentage paralysis, the contraction being measured on the kymograph to 0·25 mm. at the time when the maximum effect was attained. The doses were administered in the order represented by reading across each row successively.

TABLE I

Percentage paralysis of a rat diaphragm produced by the doses of *d*-tubocurarine A, B, C and D, in Experiment 1 (Fig. 2)

A/B = B/C = C/D = 0·9

D	A	B	C
64	25	35·5	48·25
B	D	C	A
34·5	60·0	51·5	17·75
C	B	A	D
48·5	32·25	16·5	67·0
A	C	D	B
18·75	51·75	67·0	36·5

The result of the analysis of variance is given in Table II. The rows and columns both show the effect, if any, of an alteration of response with time. The columns give a

TABLE II

ANALYSIS OF VARIANCE OF THE RESULTS PLOTTED IN FIG. 2

	N	Expt. 1 Mean square	Expt. 2 Mean square	F	P
Rows	3	7·40	109·64	6·17	< 0·05
Columns	3	1·06	65·18	3·67	< 0·2
Doses	3	1506·47	1713·51		
Error	6	11·84	17·87		
	15				
Regression coefficient of % paralysis on $\log_{10}$ of dose, (*b*)		330	348		

measure of the effect over the time taken for the application of four doses; the rows give the mean effect over the time taken for sixteen doses. In the two examples above, in Experiment 1 there is no significant effect of this kind; in Experiment 2 the variance of the rows is significant, but that of the columns, though large, is not significant. The calculation of the P 0·95 ratios gives, using only the error, with six degrees of freedom for one pair of

observations, 1·085 (Experiment 1) and 1·102 (Experiment 2) and for eight pairs of observations 1·028 (Experiment 1) and 1·034 (Experiment 2). Eight pairs of observations correspond to the division of sixteen doses between test and standard solutions. This omits that part of the error that would be introduced when the means of the doses of standard and test differ. They can at all times, by means of the preliminary doses, be placed at not more than 10 per cent. apart and the appropriate correction, though easy to calculate, will generally be very small because b is almost invariably greater than 10 times and may be over 50 times its standard error. The value of b is very high. With $b = 330$, a drop of 10 per cent. in the dose produces an arithmetical difference of 15·1 per cent. in the percentage paralysis, *e.g.*, from 60 to 44·9 per cent. Since the latter usually corresponds to about 2·5 to 3 cm. in the tracing on the drum, the effect due to error of measurement of the tracing is negligible.

TABLE III

DATA EXTRACTED FROM ANALYSES OF CONSECUTIVE ROUTINE ASSAYS OF *d*-TUBOCURARINE

(1) Assay No.	(2) b slope	(3) Vb	(4) V error $n = 10$	(5) V order $n = 3$	(6) Mean time per dose (min.)	(7) Ratio of P 95 limit to mean
1	375	101	6·3	83·4	9·5	1·017
2	307	646	55·1	755·0	12·7	1·064
3	209	106	13·2	255·0	11·4	1·046
4	263	220	27·2	745·0	15·1	1·052
5	291	422	36·0	169·0	12·3	1·054
6	310	510	31·9	103·0	7·7	1·057
7	339	37	4·6	81·5	5	1·016
8	323	2350	66·0	35·2	5	1·061
9	332	173	18·3	116·9	5	1·034
10	338	43	3·4	30·4	5	1·014
11	361	540	29·3	135·4	5	1·040
12	290	455	24·3	21·3	5	1·042

Table III shows a series of routine tests. In these a 4 × 4 Latin square design was also used, with two doses of standard and two of test, each pair of doses being in the same ratio one to another, usually about 1·3. The first six tests were read at equilibrium, that is to say, when the dose of tubocurarine had produced its maximum effect on the contractions, which remain almost constant even if the dose is left in for some hours. The table gives in the successive columns, (1) the identification number of the assay, (2) b, the coefficient of the regression of percentage paralysis on $\log_{10}$ dose, (3) Vb, the variance of b, (4) V (error), obtained by pooling the error term of the analysis of variance, the variance of columns, and the component of the mean square for elimination of lack of parallelism of the regression, neither of the latter two being significantly higher than the error term. This gives 10 degrees of freedom (6 + 3 + 1). Column (5), V (order), shows the variance eliminated for rows. Amongst the six tests under discussion, only No. 6 shows an order variance that is not significant. Column (6) gives, in minutes, the mean duration of action of the doses. Column (7) gives the ratio of the upper fiducial limit (P = 0·95) to the mean activity of test in terms of standard.

Response after fixed time—Examples 7 to 12 in Table III show the results of the analyses of assays in which, instead of waiting for each dose to produce its maximum effect, it was allowed to act only for precisely 5 minutes; the degree of paralysis at that time was used as a measure of the effect. This is similar to the procedure used by Chou[2] except that his experiments were not designed to eliminate the change in sensitivity. He used "bracketting" of doses to estimate the relative potency of the test and standard, which renders error determination difficult and uncertain.

Examination of the original records of our preliminary four-dose experiments with one solution shows that a linear regression still results with observations at this time. In using the paralysis at a fixed time it is important to ensure that the dose is injected into the bath well away from the muscle. If the concentrated solution impinges on the muscle, the local rise in concentration will markedly accelerate the rate of onset of paralysis, although the final degree of paralysis is the same when equilibrium is established. The figures given for examples 7 to 12, obtained by the standard time interval method, show that the value of b is rather

higher than for examples 1 to 6; the error term after elimination of order effects is nearly the same on the average (although this is greatly influenced by the high value, 66, for number 8). Whilst order mean square is still significant in 4 out of 6, it is considerably lower than that for examples 1 to 6. This is not suriprsing, as the total duration of the "fixed-time" test is less than half that of the other method, and the sensitivity of the preparation increases with time.

Experiments with Tyrode solution—A number of assays of irregular design in which Tyrode solution instead of Ringer - Locke solution was used, gave results for which the order effect could not be eliminated, and a value of b significantly less than that given in either of the Ringer - Locke series. Table IV gives the mean values for various elements of the variance analysis. This table shows that the mean square for (order + error) is not

TABLE IV

MEAN VALUES FROM THE ANALYSES OF THREE SERIES OF ROUTINE *d*-TUBOCURARINE ASSAYS

Series		Mean square order + error	n	Mean square error	Slope (b)	Variance of mean b
I	Tyrode irregular design ..	105·8	88	—	177	85
II	Locke equilibrium readings	102·9	78	24·3	291	34
III	Locke dose acting for 5 min. only	34·9	78	28·3	337	16·5

significantly different for experiments with Tyrode solution (I), and Ringer - Locke solution (II), using the equilibrium effect as index. The error of assay with Tyrode solution would be higher because b is significantly less ($t = 11$, $p < 0{\cdot}001$). The mean square for (order + error) is much less for series III. This is due entirely to reduction in the order term as is shown by the figures for mean square for error, order being eliminated. It seems likely that a proper design for Tyrode solution would eliminate as large a mean square for order, for the average interval between doses was about the same as for series II, but the disadvantage of the lower slope for the Tyrode would remain. The series of mean squares for error (order eliminated) in both cases is heterogeneous (χ^2 test) as are the slopes for series I and II. The series of slopes (b) for I and II are also heterogeneous, but not that for III. In other words, the variance of the slopes in series III is less than the (heterogeneous) mean variance for error. The slope for series III is larger than that for series II ($t = 6{\cdot}47$, $p < 0{\cdot}001$). On all accounts, therefore, the method of series III is to be preferred as likely to have the smaller error, and at the same time to be more rapid and convenient in execution.

SUMMARY OF THE METHOD

A Bülbring preparation of the rat diaphragm and phrenic nerve is suspended in 100 ml. of oxygenated Ringer - Locke solution at 37° C. The nerve is stimulated by approximately rectangular pulses of direct current passing down the nerve, the pulses not exceeding 1 m.sec. in duration. Doses of the standard preparation are added from a "tuberculin" syringe, with three washes with Ringer - Locke solution between successive doses, until the response becomes uniform. The assay proper then starts with doses chosen so as to keep the responses between 20 and 80 per cent. paralysis; suitable concentrations can be selected by consideration of the effectiveness of the preliminary stabilising doses. Each dose is allowed to act for 5 minutes by stop clock. Three washes with Ringer - Locke solution are used between each pair of doses, taking about 5 minutes altogether. The four doses—two of standard and two of test—are assigned at random to the elements of a 4×4 Latin square to determine the order of testing; the analysis of variance determines whether an order effect is significant and can be eliminated.

DISCUSSION

The accuracy attainable with this method is very high for a biological assay. When the test gives fiducial limits (P = 0·95) little more than 1 per cent. either side of the mean, as it does with considerable frequency, it is of some interest to examine the possible elements of the variance. We can identify: (1) errors of measurement of dose, (2) errors of measurement of volume of Ringer - Locke solution and (3) inherent variability of response of endplate, besides (4) possible errors due to variations in temperature, oxygenation, etc. Then when the fiducial limits are just over 1·0 per cent., the standard deviation will be 0·5 per cent., and the variance

0·25 on a percentage scale. This is the mean derived from sixteen observations, and the variance will be 1·0 for a single observation. If this is divided equally between the first three sources of variability, neglecting the last, the variance for each will be 0·3 and the standard deviation 0·548 per cent. This corresponds to filling the bath to a mark with 100 ml. of Ringer-Locke solution with a standard deviation of 0·548 ml., and measuring 1 ml. to 0·0548 ml. with a syringe, or with a "tuberculin" syringe of 4·0 cm. length for each ml., setting the piston with a standard deviation of about 0·25 mm. Both of these operations need some care to attain this accuracy. In fact we are approaching a situation where the "independent" variable of the regression (the dose added) suffers from an error large enough to render the ordinary methods of calculating regression coefficients not adequate. But the order of error as calculated is not far out, and it is clearly adequate for most practical needs. On the other hand, it is extremely unlikely that the fiducial limits of ±5 per cent., which are obtained with some diaphragms, are greatly affected by errors of measurement of dose.

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1. Bülbring, E., *Brit. J. Pharmacol.*, 1946, **1**, 38.
2. Chou, T. C., *Ibid.*, 1947, **2**, 1.
3. Mogey, G. A., and Trevan, J. W., *J. Physiol.*, 1948, **107**, 28P.

THE WELLCOME RESEARCH LABORATORIES
BECKENHAM, KENT

The Assay of Curare and Curare-like Substances: A Modification of the Method using the Rat's Phrenic Nerve-Diaphragm Preparation

BY G. B. WEST

MOST workers using the isolated phrenic nerve-diaphragm preparation of the rat for estimating curare-like substances have suspended the tissue in Ringer's solution at 37° C. We were unable to obtain constant contractions for many hours at this temperature when using fluid electrodes and condenser discharges from a neon-lamp circuit. In consequence, the temperature of the fluid surrounding the tissue was reduced to 20° C. The rate of stimulation was 8 stimuli per minute. The contractions of the muscle were recorded by an isotonic lever; the tension developed in most preparations varied from 1 to 10 g., and remained constant for several hours if the rate of stimulation did not alter. When a dose of tubocurarine, sufficient to produce an inhibition of 50 per cent. of the muscle contraction in about 5 minutes, was added to the bath at 20° C. and the Tyrode solution was changed after 5 minute's action, the recovery of the preparation to its original base-line required about 30 minutes. This length of time was of no use for routine work, and efforts to reduce it were made by utilising some of the constituents of the Tyrode solution.

Pure depression resulted when doses of calcium chloride or magnesium chloride were added to the bath of Tyrode solution. Potassium chloride, on the other hand, exerted two effects. In small doses, up to 45 mg., pure potentiation occurred; more than 50 mg. resulted in initial stimulation followed by depression, the latter being quite similar to that occurring with moderate doses of tubocurarine. Small doses of potassium, however, were not effective if the tubocurarine was in the bath, either before or after the potassium. The tubocurarine must be removed from the bath before the potassium (40 to 45 mg. of KCl) can aid recovery. This extra potassium was then washed out before the next dose of curare (100 μg. to a 75-ml. bath) was added. For the assay process, inhibitions of about 50 per cent. of the muscle contractions were produced, doses of the preparation under test being added at 8-minute intervals, so that a large number of estimations were made each day. With the lower temperature the diaphragm showed little sign of fatigue after 8 hours. It was possible to detect 7 to 10 per cent. difference in activity by this means.

REFERENCE

West, G. B., *Quart. J. Pharm. Pharmacol.*, 1947, **20**, 518–527.

SCHOOL OF PHARMACY
UNIVERSITY OF LONDON

The Assay of Curarizing Activity in the Conscious Mouse and Rat

By H. O. J. COLLIER, E. C. FIELLER and R. A. HALL

Introduction

At least two methods, both of quantal type, have been proposed for estimating the activity of curarising agents in the mouse. Pick and Richards[1] have proposed the intraperitoneal administration of *d*-tubocurarine to mice that have been dosed with morphine sulphate 10 minutes previously. They find that a dose of 140 ± 10 μg. of *d*-tubocurarine chloride per kg. of body weight temporarily abolishes in 50 per cent. of mice the tail erection due to morphine. From their published figures the slope of their log dose - probit line is approximately 5·5. This method may be criticised on the ground that it introduces an additional factor, morphine, into the situation. This appears to be unnecessary, since several workers have found it possible to assess the effect of *d*-tubocurarine in mice without previously administering morphine.

Skinner and Young[2] have administered *d*-tubocurarine subcutaneously to mice, using a rotating cylinder to decide whether or not a certain degree of paralysis has resulted. In a series of experiments, each involving 100 mice, these authors obtained eleven rectilinear log dose - probit lines, of which the slopes varied between 2·89 and 13·94 with a weighted mean of 7·81. The ED 50 of the hydrated salt of *d*-tubocurarine chloride in their series of experiments ranged from 295 to 538 μg. per kg. of body weight.

The object of the present communication is to report on methods that two of us (H. O. J. C., R. A. H.) have used in the mouse for the estimation of the activity of *d*-tubocurarine, and in the rat for the estimation of its dimethyl ether, for a considerable time. These methods resemble in many respects those of Skinner and Young, but differ essentially in the use of the intravenous route of administration.

Methods

The mouse—In any one experiment, batches of male mice of the same strain and of approximately equal weight were used, in dosage groups of 5 to 20 mice. Immediately before dosing, each mouse was weighed to the nearest gram on a recording balance. In most of the experiments upon which this account is based a single solution of *d*-tubocurarine was made up, and the dose was adjusted in volume to give the dosage level required and administered by tuberculin syringe. In order to obtain greater accuracy of dosing the procedure was later adopted of preparing serial dilutions of the solution to be administered and giving a volume of 0·4 ml. per 20 g. to every mouse. In most experiments the operator controlled the rate of injection to 1 ml. per minute by means of a stop-watch. During the 2 minutes subsequent to the beginning of administration, the animal was observed carefully and turned on its back from time to time. If the mouse was unable to right itself its reaction was recorded as positive.

In assays of the quantal type little information is provided by dosage groups in which all or none of the animals respond; to avoid such groups it was necessary, owing to the steepness of the log dose - probit lines and the small numbers of mice per group, to reduce the successive dosage levels by factors between 0·90 and 0·95.

The rat—Young rats of the same strain, weighing 50 to 100 g., were used. The procedure of test for the rat differed from that in the mouse only in the volumes injected and in the actual dosages employed.

Curarising drugs—The results reported here have been obtained with commercial samples of pure *d*-tubocurarine chloride, with extracts of curare and with the dimethyl ether of *d*-tubocurarine di-iodide (*d*-OO-dimethyltubocurarine). Prior to the issue of the national standard of *d*-tubocurarine we set aside one sample of this substance and one of its dimethyl ether to serve as provisional laboratory standards.

Results

Log dose - probit lines—Experiments with *d*-tubocurarine in the mouse, carried out in the way described above, provide data that can be submitted to the customary routine of

plotting the dosage - response curve, testing its linearity, and estimating its slope and the ED 50 and its fiducial limits. Typical data obtained in the series of experiments carried out during the present year are given in Table I, and illustrated in Figs. 1 and 2.

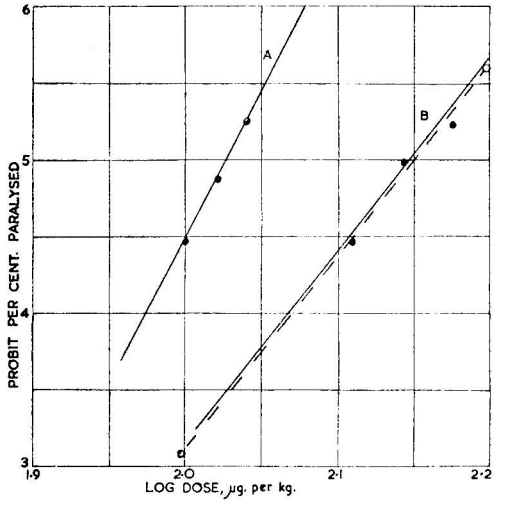

Fig. 1. Log dosage - probit paralysis lines for two specimens of *d*-tubocurarine in the mouse. The broken line is computed

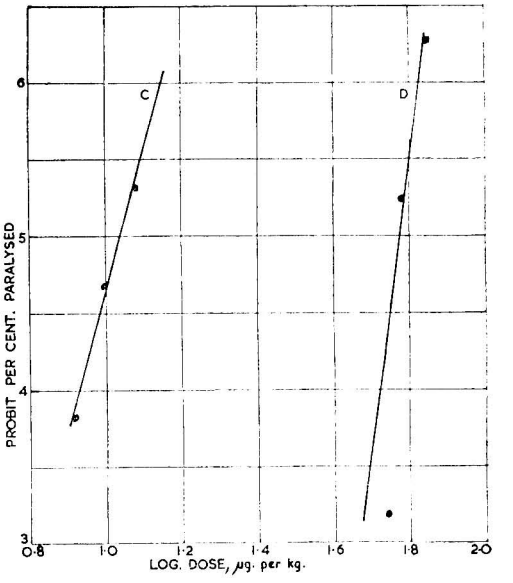

Fig. 2. Log dosage - probit paralysis lines for: (C) *d*-OO-dimethyltubocurarine and (D) *d*-tubocurarine in the rat

TABLE I

DATA UPON WHICH CURVES A, B, C AND D IN FIGS. 1 AND 2 ARE BASED

Curve	Drug	No. of animals	Dosage level μg. per kg.	No. paralysed /total No.
A	*d*-TC	60 mice	100	6/20
			105	9/20
			110	12/20
B	*d*-TC	30 mice	130	3/10
			140	5/10
			150	6/10
C	DME	24 rats	8·3	1/8
			10	3/8
			12	5/8
D	*d*-TC	22 rats	55	0/7
			60	3/5
			70	9/10

Although most of the tests supplied similarly steep estimates of slope, the standard errors attaching to these individual estimates were large, owing to the small size of the dosage groups and to the necessarily restricted ranges of doses administered. For curve A, for example (Fig. 1), the slope is 19 ± 10, so that the fiducial limits (P = 0·95) of the ED 50, if it were estimated from these data alone, would be infinite.

For these particular experiments, however, reliable estimates can be obtained by using a pooled value of the slope of the log dose - probit line. The tests cover a total of 662 mice, and provide 34 separate estimates of slope. These prove to be in good agreement with one another ($\chi^2 = 24{\cdot}1$ with 33 degrees of freedom), and supply a pooled estimate of slope 16·3 ± 2·0. There were more than two dosage groups in 14 of the series of 34, and in none of these were the deviations from linearity significant.

With *d*-tubocurarine and its dimethyl ether dosage - paralysis curves may be obtained

also in the rat in the way described above. A typical curve for each substance is illustrated in Fig. 2. These curves are based on the data given in Table I.

Whilst *d*-tubocurarine may be satisfactorily estimated in the mouse, for various reasons discussed below the rat is more suitable for the estimation of preparations of *d*-OO-dimethyltubocurarine. Experiments carried out during the present year with this substance in 261 rats have provided twelve separate estimates of the log dose - probit line. These also prove to be in good agreement ($\chi^2 = 15{\cdot}1$ with 11 degrees of freedom) and supply a pooled estimate of slope $14{\cdot}8 \pm 2{\cdot}0$. There were more than two dosage groups in seven of the experiments and in none of these were the deviations from linearity significant.

Examination of the figures on which the above conclusions are based indicates that although the slopes of the log dose - probit lines, both of *d*-tubocurarine in the mouse and of *d*-OO-dimethyltubocurarine in the rat, do not differ significantly from one test to another, their position may alter somewhat on different occasions. It is consequently necessary to employ the appropriate standard preparation in each test.

The effect of rate of injection—The question naturally arises whether or not it is necessary to inject the curarising substance at a constant rate. Experiments with *d*-tubocurarine in mice indicate that, within wide limits, the speed of intravenous injection makes little difference to the response to the curarising agent. A typical experiment is illustrated in Table II.

TABLE II

EFFECT OF RATE OF INTRAVENOUS INJECTION UPON THE RESPONSE TO *d*-TUBOCURARINE IN MICE

No. mice	Rate of injection in ml. per min.	Dosage levels in μg. per kg.	No. paralysed /Total No.
30	1	100	3/10
		105	4/10
		110	6/10
30	9–10	100	3/10
		105	5/10
		110	6/10

It is evident from these figures that it would be difficult to show a difference between the responses to injections at a rate of 1 ml. per minute and at a rate of 9 to 10 ml. per minute. Consequently no serious error is likely to arise if we fix a rate of injection at 1 ml. per minute.

The effect of the body weight of mice—As mice in any batch may vary slightly in body weight, we thought it important to investigate whether or not variation in the weight of mice used affected the response to *d*-tubocurarine. The results of an experiment set out in Table III indicate that it would be difficult to show a significant difference in response between mice weighing 14 to 17 g. and those weighing 20 to 26 g. We concluded therefore, from this and similar experiments, that no serious error was likely to arise owing to differences in the weights of mice in an experimental batch if the weight range was less than 5 g.

Repeated use of animals—At the dosage levels used in the experiments described above the loss of righting reflex seldom lasts long, and deaths are very infrequent. Recovery appears to be complete, for we found that the same batch of mice or rats, when used once weekly for two or more successive weeks, continued to give responses suitable for assay purposes.

The fact that mice or rats may be used repeatedly suggests that the accuracy of our assay might be increased by adopting a cross-over design. Such a design, however, might entail an unacceptable delay in the completion of the test.

Effect of strain of mice—During these tests we used on different occasions two different strains of white mice obtained from two suppliers. It was evident from a number of tests that one strain was more sensitive than the other. This fact is exemplified by comparison of Table II with Table III. The same sample of *d*-tubocurarine was used on both occasions, but a different strain of mice was employed. If a standard *d*-tubocurarine and a single strain of mice is employed in each test, no error is likely to arise as a result of strain differences.

Duration of paralysis as a basis of assay—In a previous publication[3] it was suggested that the mean duration of paralysis, as indicated by the loss of righting reflex, is a continuous variate which may be usable for the estimation of curarising activity. From the slopes of the various log dose - response lines obtained for *d*-tubocurarine and its dimethyl ether in the mouse, rat and rabbit, the response of the rat to the dimethyl ether appeared to offer the

TABLE III

EFFECT OF WEIGHT OF MICE UPON THE RESPONSE TO *d*-TUBOCURARINE

No. mice	Range of weight in g.	Dosage level μg. per kg.	No. paralysed /Total No.
40	14–17	71	5/20
		79	15/20
40	20–26	71	5/20
		79	10/20

best possibilities of use. Analysis of these and subsequent results, however, shows that the two following objections make this response unsatisfactory for assay purposes—

(1) The variance of the response at each dosage level is high.
(2) At higher dosage levels deaths of animals not infrequently occur. In practice this is wasteful, and in theory it is difficult to know how to treat deaths.

DISCUSSION

Experimental considerations—Use of the subcutaneous or intraperitoneal routes of administration of a drug introduces variations in the responses of individual animals due to (1) different rates of absorption into the blood stream from the site of injection and (2) different rates of elimination of the drug either by its excretion or destruction. If the intravenous route of administration is employed, variation due to different rates of absorption becomes negligible. Since *d*-tubocurarine takes effect, if at all, within 1 minute of its intravenous administration to the mouse, variation due to individual differences in its elimination from the body must be slight. Since we have shown[4] that the dimethyl ether is slowly eliminated by the rat, this argument must apply with even greater force to this compound in this animal. For these reasons it seems probable that the intravenous administration of curarising drugs, followed by observation of whether or not the righting reflex is lost, reduces variation between individuals to that based upon the conveyance of the drug to and the responses of the neuromuscular junctions themselves. The loss of the righting reflex is itself a mean effect, based upon the responses of a large number of neuromuscular junctions. In effect, therefore, we appear to be studying in each animal the mean response of a large number of neuromuscular junctions to the curarising agent. It may be for these reasons that the dose - response curves obtained by our method are particularly steep, the slopes being two to three times as great as those obtained by Skinner and Young or by Pick and Richards. It is worth noting that use of the duration of paralysis as a basis for assay would introduce variation due to differing rates of eliminating the drug.

When the slope of the dose - response curve is steep it is evident that the accuracy of administration of the dose becomes extremely important. It is for this reason that we have adopted the procedure of giving to the mouse 0·4 ml. per 20 g. and using dilutions of the drug made serially by a factor of 0·9 to 0·95.

The question of the choice of animal for assay of curarising activity depends partly on the relative sensitiveness of each animal to the substance concerned and partly upon the cheapness and availability of each species. It is well known that the mouse is more sensitive to *d*-tubocurarine than to its dimethyl ether. When the latter substance is to be estimated the mouse does not appear to be suitable, since the ether is derived from *d*-tubocurarine and the activity measured might be merely that of an impure sample of *d*-tubocurarine. The rat, however, is about five times as sensitive to *d*-OO-dimethyltubocurarine as it is to *d*-tubocurarine itself, and is consequently a suitable animal for assay of the ether. When *d*-tubocurarine itself is to be estimated the mouse appears to be more suitable, as it is more readily available, and more sensitive to this substance than to its derivative.

If there is any question of one substance being contaminated with the other, assay in both the mouse and the rat should be sufficient to reveal this and to indicate the approximate proportions of each in the mixture (see Chang and Gaddum[4]).

The planning of assays—The rabbit head-drop test elaborated by Dutta and MacIntosh,[5] which is alone regarded as acceptable for the biological assay of impure preparations of *d*-tubocurarine chloride,[6] provides fiducial limits ($P = 0{\cdot}95$) lying within ± 10 per cent. of the estimated activity. It can easily be calculated from the standard formula giving the fiducial limits that, if we were prepared to assume that in all future assays the slope β of the log dose - probit line would have the value 16·3 indicated by the series of tests discussed above, then,

subject to one restriction, the same degree of accuracy as in the official rabbit head-drop test would be attained by using in an assay a total of 64 mice, 32 for the standard and 32 for the test preparation. The restriction is that all the doses administered should be within the dosage range corresponding to reaction percentages lying between 25 and 75 per cent. It is necessary to make a proviso of this sort because the weight nw ($= nz^2/pq$ in the usual notation) attaching to a dosage-group of n animals varies with p, the expected proportion responding; if p lies between 25 and 75 per cent., w is at least 0·54.

In fact, of course, we can never be certain that the slope β will remain constant, and it is preferable to estimate it afresh in each assay, that is, to treat each assay as self-contained. From this point of view it is impossible to predict accurately the number of mice that should be used to obtain the required degree of accuracy, since we now have to admit that the true slope might in future prove flatter than in the past by an unknown amount. If we exclude this possibility, and assume as above that the true slope β will remain fixed at 16·3, it would appear from the following idealised examples (in which the dosage ratios and percentage responses have been chosen as arithmetically convenient) that the decision to interpret each assay as a self-contained one entails, if it is to retain the desired degree of accuracy, an average increase in size of up to 50 per cent.—

(i) If the true slope β is known to be 17·03, P = 0·95 limits of ±10 per cent. will be obtained by using a total of 58 animals, provided that all doses administered lie within the 25 to 75 per cent. response range.

(ii) If we interpret as a self-contained experiment a four-point assay with equal dosage groups in which the dose-ratio is 0·89 : 1 and the proportions responding to the lower and higher doses are respectively one-third and two-thirds, the estimated slope is 17·02; the fiducial limits (P = 0·95) are ±9·5 per cent. if the test has been carried out with 84 animals, and ±7·0 per cent. with 96 animals.

(iii) Similarly, if the dose-ratio is 1·0 : 1·2 and the proportions responding are one-quarter and three-quarters, the estimated slope is 17·04; the fiducial limits (P = 0·95) are then ±10·9 per cent. with a total of 80 animals, and ±9·4 per cent. with 96.

(iv) If it is desired to test the linearity of the log dose - probit curves, we can in place of the four-point assays considered in (ii) and (iii) adopt six-point designs with dose-ratios 0·89 : 0·94 : 1 and 1·0 : 1·1 : 1·2 approximately, keeping the total numbers of animals at 96 by using for each preparation dosage-groups of sizes 21, 6, 21 and 20, 8, 20 respectively. If the central groups give 50 per cent. response, as they ideally should, the limits (P = 0·95) will be ±8·8 per cent. in the former case and ±9·8 per cent. in the latter.

The steepness of the slope in the assay methods under discussion implies that an accurate result can be obtained from a relatively small-scale test; but it has the attendant disadvantage that in order to do so the experimenter must know fairly well, before carrying out the assay, what its result is going to be—otherwise he may easily administer a range of doses that will each produce 0 or 100 per cent. response. One possible method of avoiding this difficulty is to carry out a preliminary pilot assay, using small dosage-groups and covering a wide range of doses, in order to locate roughly, for each preparation, the effective portion of the dosage range. As an alternative, which we hope to discuss more fully on some subsequent occasion, it should be possible to adopt in a comparative assay the sequential procedures developed in recent years for the economical assessment of the sensitivity of explosives.[7,8]

Summary

1. If a solution of *d*-tubocurarine of suitable concentration is administered intravenously to a number of mice a proportion of them temporarily lose the righting reflex.

2. Analysis of a number of experiments indicates that the log dose - probit line obtained by administering intravenous doses at several levels to groups of mice is both steep and straight. This response may be made the basis of an assay method.

3. Analysis of a number of experiments indicates that the log dose - probit line obtained in a similar way with *d*-OO-dimethyltubocurarine in rats is also steep and straight and that this response may also be made the basis of an assay method.

4. The relation of these results to those of previous authors, the convenient form of assay, and the merits of the mouse and the rat for assay of *d*-tubocurarine and its dimethyl ether are discussed.

We should like to thank the Directors of Messrs. Allen and Hanburys, Ltd. for permission to publish this work.

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Assay of Curare Preparations by the Rabbit Head-drop Method

BY N. K. DUTTA AND F. C. MACINTOSH

A RABBIT head-drop method for standardising curare preparations was first used by Holaday in America about 1940, but so far as we are aware no description of his procedure has yet been published, except a sentence or two in a paper[1] on the use of curare to soften therapeutic convulsions. Modifications of Holaday's test have been briefly employed by Chase, Smith and Bhattacharya[2] and by Everett.[3] We describe below a method of assay based on the same principle, which has been found convenient for checking the potency of *d*-tubocurarine preparations and for assessing the activity of new curarising compounds (*cf.* Barlow and Ing,[4,5] Paton and Zaimis[6]).

PRINCIPLE—A solution of the drug is infused at a steady slow rate into the ear vein of a rabbit until the animal is so weakened that it can no longer hold up its head. The volume of solution which must be injected to produce this end-point is noted. The unknown preparation is compared with the standard in a two-day cross-over test.

APPARATUS—We have used a simple apparatus, made for us by Dr. B. Wheeler Robinson, which enables us to treat four rabbits simultaneously. The barrels of four 5-ml. syringes, lubricated with liquid paraffin and containing the solution to be injected, are clamped in a rack and their plungers are attached to a movable carriage, which is propelled along guide rails by a gramophone motor and reduction gearing. The displacement of the plungers is measured by means of a Veeder counter operated by a cam on the motor spindle. Syringes of nearly the same diameter are chosen, and each of them is calibrated in terms of the counter reading. Repeated calibrations showed that the volume injected could be measured with an error of less than $\pm 0{\cdot}5$ per cent. In most of our tests the infusion rate was $0{\cdot}4 \pm 0{\cdot}01$ ml. per minute, and the volume infused per rabbit 3 to 5 ml. Small variations in the speed of infusion are unimportant so long as the total volume injected can be measured accurately.

The rabbits sit side by side in wooden stalls fitted with wire yokes (Fig. 1). Their heads, which project over the table edge, can move freely up and down, but not far in any other direction. Each rabbit occupies the same stall on the two days of the test and receives the drug from the same syringe. The needle in his ear vein is connected by polythene tubing (2-mm. bore) to the syringe and is kept in place by a bulldog clip fixed just peripheral to the puncture; a strip of adhesive tape binds the plastic tubing to the tip of the ear. The plastic tubing is less distensible than rubber tubing; its capacity is hardly altered when it is coiled or straightened and its transparency makes it possible to ensure that no air bubbles are present. Light-weight buttless needles with a short bevel, such as those of the Solila pattern ($\frac{7}{8}$-inch: Amalgamated Dental Co.), are most satisfactory.

PROCEDURE—The rabbits having sat for 10 minutes or more in their stalls, the apparatus is set running and the needles are successively inserted and bound in place. The operator calls out the moment of insertion and an assistant notes the reading of the counter. The rabbit appears normal until about two-thirds of the head-drop dose has been given. The

onset of curarisation is usually heralded by a period of restlessness; the pupils may dilate; and the rabbit's head begins to fall, but can be jerked up again. When the true end-point is reached the neck is fully stretched and toneless, and there is no movement of the head in response to a light touch on the snout. At this moment—it can usually be judged within 10 seconds or so—the needle is pulled out, and the counter reading is noted for a second time. From the difference between the counter readings the volume of solution injected is calculated.

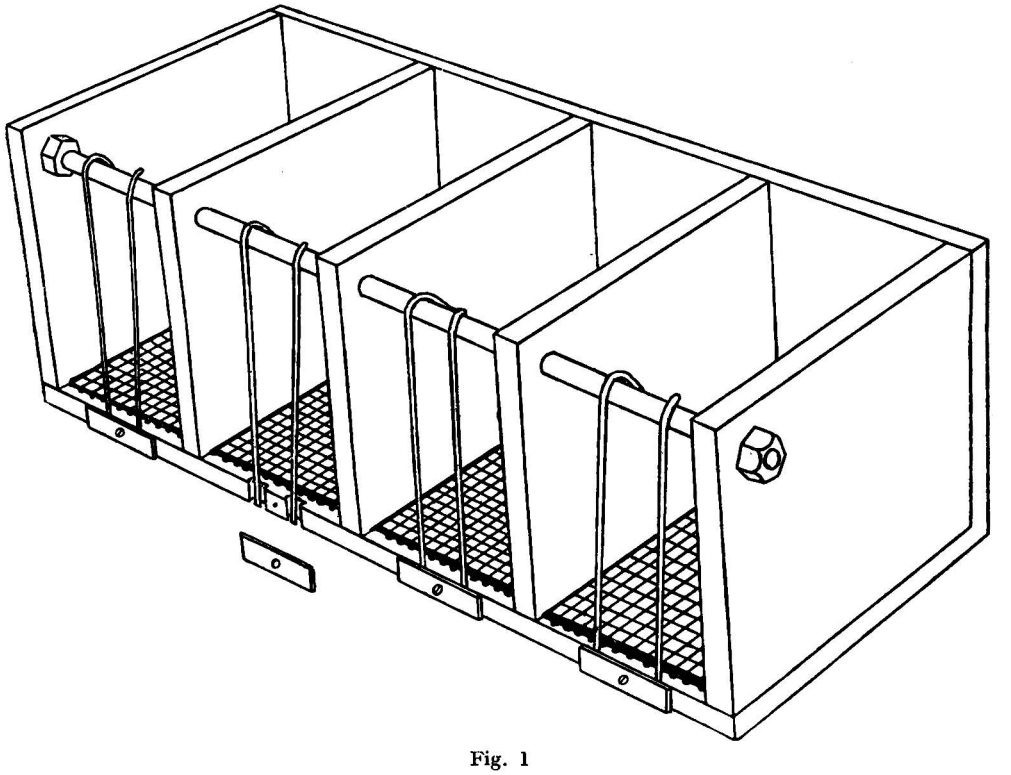

Fig. 1

The rabbits soon recover and seem quite normal 15 minutes later. Occasional animals, especially those unaccustomed to the procedure, suffer severe respiratory embarrassment: they recover quickly if a solution of Prostigmine and atropine (Prostigmine methylsulphate 0·5 mg., atropine sulphate 0·5 mg.) is promptly injected, through the original puncture site, into the vein. It is very important not to excite the rabbits when they are becoming curarised, or after the end-point is reached, because any struggling deepens the curarisation. For this reason docile animals should be selected: we have found both sexes of the Himalayan variety suitable.

On the first day of the test four or more rabbits receive the unknown preparation, and an equal number the standard preparation. The test is completed one to three days later, the groups being crossed over, so that the rabbits that received the unknown on the first day now receive the standard, and *vice versa*.

FACTORS INFLUENCING THE ACCURACY OF THE ASSAY—We have examined the effect of varying the rate of infusion, and the significance of day-to-day and rabbit-to-rabbit variation. In each of two tests on 12 rabbits, three solutions containing 80, 100 and 125 μg. per ml. respectively of *d*-tubocurarine chloride were administered, each rabbit receiving a different solution on each day of the test, according to a replicated Latin square design. The volume of solution injected per minute was kept constant. The variance analysis for one of the tests is shown in Table I. In this test the variance between days was significant, but this was not usual. With rabbits that are accustomed to the procedure there is usually no significant increase or decrease in sensitivity with repetition of the treatment. Untrained rabbits, or those that have not been used for some weeks, are apt to be more resistant to

tubocurarine, but a single test increases their sensitivity to a level which is maintained with minor fluctuations through succeeding tests. It is best if all the rabbits in a cross-over assay have been tested within the preceding week; if fresh rabbits have to be used, they should be divided equally between the two groups.

TABLE I

SOURCES OF VARIATION IN THE HEAD-DROP DOSE: ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom, DF	Mean square	F	P
Infusion rate:				
Slope	1	15·0416	—	—
Linearity	1	833·6805	3·03	> 0.05
Days	2	1344·5278	4·89	$> 0.01 < 0.05$
Unconfounded interaction ..	2	264·4456	—	—
Between rabbits	11	2318·6566	8·44	< 0.001
Within rabbits	18	274·8519		
Total	35	34529·2222		

Variance between rabbits is highly significant, whether dosage is recorded on a body-weight basis or directly, and the cross-over design is therefore necessary for maximum efficiency.

The curarising dose is nearly independent of the time taken to administer it unless the time varies widely, as is shown in this test by the insignificance of the variance due to change of infusion rate. The average time required to curarise was here 7 minutes for the strongest solution and 12 minutes for the weakest solution; and within these limits differences in the mean duration of the infusion as between standard and unknown solution can probably be disregarded with safety. With infusion rates much slower or faster than these the amount of tubocurarine required to produce the end-point is somewhat increased (Fig. 2): with a slow injection because an appreciable part of the dose has been inactivated before the injection is completed, and with a fast injection because the drug does not become fully effective immediately on entering the ear vein, so that the curarisation continues to get deeper after the end of the infusion.

CALCULATION OF RESULTS—The dose of each preparation given to each rabbit is recorded in mg. (or ml.). The potency ratio and its limits of error are then conveniently calculated as shown in Table II, where $n/2$ is the number of animals per group, and the standard is administered on the first day to group A and on the second day to group B.

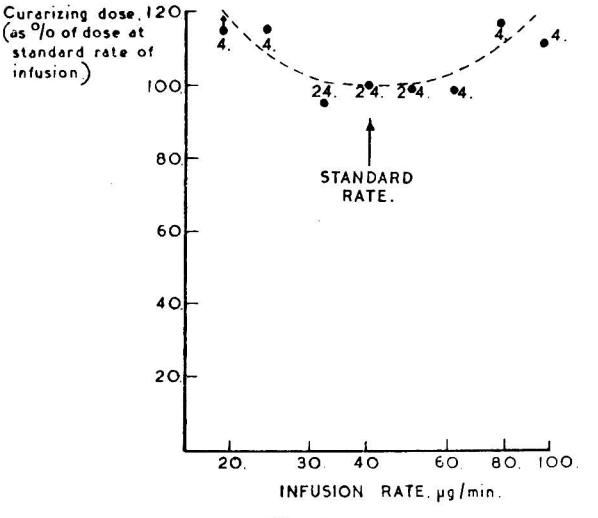

Fig. 2

It will be noted that with this treatment the variance between days does not contribute to the error. It is assumed that the curarising dose is independent of the time taken to administer it and, as has been seen, this assumption is legitimate provided that the mean

infusion time is not too different for the two solutions. Where the mean times with standard and unknown differ by more than 20 per cent. it is wise to repeat the test.

EXAMPLE—Table III gives the data obtained in a typical cross-over test, in which two preparations of *d*-tubocurarine chloride were compared. Each preparation was administered in the form of a 0·01 per cent. solution in 0·9 per cent. sodium chloride solution and the infusion rate was 0·4 ml. per minute.

TABLE II

	Rabbit No.	Log doses: Day 1	Log doses: Day 2	Differences ($X_s - X_u = x$)
Group A ..	1	$X_{s(1)}$	$X_{u(1)}$	x_1
	2	$X_{s(2)}$	$X_{u(2)}$	x_2
	3	$X_{s(3)}$	$X_{u(3)}$	x_3
	|	|	|	|
	|	|	|	|
		(Mean for Group A)		$\bar{x}_A$
	|	|	|	|
	|	|	|	|
Group B ..	$n-2$	$X_{u(n-2)}$	$X_{s(n-2)}$	x_{n-2}
	$n-1$	$X_{u(n-1)}$	$X_{s(n-1)}$	x_{n-1}
	n	$X_{u(n)}$	$X_{s(n)}$	x_n
		(Mean for Group B)		$\bar{x}_B$

M = log potency ratio, unknown : standard = $\frac{1}{2}(\bar{x}_A + \bar{x}_B)$

VM = variance of M = $\dfrac{S(x-\bar{x}_A)^2 + S(x-\bar{x}_B)^2}{n(n-2)} = s_M^2$

Limits of error of M are M $\pm$ ts_M with t for $(n-2)$ degrees of freedom.

TABLE III

	Rabbit No.	Weight kg.	Curarising dose (μg.): Standard		Curarising dose (μg.): Unknown	
Group A	1	2·0	287	on Day 1	276	on Day 2
	2	1·8	285		303	
	3	2·0	286		314	
	4	1·7	341		378	
Group B	5	1·7	294	on Day 2	312	on Day 1
	6	1·8	311		320	
	7	1·9	391		315	
	8	1·8	443		401	

These results, when treated by the method already described (Table II), give the following results—

Potency (unknown : standard) .. 1·001
Limits of error (P = 0·05) .. .. 0·917 and 1·093

ACCURACY OF THE ASSAY—From the data of the Latin square tests it was calculated that if 8 rabbits were used in a cross-over assay the limits of error (P = 0·95) of the potency ratio, determined by the formula given above, could be expected to lie within ±10 per cent. This expectation has been verified in 10 out of 13 routine assays of this sort; in the other 3 the fiducial limits were slightly wider. In 10 consecutive assays of known solutions of *d*-tubocurarine chloride the largest error in estimating the mean potency ratio was 8 per cent.

CHOICE OF METHODS FOR THE ASSAY OF CURARE PREPARATIONS—We think that this depends, to some extent, on the sample to be examined. If the material purports to be, for instance, pure *d*-tubocurarine chloride, and has to be compared with an authentic specimen of the salt in order to check its freedom from impurities of higher or lower activity, then it probably matters little which method is chosen, so long as it is convenient and gives reproducible results. If the material to be tested is an impure preparation that is supposed to owe its activity to a particular alkaloid, there may be some advantage in the use of an *in vivo* rather than an *in vitro* method of comparison. It is possible that surface-active material or other impurities might affect the rate of curarisation when the drug is applied directly

to the muscle, whilst having less effect when the drug has to be transported to the muscles by the blood; though we have no evidence that this possibility is realised in practice. In any event there is something to be said for having the whole animal under observation and not merely a piece of muscle, particularly where one is testing a preparation intended for clinical use. It must be remembered, however, that the impurities may also be toxic: neither death, head-drop nor immobilisation is a specific test for curarising activity. Within these limitations the rabbit head-drop procedure has the advantages of being simple to carry out and of producing data from which the precision of the result can be calculated.

In comparisons of the kind referred to above it is presumed that the activity being measured is due to a single active principle. When new curarising compounds are examined an attempt is made to state their potency in terms of a reference substance. Here it is certainly too early to name any single test as pre-eminently reliable for predicting potency in clinical application. Recent work[4,5,6] has emphasised the remarkable variability in the relative activity of curarising compounds, depending on the species and the muscle used for the comparison. Until the significance of these discrepancies is better understood it seems wisest to recommend that whatever screening test be used the most active compounds at least of any series should be tested on more than one muscle or more than one species.

SUMMARY—A procedure is described for the assay of curarising drugs by means of the head-drop reaction in the rabbit. The error of a cross-over test on eight animals is usually less than ±10 per cent. (limits for P = 0·95).

We are indebted to Dr. C. W. Emmens for statistical advice, and to Mr. C. Pergande for technical assistance. Permission to administer curarising drugs to unanaesthetised animals was granted by the Home Office.

One of us (N. K. D.) is grateful to Sir Charles Harington, F.R.S. for permission to work at the National Institute for Medical Research, and to the Government of India for a grant.

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Use of a Rotating Drum in Assessing the Activities of Paralysant, Convulsant and Anaesthetic Drugs

BY H. O. J. COLLIER, R. A. HALL AND E. C. FIELLER

(*Read at a meeting of the Biological Methods Group, May 26th,* 1949)

THE assay of curarising activity by visual observation of the righting reflex, described in a preceding communication,[1] although rapid, simple and in our opinion reliable, suffers from the defect that it depends upon a subjective judgment. We have therefore devised a rotating drum apparatus in order to render objective the decision whether or not a certain degree of paralysis has been effected in a mouse.

The objects of the present paper are—

(1) To compare tests of curarising activity carried out with the rotating drum with those based on visual observation of the righting reflex; and
(2) To describe the use of the drum in estimating the activities of various compounds, namely *d*-tubocurarine, decamethonium iodide and its relatives, "myanesin," thiopentone and leptazol.

APPARATUS AND METHODS

The apparatus, which is illustrated in Fig. 1, consists essentially of a 6-inch drum, such as is commonly used for smoked paper recording, arranged so that it can be rotated at a

definite speed on a horizontal spindle. The surface of the drum is fitted with wire-mesh to enable mice to grip and run upon it. A smooth wall is fitted on each side of the drum to prevent mice crawling over the edge. A tray of sawdust is placed below the drum in order to catch mice that fall off. Dimensions and specifications of these fittings are given below the figure.

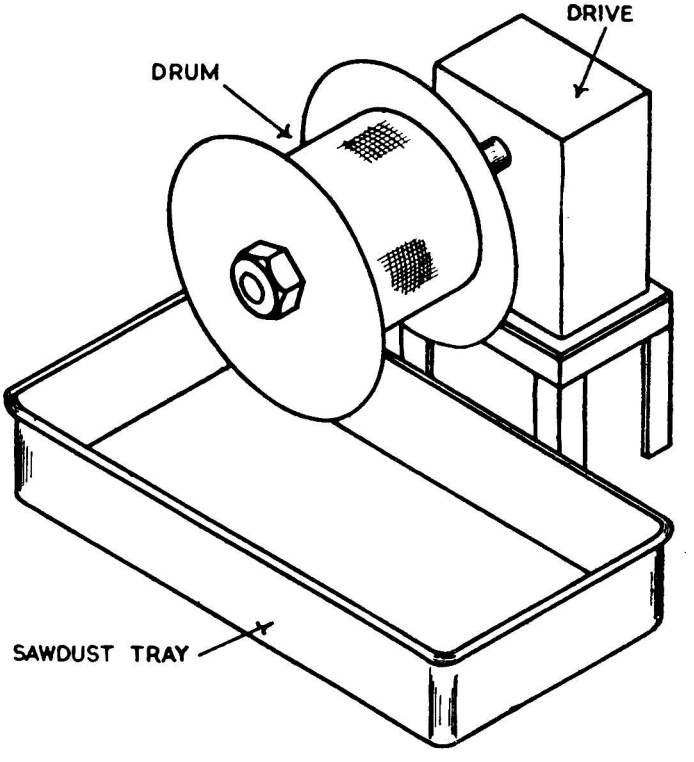

Fig. 1. The cylindrical drum surface, 6⅛ in. wide and 6¼ in. in diameter, is covered with wire gauze of 6 mesh, 21 S.W.G. (0·032 in.) wire. The end walls of the drum are of sheet metal 10 in. in diameter, projecting 1⅞ in. beyond the wire gauze surface all round, as smooth flanges. The central axis of the drum is mounted 10¼ in. above the floor of the sawdust tray. The tray is 20 in. long, 10½ in. wide and 3 in. deep.

In order to find the optimal speed of rotation the drum was connected to a motor by two pulley systems through a reducing gear box. Investigation of the behaviour of normal mice on the drum, rotating at speeds ranging from 2 to 10 r.p.m., showed that a speed of 2·1 r.p.m. (corresponding to a surface speed of 19 mm. per second) was satisfactory for our purposes. At this speed many mice will continue to run round the drum for half an hour or more.

In order to observe the behaviour of saline-treated mice upon the drum, 100 animals were injected intravenously in turn with 10 ml. of normal saline per kg. of body weight. Each was placed on the drum immediately after injection and allowed to remain on it for 3 minutes. Of these 100 animals, 92 ran against the direction of rotation of the drum and retained their hold for the full period; 8, on the other hand, allowed themselves to be carried round on the moving surface and leaped from the drum into the sawdust tray below. It is therefore necessary to take precautions, which will be described below, against counting such mice as positive reactors in a test.

The routine of performing a test on the rotating drum was in most respects similar to that described previously.[1] After intravenous injection of the drug the mouse was immediately placed on the upper surface of the rotating drum, facing against its direction of movement. Those mice that were unable, through the action of the drug, to retain their hold on the

moving surface, slid off and fell into the tray below. These were counted as positive reactors. Those that, in spite of the administration of the drug, remained on the drum for longer than the time taken for the action of the drug to pass its peak (up to 3 minutes), were counted as negatives. The occasional mouse that 'stayed put' on the surface, rotated with the drum and leaped into the tray could quite readily be distinguished from the affected mouse that slid off from the upper part of the surface. Any doubts on this point were settled by putting the mouse in question once more on to the drum.

Results

Responses to curarising drugs—As might be expected, administration of adequate doses of the drugs listed in Table I, which have a curarising action, prevented a mouse from retaining its hold on the drum surface. Intravenous administration of a drug at various dosage levels to groups of mice, followed by observation of the ability of each animal to retain its hold on the drum, provided data which could be submitted to the customary routine of plotting the dosage - response curve, testing its linearity and estimating its slope, the ED 50 and its fiducial limits. Tests of linearity showed the log dose - response lines obtained with each compound in Table I to be satisfactory.

Table I

Mean slopes of log dose - probit response lines obtained with the rotating drum and by observation of the righting reflex in mice, using four substances having curarising activity

Drug	Rotating drum method			Righting reflex method		
	Total No. of mice	Mean slope	Standard error	Total No. of mice	Mean slope	Standard error
d-Tubocurarine chloride	110	17·6	± 3·7	662	16·3	± 2·0
Decamethonium iodide	260	13·7	± 1·8	235	10·8	± 1·6
Hexamethonium iodide	40	15·2	± 6·0	40	17·4	± 7·8
Pentamethonium iodide	220	16·3	± 2·4	20	33·9	± 15·0

The question whether the rotating drum method provides a measure of activity more accurate or less accurate than observation of the righting reflex can be answered by comparison of the slopes obtained with these two methods of estimating the degree of paralysis. It will be seen that, for any one compound, there is no significant difference in the slopes obtained by the two methods. For practical purposes, however, the rotating drum is to be preferred, since it does not require trained judgment.

The relative sensitiveness of our two methods of estimating paralysis provides a second point of interest. With all curarising compounds for which this point has so far been investigated it has been found that a positive reaction on the rotating drum may be obtained at a lower dosage level than is required to abolish the righting reflex. This point is illustrated in Fig. 2, in which the ED 50 for decamethonium iodide obtained with the rotating drum was in one experiment 83, and in another 91 per cent. of the ED 50 required to abolish the righting reflex.

Fig. 2 also illustrates the point mentioned in our previous paper (p. 583), that the position of the ED 50 may vary from experiment to experiment, although the slope of the curve does not normally do so significantly.

If fiducial limits are calculated directly from the data of these assays, chance variations in the observed value of the slope will of course have a considerable effect on the values obtained, although the true slope is constant. The example illustrated in Table II, which is based on the assumptions that the value found for the slope will be 15 and that the percentages of animals responding in the different dosage groups will approximate to 20 and 80, may serve as a guide to the accuracy to be expected.

Responses to other drugs—The question arises how far the rotating drum method may be used for the estimation of substances other than those with curarising activity. One limitation is obviously imposed by the present form of the apparatus; the compound must be one to which the animal can respond fairly rapidly. For this reason, and for another stated in the previous paper, we believe that the intravenous route of administration of a drug should be used.

Provided the substance can be administered intravenously we might expect that drugs impairing nervous co-ordination in various ways would give a positive response in mice on the rotating drum. Accordingly we obtained trial dose - response curves with intravenous "myanesin," thiopentone and leptazol. Each of these curves, which are illustrated in Fig. 3, are steep and rectilinear.

TABLE II

EXAMPLE OF THE RELATION OF FIDUCIAL LIMITS TO NUMBERS OF MICE EMPLOYED

Fiducial limits (P = 0·95)	No. of animals dosed with standard preparation	No. of animals dosed with test preparation
± 10%	40	40
± 7·5%	70	70
± 5%	150	150

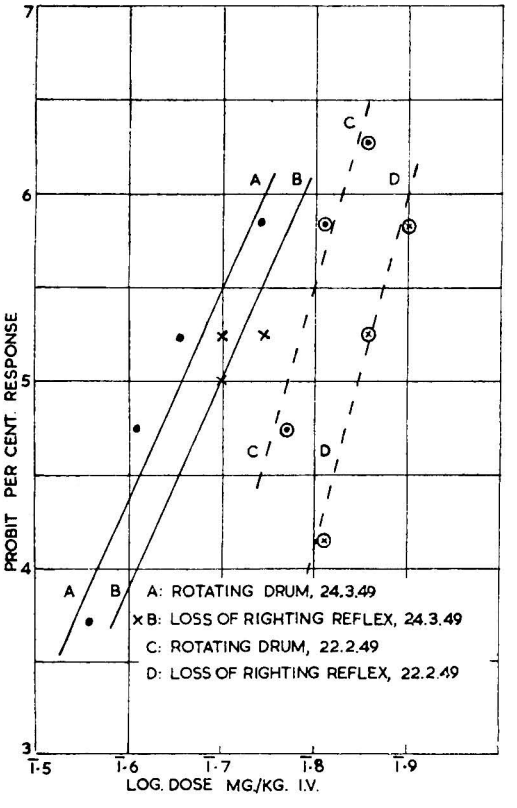

Fig. 2. Log dose - probit response lines obtained with a sample of decamethonium iodide on two different days. On each occasion the loss of righting reflex and the ability to hold on to the rotating drum were noted

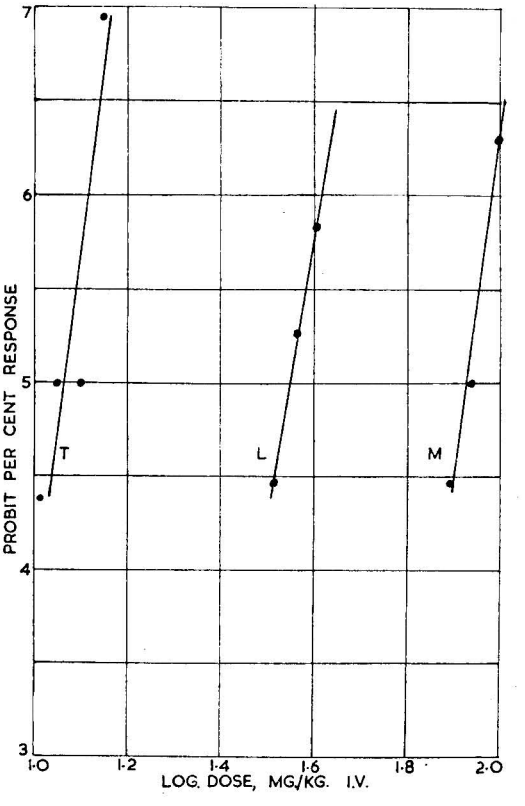

Fig. 3. Log dose - probit response lines obtained with Thiopentone (T), Leptazol (L) and "Myanesin" (M) by means of the rotating drum. Ten mice were used at each dosage level

Although we have not performed a sufficient number of experiments to draw a final conclusion, our results suggest that the type of drum described may be used for estimating convulsant and anaesthetic, as well as paralysant substances.

SUMMARY

1. A rotating drum apparatus, which may be used for estimating the degree of paralysis of a mouse after intravenous administration of a curarising drug, is described.

2. The results obtained with some substances having curarising activity, namely deca-, hexa- and penta-methonium iodides and *d*-tubocurarine chloride, are reported.

3. Comparison of the dose - response curves obtained with the drum and by observation of the righting reflex show that there is little difference in accuracy between the two methods of measuring degree of paralysis.

4. Mice on the rotating drum respond to a somewhat slighter degree of paralysis than is required to abolish the righting reflex. The drum method is therefore more sensitive.

5. Satisfactory dose - response curves have also been obtained with the drum after intravenous administration of other drugs impairing nervous co-ordination, namely "myanesin," thiopentone and leptazol.

We should like to thank the Directors of Messrs. Allen and Hanburys, Ltd., for permission to publish this work, Miss J. E. Pryke for assistance in biological experiments and Mr. A. Marshall for drawing Fig. 1.

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DISCUSSION ON THIS PAPER

Dr. M. R. A. CHANCE asked whether the exceptional behaviour of 8 per cent. of the mice could arise from the fact that many rodents in captivity do not obtain sufficient exercise in scratching to keep their claws to the normal length, and Mr. A. L. BACHARACH enquired whether it was always the same individual mice that behaved differently. Dr. COLLIER replied that the anomalous mice were characterised by unwillingness to run on the drum rather than by inability to hold on to the surface. Mr. HALL pointed out that the wire of the mesh was too thick in gauge to permit the mice to enclose it completely in their claws. He added that normal mice that had fallen off the drum would remain on when replaced.

Dr. CHANCE also asked whether the effect of leptazol, which caused the mice to fall off the drum, appeared at dose levels much below that necessary to produce obvious signs of central nervous stimulation, such as convulsions. Mr. HALL said that this was so.

Finally, Dr. CHANCE asked whether the relative activities of a substance tested by the rotating drum and righting reflex methods would give information on the different dose required to stimulate different parts of the central nervous system. Dr. COLLIER replied that as the different peripheral actions of curare on the neuro-muscular junctions of the digits and of the limbs might be studied by the method suggested, he thought the same might apply to drugs that had an action on different parts of the central nervous system at different dose levels.

Mr. R. F. SELLAR suggested that some of the "unco-operative" 8 per cent. of mice might be deterred from jumping voluntarily from the revolving drum by raising the latter and Dr. G. A. MOGEY pointed out that he had not found this manoeuvre to be very successful. He had been using a horizontal wire grid on which the mice were suspended upside down, and had raised it to a height of 4 feet above the sawdust pit, but some mice still dropped, although the drop did not appear to have been occasioned by the tubocurarine. He described how mice tended desperately to cling on to the grid with their fore-paws after they had relaxed the grip of the hind paws as the action of the paralysing agent asserted itself—whereas when about to jump off deliberately they let go first with their fore-paws and groped about in the air with them as if searching for a suitable landing place. In conclusion, Dr. MOGEY reminded the meeting of the necessity of paying very strict attention to the point that curarised animals must not be hurt.

Dr. G. E. FOSTER asked whether Dr. Collier had experienced any difficulty in carrying out tests on *d*-tubocurarine preparations so that the results would conform with the limits required by the Therapeutic Substances Act. Dr. COLLIER replied that the precision attained depended on the number of animals used: a large number was required to conform with the T.S.A. limits.

The Extraction of Growth Factors from Natural Products prior to Microbiological Assay

By J. S. HARRISON

(*Read at the Annual General Meeting of the Biological Methods Group, December 17th, 1948*)

THE methods used for the extraction of growth factors of the vitamin B complex from their bound forms in natural products are essentially empirical. Treatments with acid, alkali or enzyme preparations are usually employed and the highest valid result from a particular material is taken as the best available value. The extraction of pantothenic acid and the vitamin B_6 complex are discussed below.

Pantothenic acid

The accepted method of extracting pantothenic acid from natural products is a combination of heat and enzyme treatments.[1,2] Until recently the following technique was used in this laboratory for dried yeast and other products prior to assaying with *Lactobacillus arabinosus*—

> Steam 1 g. of material in 25 ml. of 0·1 *M* sodium acetate buffer solution for 30 minutes at pH 6·8, cool, and incubate at pH 4·5 for two days at 37° C. with 0·5 g. each of papain and takadiastase of known pantothenic acid content.

Recently we found that in certain cases, notably with malt extract, a simple treatment with 1·67 *N* sodium hydroxide for 5 minutes at room temperature would release two and a half times as much pantothenic acid activity as the orthodox enzyme treatment. This high value was not increased by further enzymic treatment. Malt, on the other hand, gave a value of 5 μg./g. by enzyme treatment, which was increased to about 10 μg./g. by sodium hydroxide and further to 15 μg./g. by a combination of alkali and enzyme treatments. Malt wort and various other cereal products showed similar increases. Optimum conditions for extraction were found by experiment and the standard treatment finally adopted is given below. Control experiments with calcium D-pantothenate showed that under these conditions less than 5 per cent. of the activity was destroyed by the alkali.

> Dissolve or suspend 1 to 5 g. of material, according to its expected pantothenic acid content, in 25 ml. of 0·1 *M* sodium acetate at pH 6·8, and steam for 30 minutes. Cool, add 5 ml. of 10 *N* sodium hydroxide and allow to stand for exactly 5 minutes, then add 5·1 ml. of 10 *N* sulphuric acid or its equivalent and adjust to pH 4·5 by addition of sodium hydroxide. Add exactly 0·5 g. of papain and 0·5 g. of takadiastase, both of known pantothenic acid content under the conditions of the test, cover with a layer of toluene, incubate at 37° C. for 3 days, filter (or preferably centrifuge if filtration is difficult), and make up to a suitable known volume from which the assay dilutions can be prepared. Extract a portion of the dilution twice with light petroleum before testing, unless this treatment has been proved to be unnecessary, as the effect of certain fats on the growth of *L. arabinosus* is considerable.

In assays for which less than the complete treatment was necessary, the appropriate stage in this schedule was omitted. An interesting point which may have some connection with the effect produced by the alkali was that, almost invariably, the soda plus enzyme treatment gave a perfectly clear solution, whilst the enzymes alone and soda alone gave solutions that were difficult to filter and remained cloudy.

Table I gives the results obtained with various materials. Heart muscle is included on account of its high content of co-enzyme A. The greatest increases in apparent pantothenic acid content were obtained with malt products. With malt itself the maximum liberation was obtained with soda plus enzymes, whilst for malt extract it was sufficient to use soda only. This can probably be explained by prior action of the natural enzymes on the raw materials, and it is not surprising that additional incubation with further enzymes does not liberate any more of the growth factor. As the apparent pantothenic acid content of heart muscle was not significantly increased, it was concluded that co-enzyme A is not affected by cold alkali treatment.

All the values so far quoted were obtained with *L. arabinosus* as the test organism. In a smaller number of tests the same effects were observed in using the *Saccharomyces carlsbergensis* assay. As examples of the similarity of the results, a particular sample of malt extract gave, untreated and soda-treated respectively, 6·5 μg./g. and 19·2 μg./g. by the lactic assay and 5·0 μg./g. and 20·4 μg./g. by the yeast method. The large increase which has been obtained over the previously accepted values makes previous figures for pantothenic acid balances in malt wort fermentations meaningless.*

Vitamin B_6

The microbiological assay of the vitamin B_6 group has received considerable attention recently, and there have been interesting developments in respect of the extraction and the different behaviour of the various members of the complex.

Table I

Pantothenic acid activity (μg./g.) of some natural products

Sample	Method of extraction			
	Water	Enzymes	Soda	Soda + Enzyme
Malt	3·0	5·4	10·3	14·6
Malt extract	7·8	9·0	25·2	25·1
Grain wort	0·4	0·9	—	1·2
Wheat germ	—	19·4	19·9	19·0
Cane molasses	8·0	8·4	9·0	9·4
Dried bakers' yeast	66	111	65	116
Sheep's heart	—	27·7	—	32·9
Mutton	—	14·1	—	13·1

Acid extraction—In the early published work on this subject alkaline hydrolysis was rejected presumably because of the known destruction of vitamin B_6 by alkali, and heating with mineral acid became the standard treatment. In the early acid extractions, when *Neurospora sitophila* mutant 299 was used as the test organism, *N* hydrochloric acid was used,[3] 1 to 5 g. of the sample being heated with 40 ml. of *N* hydrochloric acid. In 1947, Rubin, Scheiner and Hirschberg,[4] using sulphuric acid in the extraction of dried brewer's yeast, demonstrated that the efficiency of extraction of vitamin B_6 activity for *S. carlsbergensis* during an extraction time of 1 hour at 20 lb. pressure, was greatest in the relatively narrow range of pH 1·5 to 2·0, and diminished progressively on either side of these limits. The lower response of the test organism to extracts prepared at other pH levels could be raised to maximal or near-maximal by further hydrolysis in 0·055 *N* sulphuric acid for 8 hours, or by enzymic treatment. Similar release of activity was shown for vitamin B_6 in liver. Since the publication of Rubin, Scheiner and Hirschberg, other papers have been presented to explain the chemical mechanism involved in this phenomenon. It is related to the presence of pyridoxamine phosphate in natural products such as dried yeast, as demonstrated by Rabinowitz and Snell.[5] The same workers showed that the acid extraction could be improved considerably by extending the 1-hour hydrolysis to 5 hours.

It was shown by Atkin, Schultz, Williams and Frey[6] that the optimum pH for liberating vitamin B_6 from wheat or wheat products was lower than for yeast and many other materials. We have confirmed this, and in experiments in our laboratories with dried baker's yeast and two different types of molasses, in which 100 mg. of the sample were autoclaved for 2 hours at 20 lb. pressure with 25 ml. of sulphuric acid of various strengths, the optimum normality for yeast was shown to be 0·055 (pH 1·7), whereas that for molasses was 0·5 (pH 0·7).

Extraction by enzymes—The efficiency of a method of extraction can be assessed by comparing the results with those obtained by a second method. Rabinowitz and Snell[7] used an enzyme preparation, clarase, for the extraction of vitamin B_6. Using this method we first worked out the best conditions for extraction with dried baker's yeast as the test substance. Maximum extraction was obtained in 3 days when 100 mg. of dried yeast,

* Since this paper was read a communication by Neilands and Strong (*Arch. Biochem.*, 1948, **19**, 287) has appeared that describes an animal enzyme technique by which the apparent pantothenic acid content of various natural products can be considerably increased. Among the substances tested was dried yeast, the pantothenic acid content of which was increased fourfold by the new treatment, whereas alkali treatment has proved to be without appreciable effect.

100 mg. of clarase of known B_6 content, and 10 ml. of *M* sodium acetate at pH 5, covered by 2 ml. of sulphur-free toluene, were incubated at 37° C. With this technique good agreement was obtained between the enzyme and acid extractions. Actual values are given in Table II.

TABLE II

COMPARISON OF ACID AND ENZYME EXTRACTION OF VITAMIN B_6 ACTIVITY FROM YEAST AND MOLASSES

Sample	Acid extraction 5 hr. at optimum pH	Clarase extraction 3 days at 37° C.
Dried bakers' yeast	[0·055 *N*] 20·3 μg./g.	20·7 μg./g.
Beet molasses	[0·5 *N*] 5·57 "	5·25 "
Cane molasses	[0·5 *N*] 11·6 "	11·4 "

The foregoing results were obtained with the Carlsberg yeast assay. Substantially similar results were obtained in a shorter series of experiments with *N. sitophila* mutant 299.

Available vitamin B_6—The ideal method of determining the amount of available growth factor in a natural product is to carry out assays under the precise conditions to be studied. This is often impossible where large-scale processes are involved, but the assay method should be designed to approach as nearly as possible to the practical conditions. In a study on these lines of the availability to yeasts of the vitamin B_6 complex in molasses, it was found that the amount of available growth factor measured by microbiological assays with two different yeasts gave different values. This is understandable because different organisms are known to vary in their capabilities of utilising bound or modified forms of growth factors. It followed that more of a factor might become available to the same organism with increased time of growth, if cell enzymes took an appreciable time to attack the bound factor, or if the growth had to reach a certain value before a sufficient concentration of enzyme was reached. Hence the apparent B_6 content of molasses might increase with longer incubation time of the assay. Two strains of *S. cerevisiae* and one of *S. carlsbergensis* were tested, with the surprising results that the values obtained with *S. carlsbergensis* decreased with time, whereas the *S. cerevisiae* values increased, but to a figure much higher than the maximum

TABLE III

EXTRACTION OF GROWTH FACTORS FROM NATURAL PRODUCTS

Growth factor	Method of extraction: Enzymes	Acid	Alkali
Vitamin B_6	Maximum with clarase	Maximum at correct pH	Sub-maximum
Pantothenic acid ..	Maximum with animal enzyme*	Destroyed hot	Destroyed hot, maximum cold, in certain cases with enzymes
Biotin	Sub-maximum	Maximum 126° C. 3–6 *N* H_2SO_4, 1 hr.	—
Inositol	—	Maximum 100° C. 6 *N* HCl, 20 hr.	—
Aneurine	Maximum with prior acid treatment	Maximum 100° C. when followed by enzymes	Destroyed
Nicotinic acid	Mostly sub-maximum	Maximum 120° C. *N* HCl, 20 min.	Maximum for cereals
Riboflavine	Useful for tissues	Maximum 120° C. 0·25 *N* HCl, 15 min.	Destroyed

* See Neilands, J. B., and Strong, F. M., *Arch. Biochem.*, 1948, **19**, 287.

obtained by acid hydrolysis. No similar variation in results with time of incubation was found when acid-hydrolysed samples were used. It would appear that values higher than the "complete" figure must be invalid as a measure of the true B_6 complex, although no great "drift" was observed at various levels. No satisfactory explanation of this phenomenon is yet forthcoming.

CONCLUSION

In Table III a summary is given of the methods of extraction that have been found to be most satisfactory for the liberation of members of the B complex of growth factors from natural materials. The table only represents general findings and may need considerable

modification as more experimental results become available. In the case of pantothenic acid, two improved methods are immediately introduced. It is, in any case, quite certain that any new test substance must be examined under various conditions to find the most suitable extraction technique. At present no general method is satisfactory for all materials because the forms of the chemical combinations within the living cell are not understood. Until these problems are solved empirical procedures must, of necessity, be used for the extraction of growth factors from natural products.

Summary

A method is described for the extraction of pantothenic acid from natural materials by treatment with cold alkali which gives considerably higher values for the pantothenic acid content of malt products and other substances than the orthodox enzyme treatment. Methods of estimating vitamin B_6 in both free and bound forms are discussed.

The author thanks the Directors of the Distillers Company Limited for permission to publish this paper.

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Discussion

Dr. A. J. AMOS congratulated the author on his interesting paper. The method of extraction of test materials, he said, was an aspect of microbiological assay that did not always receive sufficient attention.

Dr. MORRIS stated that in his experience the use of *N* NaOH for the extraction of pyridoxine caused no loss of the vitamin at all and the method could be used for assay purposes. Subsequent treatment with sulphuric acid gave no increased response.

Mr. S. A. PRICE said that in his experience a frequent result of unsuitable extraction procedures for pantothenic acid was non-valid assays in which the liberation of the vitamin could not be evaluated precisely. Did Mr. Harrison not have this difficulty, or was he using a better medium less prone to give such troubles?

Mr. HARRISON replied that he used one of the published media for pantothenic acid assay, and the validity of the assays was usually satisfactory.

Notes

AN AUTOMATIC BURETTE—VACUUM OPERATED

AUTOMATIC burettes in which a burette is attached to a reservoir and can be filled from it when necessary, are being increasingly used in chemical and other laboratories. Generally they can be divided into two classes. In those intended for use with solutions relatively stable in presence of air the burette is fixed above the reservoir and the solution is pumped into the burette by air pressure, from a blow-ball or rubber bellows. In the other type, which is more often used for solutions liable to alteration of strength by access of air (*e.g.*, solutions of alkali hydroxides, ferrous ammonium sulphate, titanous chloride or sulphate, etc.), the reservoir is kept at a higher level and the solution is fed into the burette near the bottom by means of gravity; and the top of the burette and the reservoir are connected to a source of inert gas. Zintl and Reinacker[1] (1926) designed a special automatic burette for use with solutions unstable in contact with air, and this was further modified by Smith (1935) but it is rather complicated.

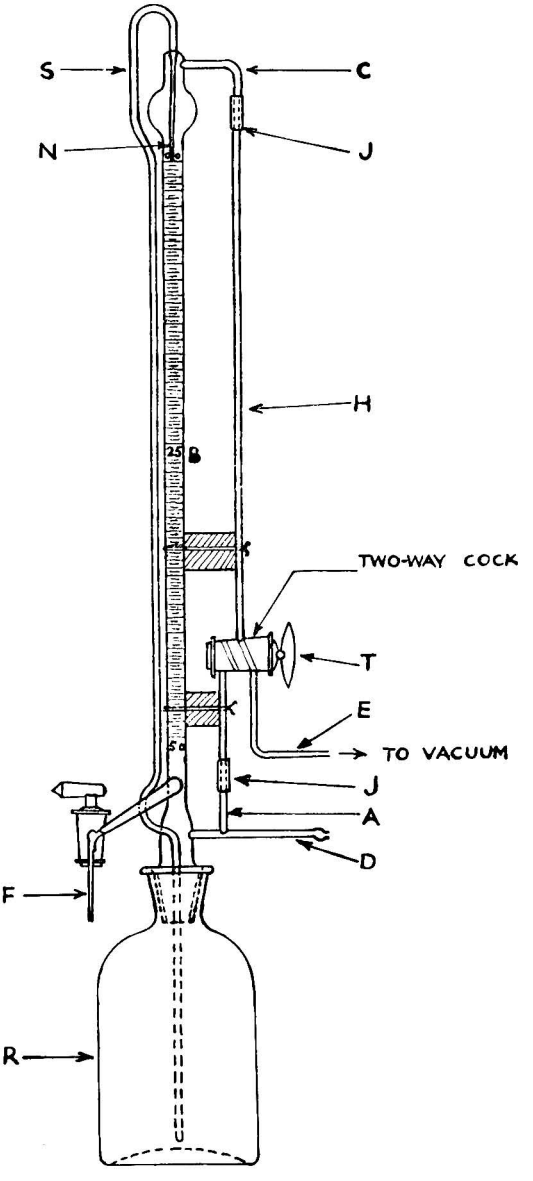
In our laboratory we had an apparatus intended for use with a blow-ball, but we wanted our standard solution not to come in contact with air. So we modified it and found that the new apparatus worked more smoothly than the original and had the additional advantage that it could be directly connected with a source of inert gas and used for solutions unstable in presence of air. A diagram of the modified apparatus* is given in Fig. 1.

The apparatus shown, except the connections between the tubes A and C, is one of the several types available on the market. It has a reservoir, R, burette, B, side tubes, A and D, and a tube, S, which

* The design is the subject of a patent application.

narrows down at the extremity N, and acts as a siphon for adjusting the solution in the burette to zero point automatically. Normally a rubber bellows is attached to the point A and a rubber tube with a pinch cock to the tube D. With the help of the rubber bellows, air is blown into the reservoir, R, which forces the liquid into the burette, B; when the pressure in the reservoir is released by opening the pinch cock at D, the liquid near the top of the burette is sucked back into the reservoir till the level is at zero.

For working the apparatus with a vacuum we have connected A to C through a two-way stop-cock T, as shown in the drawing. The joints J and J may be two glass tubes joined by rubber tubing or one



continuous glass tube. For use, E is connected to the vacuum. This reduces the pressure in the burette and the solution is sucked up from the reservoir, R, through the tube S. After the burette is filled to above the zero mark, the two-way stop-cock T is turned round to make connection between A and C. Thus both the reservoir and the burette are connected with the atmosphere and the siphon tube S adjusts the level of the solution in the burette to zero. The burette is now ready for use and the solution can be taken from F without any difficulty, as the top of the burette is connected to the amosphere through C, H, A, and D.

For obtaining the reduced pressure, E can be connected to a vacuum line, or a vacuum pump, or air

can be merely sucked out by the mouth. The burette can be connected either to the vacuum or to the atmosphere by simply turning the stop-cock T.

For working under an inert atmosphere, D can be connected directly to a small Kipp's apparatus or to a gas cylinder. It is not necessary to have a stop-cock between the Kipp's apparatus and tube D, as the pressure adjusts itself automatically.

For using the apparatus for standard alkali solution, D is connected to a gas washing tower containing a concentrated solution of sodium hydroxide; thus the air entering the reservoir or the burette is always free from carbon dioxide, and there will be no danger of the standard alkali solution being affected.

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March, 1949

NOTES ON THE DETERMINATION OF COPPER IN FOODS BY HIGH'S METHOD

THE method described by High[1] for the determination of copper in foods has been found very convenient and reliable. The following comments may be of service to other users of the method.

1. High ashes the sample in a silica basin. We have found that with certain materials, particularly when much phosphate is present, the silica is attacked and retains some of the copper. Experiments in which known amounts of copper were added to a mixture of 50 parts of sucrose and 1 part of potassium dihydrogen phosphate gave from 70 to 92 per cent. recovery of the added copper. A porcelain basin, however, is quite satisfactory as long as the internal glazing is undamaged, provided the temperature of ashing is not allowed to rise above 600° C. Ashing should be continued until the ash is nearly white, but traces of carbon can be filtered off after extraction of the ash without effect on the result.

2. High adds 0·5 g. of his solid reagent (a 1 : 25 mixture of sodium diethyldithiocarbamate and sodium chloride) to an unstated volume of the solution of the ash of the sample. If the total available volume of solution is only 50 ml. (as is necessary when the total copper in the ash is small) the solid reagent may be added to 20 ml. or less, to permit duplication, and we find that 0·25 g. of solid reagent is then amply sufficient for the development of maximum coloration. It is now our practice to take 20 ml. of the solution on all occasions, and economy of reagent is thereby effected.

3. Sodium diethyldithiocarbamate appears to decompose with time even in the solid state, and this is particularly true when ground finely with sodium chloride (perhaps because of the greatly increased surface area). We have found it advisable to purchase small quantities of the reagent frequently, and to keep the mixture with sodium chloride not longer than six weeks. The solutions to which the solid mixture is added must be watched for the slightest sign of turbidity as distinct from coloration; if turbidity is noticed it is almost certainly due to decomposition of the solid reagent. It is advisable to put up a "blank" on the reagents with each set of determinations, and if a previously plotted standard curve is to be used, at least one point on it should be freshly checked. Using the Spekker absorptiometer with Ilford 601 (Violet) filters, and setting the drum reading to 1·0 against the "blank" *without* solid reagent, the reading of the "blank" *with* solid reagent should be not less than 0·98. A solution containing 5 μg. of copper per ml. gives a drum reading of about 0·4.

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ERIC C. WOOD
Miss E. M. AULT

March, 1949

LABORATORY INVESTIGATION OF LEAD POISONING IN CATTLE

THE following is a report on the analytical results obtained from the examination of viscera and visceral contents removed from cattle suspected of having been poisoned by ingestion of lead. Reports received showed that the chief offending objects to which cattle gained access were abandoned tins of paint left in fields; pipes that had been re-jointed with red lead were another source of danger. Old military sites re-opened for cattle grazing were also a potential danger unless all metallic refuse had been carefully removed.

In all cases of cattle affected, the following symptoms and post-mortem findings were recorded by the veterinary surgeons who submitted the samples for analysis: evidence of abdominal pain, grinding of teeth, staggering, salivation, convulsions, blindness, coma, then death. Blindness itself in most instances appeared to be suggestive of lead poisoning having occurred, but a few samples examined in this laboratory from cases where there was a report of blindness sometimes gave negative results by chemical analysis and no mineral poisons of any description were found. It is possible that death in such cases was due to poisonous plants or bacterial infection.

Post-mortem appearances in all cases included detachable mucous membrane from the stomachs and inflammation of the abomasum.

It might be of some interest to describe briefly the physiological action said to take place in the bovine body after ingestion of lead.[1] Absorption from the stomachs in such cases is said to be slow or of the cumulative type, and the liver and kidney are the organs chiefly concerned in arresting lead salts during the period of absorption and elimination; it is possible that the absorbed lead is in the form of some complex albuminate. Like most metallic poisons, lead is eliminated slowly, by the urine and faeces, although a portion may be re-absorbed and carried back to the liver.

Prior to chemical analysis and estimation, an examination was made of all stomach contents received. In three of the cases under review, lead paint was found in the form of small particles which were seen to be black externally and white internally.

METHOD—

Reagents—A. Chloroform A.R. *B*. Aqueous citric acid solution, 5 g. per 100 ml. *C*. Aqueous potassium cyanide solution, 5 g. per 100 ml. *D*. Dithizone solution in chloroform, 10 mg. in 400 ml. *E*. Lead-free reagent, prepared by mixing 30 ml. of reagent *C*, 15 ml. of reagent *B* and 5 ml. of aqueous ammonia (sp.gr. 0·880) and diluting to 450 ml. with distilled water. *F*. Lead-free reagent, prepared by mixing 10 ml. of reagent *C* and 5 ml. of aqueous ammonia (sp.gr. 0·880) and diluting to 500 ml. with distilled water. If necessary, reagents *E* and *F* can be freed from lead by extraction with dithizone solution.

Procedure—Ignite 20 g. of sample in a crucible after adding 1 ml. of diluted sulphuric acid (1 : 5). Then ash at 500° C. for 2 hours. (If the sample is in liquid form add the sulphuric acid, evaporate to dryness on a water-bath and then ash at 500° C.) Cool and add 1 ml. of concentrated hydrochloric acid and place the basin on a steam-bath for several minutes; add 2 ml. of diluted nitric acid (1 : 5). Filter into a 100-ml. graduated flask, wash well with distilled water and make up to the mark; mix thoroughly. Pipette an aliquot of the solution into a 100-ml. beaker and neutralise with diluted aqueous ammonia (1 : 3). Then make acid with 5 per cent. citric acid solution and finally alkaline with ammonia. Transfer the solution from the beaker to a 250-ml. separating funnel, add 15 ml. of lead-free reagent *E* and 10 ml. of dithizone solution *D*. Shake well and allow to settle. Run off the dithizone layer into another funnel and re-extract the aqueous layer with 10 ml. of dithizone solution, or until the added dithizone solution remains green.

To the combined dithizone extracts add 20 ml. of lead-free reagent *F*. Shake well, allow to settle and repeat until the added reagent *F* remains colourless.

Run the dithizone extract into a 50-ml. flask and make up to the mark with chloroform. Match in a colorimeter against a standard solution which has been treated in exactly the same manner.

Photometric estimations can be carried out by preparing a curve from a series of standards prepared from standard lead acetate solution.

Quantitative estimations carried out by the above method gave the following results. For the purposes of comparison the figures have been converted to mg. per 100 g. of material as received.

Case No.	Subject	Rumen contents	Reticulum contents	Abomasum contents	Liver	Kidney
C.32	Heifer	1·03	56·9	10·14	4·36	16·35
C.34	Calf	8·27	11·02	149·7	7·36	28·7
C.37	Roan cow	1·65	*	*	7·18	48·2
C.38	Heifer	*	*	8·3	8·35	nil
C.123	Calf	84·0	*	35·0	*	*
C.155	Stirk	68·6	178·0	106·0	12·1	21·7
E.58	Calf	38·4	*	70·2	11·8	23·2
E.59	"	42·0	*	*	10·7	nil
E.75	Stirk	72·0	*	*	11·3	*
E.100	Heifer	*	*	89·0	10·6	25·1
E.101	"	*	*	38·7	11·2	26·3
E.103	Calf	*	*	*	9·7	*

* Denotes no sample received.

The data show that considerable amounts of lead were found in stomach contents except in No. C.38 and that the figures for kidney are much higher than those for the liver.

There is no evidence that lead is a normal constituent of the body in anything more than insignificant amounts; and the estimation of lead in liver and kidney proved those organs to be valuable material in the investigations carried out in this laboratory. It might, therefore, be safe to say that the figures given for these organs are of considerable diagnostic value, but on the other hand it is difficult to arrive at a safe and definite figure for a toxic dose.

REFERENCE

1. Lander's *Veterinary Toxicology*, 3rd Edition, 1945.

DEPARTMENT OF VETERINARY HYGIENE
ROYAL (DICK) VETERINARY COLLEGE
EDINBURGH, 9

D. J. BROWN
December, 1948

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1949, **74**, 464).

Public Analyst	*Appointments*
ALCOCK, Arthur	County Borough of Salford.
BUTTON, Donald Frank Harrington ..	Metropolitan Borough of Islington.
JENKINS, Daniel Ceiriog Evans ..	Borough of Hereford.
JONES, Daniel Evans	County Borough of Merthyr Tydfil.
WORDSWORTH, Charles Harcourt ..	Metropolitan Borough of Paddington.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENT

NOTIFICATION of the following appointment has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1949, **74**, 464).

Official Agricultural Analyst	*Appointment*
ALCOCK, Arthur	County Borough of Salford.

Ministry of Food

STATUTORY INSTRUMENT*

1949.—No. 1656. The Food Standards (Table Jellies) Order, 1949. Price 1d.

This Order, which came into force on September 11th, 1949, and which should be read with the Food Standards (General Provisions) Order, prescribes standards for Table Jelly Tablets, Table Jelly Crystals and Table Jelly Compounds.

The Schedule to the Order sets out the standards as below :—

1. The standard for table jelly tablets shall be as follows:—
   (*a*) Table jelly tablets shall consist of sugar, gelatine or other jelly-forming material (other than farinaceous products) with other ingredients (not being farinaceous products) in tablet form. When made up with water, the tablet shall produce a jelly table sweet complying with the setting test specified in paragraph 5 of this Schedule.
   (*b*) The percentage of soluble solids contained in the tablets shall be not less than 72 per cent. by weight.
   (*c*) The percentage of sugar contained in the tablets (all sugar contained in or added to the product being taken into account in whatsoever form the same may have been introduced) shall be not less than 63 per cent.
2. The standard for table jelly crystals shall be as follows:—
   (*a*) Table jelly crystals shall consist of sugar and gelatine or other jelly-forming material (other than farinaceous products) with other ingredients (not being farinaceous products) in crystal form. When made up with water, the crystals shall produce a jelly table sweet complying with the setting test specified in paragraph 5 of this Schedule.
   (*b*) The percentage of sugar contained in the crystals (all sugar contained in or added to the product being taken into account in whatsoever form the same may have been introduced) shall be not less than 84 per cent.
3. The standard for table jelly compounds shall be as follows:—
   (*a*) Table jelly compounds shall consist of sugar and gelatine or other jelly-forming material with other ingredients to form a product which, when made up with milk, produces a jelly table sweet complying with the setting test specified in paragraph 5 of this Schedule.
   (*b*) The percentage of sugar contained therein (all sugar contained in or added to the product being taken into account in whatsoever form the same may have been introduced) shall be not less than 50 per cent.
   (*c*) The percentage of starch if any contained therein shall not exceed 20 per cent. by weight.
4.—(1) "Percentage of soluble solids" means the percentage by weight of soluble solids ascertained at 20° C. by means of a refractometer on the sucrose scale, no correction being made for insoluble solids.
   (2) The percentage of sugar shall be determined by adding the percentage by weight of sucrose to the percentage by weight of the total reducing sugars expressed in terms of dextrose.

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

5. The setting test referred to in paragraphs 1 to 3 of this Schedule shall be as follows:—

If the product is sold in a container with a content intended to produce one pint of a jelly table sweet, that content, or in any other case 3·75 oz. of the product, shall be made into a jelly table sweet—

(*a*) in the case of table jelly tablets or table jelly crystals by the addition of water at 88° C. approximately;

(*b*) in the case of table jelly compounds by the addition of milk at 88° C. approximately;

the total volume to amount in each case to one pint.

85 mls. of the solution shall be immediately introduced into each of six beakers of approximately 5 cm. internal diameter and the beakers cooled for 18 hours in a water-bath maintained at 16° C. ± 1° C. At the end of this period the contents of the beakers shall be turned out on to a plate or dish by the following method. Each beaker shall be immersed in a water bath at approximately 50° C. for 8 seconds. Upon removal each beaker shall be immediately dried and the contents transferred to a plate or dish by inversion of the beaker. If not less than four out of the six jelly table sweets shall retain for 30 minutes the general shape of the beaker and shall not at the end of such period have collapsed or split so as to alter their shape, then the setting test shall be deemed to have been satisfied.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Preservation of Dairy Products for the Phosphatase Test. G. P. S. Sanders and O. S. Sager (*J. Dairy Sci.*, 1949, **32**, 166–174)—The following substances were tested for use as preservatives of milk intended for the Sanders-Sager phosphatase test (*Ibid.*, 1947, **30**, 909–920):—1·5 to 2 per cent. of chloroform, 3 to 3·5 per cent. of toluene, 1·5 to 2 per cent. of borax, 0·01 to 0·03 per cent. of formaldehyde, 0·05 to 0·1 per cent. of mercuric chloride, and 0·06 to 0·15 per cent. of hydrogen peroxide. All preserved fresh milk for 10 to 21 days at room temperature, but the last three produced serious chemical inhibition of the phosphatase activity and the borax interfered with the pH of the test. Chloroform was more effective than toluene as a preservative in the same concentration: neither inhibited the enzyme appreciably. Phenolic compounds should not be used as preservatives in the test because they give rise to interfering colours. The use of 1·5 to 2 per cent. of chloroform is recommended. A. H. ADAMS

Quick and Simple Method for Determination of Quinine and Total Alkaloids in Cinchona Bark. A. J. Loustalot and C. Pagan (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 153–159)—*Procedure*—To 2 g. of dried *Cinchona* bark ground to pass a 100-mesh sieve add about 0·5 g. of finely powdered calcium oxide and enough water (7 to 10 ml.) to make a smooth paste. After 10 min., transfer the paste to a 200-ml. flask with 100 to 150 ml. of alcohol. Shake the suspension vigorously at first and then occasionally during 1 hr., after which adjust its volume to 200 ml. and filter through a Whatman 24-cm. No. 5 paper, placing a watch glass over the funnel and a cotton wool plug in the neck of the receiving flask to minimise evaporation.

Pipette 25 ml. of the extract into a 50-ml. flask, add 25 mg. of Norit A (decolorising carbon), shake for 15 to 30 sec., and filter. To 8 ml. of the clarified extract (≡ 80 mg. of bark) add 5 ml. of 0·1 *N* hydrochloric acid, dilute to 100 ml., and determine the percentage transmittance at 380 mμ. in a photometer. Ascertain the percentage of quinine sulphate directly from a standard curve prepared as follows.

Pipette 5 ml. of each quinine sulphate stock solution (*infra*) into 100-ml. flasks, add 8 ml. of 95 per cent. alcohol, dilute to volume with water, and determine the percentage transmittance at 380 mμ., using a blank solution containing the acid and alcohol as reference solution. Plot the logarithms of percentage transmissions against the concentrations. Prepare a new curve for each set of determinations.

To determine the total alkaloids, pipette 100 ml. of the alcoholic bark extract (≡ 1 g. of bark) into a 400-ml. beaker, add 10 ml. of 0·1 *N* hydrochloric acid and 100 ml. of water, and titrate the excess of acid to pH 6·2 with 0·05 *N* sodium hydroxide and a pH meter. Make a blank determination by titrating the alcohol and acid. Subtract the value obtained with the unknown solution from the blank determination. Each millilitre of 0·05 *N* sodium hydroxide ≡ 0·0155 g. of total alkaloid.

To prepare the standard stock solutions, dissolve quinine sulphate in 0·1 *N* hydrochloric acid so that 5-ml. aliquots contain 1·6, 3·2, 4·8, 6·4, and 8·0 mg. of anhydrous quinine sulphate. This series is equivalent to 2, 4, 6, 8, and 10 per cent. of quinine sulphate when an 80-mg. sample of *Cinchona* bark is used. The solutions are stable for 6 months or more in tightly stoppered, black bottles.

At a wavelength of 380 mμ, cinchonine and cinchonidine have no effect on the absorption of light by quinine and quinidine. Solutions containing quinine alone and quinidine alone at the same concentrations give equal transmission values at 380 mμ. Since quinidine resembles quinine in its physiological effects, no serious error is incurred by its inclusion as an anti-malarial constituent. Also, quinidine is seldom present in large amount in *Cinchona* bark.

When the logarithmic percentage transmittance at 380 mμ. is plotted against concentrations of

quinine sulphate ranging from 8 to 92 p.p.m., the deviation from Beer's law is small.

The method may be useful for the rapid evaluation of *Cinchona* seedlings in breeding and selection investigations and for the commercial assay of *Cinchona* bark. A. O. JONES

Determination of Nitrogen in Milk by Direct Nesslerisation of the Digested Sample. J. H. Hettrick and R. M. Whitney (*J. Dairy Sci.*, 1949, **32**, 111–112)—A rapid method for determining protein in milk by direct nesslerisation of the digested sample is described. The ammonia complex formed with coloured Nessler reagent is measured on the spectrophotometer and referred to a standard curve. The wavelength for maximum absorption is a function of the Nessler reagent used and for the most accurate work, should be determined by each investigator for the reagent he uses. The point of maximum absorption found by the authors was at 420 mμ. Small differences in the amounts of the reagents used do not affect the accuracy of the method, but the temperature must be controlled to at least ±1° C. and the time for colour development to ±3 min. Results obtained average 2·5 per cent. low, but are within experimental error, when compared with a macro-determination of nitrogen.

Method—Reagents—Ammonia-free water—Re-distil distilled water in a 2-litre glass still in presence of 10 ml. of concentrated sulphuric acid. Test for ammonia as below and discard if not ammonia-free. *Gum acacia solution, 2 per cent. w/v*—Dissolve 2 g. of good quality gum acacia in ammonia-free water and dilute to 100 ml. Add 1 ml. of carbon tetrachloride as preservative. This solution should be clear. *Nessler's reagent*—Dissolve 50 g. of potassium iodide in 35 ml. of cold water (ammonia-free) and add a saturated solution of mercuric chloride until the orange precipitate persists. Add 400 ml. of approximately 9 *N* sodium hydroxide solution (360 g. of sodium hydroxide in 1 litre of ammonia-free water) and dilute to 1 litre with ammonia-free water. Allow to stand several days.

Procedure—Dilute about 1 g. of milk, weighed accurately, to 500 ml. with ammonia-free water and transfer a 12·5-ml. aliquot to a micro-Kjeldahl flask. Add 0·4 g. of potassium sulphate and 0·5 ml. of concentrated sulphuric acid and digest for exactly 3 min. after the appearance of sulphur trioxide fumes. Cool and add 3 drops of 30 per cent. hydrogen peroxide directly into the liquid in the flask. Digest for exactly 3 min. more, cool to room temperature, dilute with ammonia-free water, add 1 ml. of 2 per cent. gum acacia solution, and make up to 50 ml. with ammonia-free water. Transfer a 10-ml. aliquot to a test tube and place the tubes in a thermostat at a convenient temperature ±1° C. Add exactly 2 ml. of Nessler reagent and measure the percentage transmission on the spectrophotometer at 420 mμ. after 10 min., using a permanent colour blank. Determine the amount of nitrogen present in the aliquot from the standard curve.

Permanent colour blank—To a series of test tubes containing 10 ml. of distilled water, add 0·1, 0·2, 0·3, and 0·5 ml. of 1 per cent. potassium dichromate solution and measure the percentage transmission at 420 mμ. on the spectrophotometer, using distilled water as a blank. Plot the logarithms of the percentage transmissions against concentrations and from this curve determine the concentration of potassium dichromate necessary to yield the same percentage transmission as the average of five reagent blanks measured against distilled water. Prepare a potassium dichromate solution of this concentration to use as a permanent colour blank.

Standard curve—Transfer 0·4 g. of potassium sulphate and 0·5 ml. of concentrated sulphuric acid to each of a series of ten micro-Kjeldahl flasks containing 2, 6, 8, 10, 11, 12, 13, 14, 15, and 16 ml. of ammonia solution (1 ml. equivalent of 0·01 mg. of nitrogen) and proceed as in the method above. Plot the logarithms of the percentage transmission values obtained against the milligrams of nitrogen per 50 ml. of digested solution. A. H. ADAMS

Determination of Copper in Foods by a Two-Colour Dithizone Method. C. A. Greenleaf (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 144–152)—*Reagents*—To prepare and purify the reagents used in the method proceed as follows. Re-distil water, hydrochloric acid, nitric acid, and ammonia solution from Pyrex glass. *Carbon tetrachloride*—Shake thoroughly with 10 per cent. sodium hydroxide solution, dehydrate with anhydrous sodium sulphate, and re-distil from calcium oxide, rejecting the first portion containing moisture. *Dithizone solution*—Dissolve 30 mg. in 20 ml. of chloroform and shake out separately with a 100-ml. and a 50-ml. portion of diluted aqueous ammonia (1 : 100). Discard the chloroform, extract the ammonia solutions in series with small portions of carbon tetrachloride until the extracts are practically colourless, combine and filter the ammonia solutions, add 10 ml. of 3·2 per cent. sodium bisulphite solution (*infra*) and a slight excess of 6 *N* hydrochloric acid. Extract the dithizone with several portions of carbon tetrachloride and make it up to 1 litre. Dilute 300 ml. of this solution to 500 ml. with carbon tetrachloride. Store both solutions (designated as 25 mg. per litre and 15 mg. per litre, respectively) in a refrigerator in dark bottles under a layer of 0·1 *M* sulphurous acid (sodium bisulphite and hydrochloric acid). *Ammonium citrate solution*—Dissolve 150 g. of citric acid in 600 ml. of water, make just alkaline to cresol red, used externally, with ammonia solution, add 10 ml. in excess and dilute to 1 litre. Extract with dithizone solution in chloroform until the extracts are green, then with chloroform until the extracts are colourless and, finally, with carbon tetrachloride, and filter. *Potassium iodide solution* (10 per cent.)—To 50 g. of potassium iodide dissolved in 500 ml. of water in a separator add dithizone solution in chloroform and add ammonia solution drop by drop with shaking until the dithizone begins to enter the aqueous phase. Extract with further portions of dithizone solution until the extracts are green, then add hydrochloric acid drop by drop until the dithizone is precipitated from the aqueous phase. Extract with carbon tetrachloride and filter. If free iodine appears on standing, discharge

the colour with dilute sodium thiosulphate or bisulphite solution. *Acid potassium phthalate* (0·1 *M*)—Dissolve 10·2 g. of the salt in 500 ml. of water, extract with dithizone solution in carbon tetrachloride, then with carbon tetrachloride and filter. Purify 3·2 per cent. *sodium bisulphite solution* in the same manner. *Sulphuric acid* is the least controllable reagent contributing copper to the blank determination; it usually contains 1 or 2 p.p.m.

Procedure—Prepare the sample by wet combustion with nitric and sulphuric acids. On the macro-scale take an amount of sample equivalent to 20 to 100 μg. of copper and use 20 ml. of sulphuric acid. When the digest remains yellow and does not char on evolution of sulphur trioxide add a little more nitric acid and 5 ml. of 60 to 70 per cent. perchloric acid, heat until the perchloric acid is expelled, cool, add 50 ml. of water and re-heat to fuming-point, cool, and dilute to 200 ml. With micro-digestion apparatus reduce the amounts of sample and acids proportionately and dilute the digest with 10 to 20 ml. of water before transferring to a separator.

To 10 ml. of ammonium citrate solution in a separator add the digested micro-sample or a 20-ml. aliquot of the macro-digest, dilute to about 85 ml., add 0·5 ml. of bromophenol blue indicator, and aqueous ammonia solution until the bluish tinge of the indicator appears and then *N* hydrochloric acid, drop by drop, until the colour just changes to yellow (pH 3·0 to 3·3), avoiding excess of acid. Add 10 ml. of 10 per cent. potassium iodide solution and extract with 20 ml. of dithizone solution in carbon tetrachloride, shaking vigorously for 2 or 3 min. If the extract is red add more dithizone solution and shake again. Rinse down the separator walls with carbon tetrachloride, displace floating drops of dithizone solution, draw off all but about 5 ml. of the aqueous layer by suction, rinse the stopper and walls with 25 ml. of water, but do not shake, and again remove the upper layer by suction. Add 2·5 ml. of 2 per cent. potassium iodide solution, shake vigorously and draw off the dithizone solution into a 125-ml. separator, using small amounts of carbon tetrachloride to displace floating drops and to wash dithizone solution from the tap bore and stem. Discard the aqueous layer. To the extract add 25 ml. of 0·01 *N* hydrochloric acid and saturated bromine water, drop by drop, with shaking until the dithizone layer is yellow. Shake vigorously for 1 min. to transfer the copper to the hydrochloric acid and then draw off the carbon tetrachloride layer. Wash the hydrochloric acid extract with 10 ml. of carbon tetrachloride, discard this washing, and remove floating drops and the carbon tetrachloride in the tap bore and stem by means of more carbon tetrachloride. Finally, apply suction to the top of the separator and admit a stream of air through the tap until all the carbon tetrachloride remaining in the liquid has been removed by aspiration. If the amount of copper is less than 12 μg. (as shown by absence of appreciable colour change in the preliminary extraction) use the entire solution for the estimation. If purplish hues develop draw off the copper solution, rinse the separator with 0·01 *N* hydrochloric acid and make the solution and rinsings up to a definite volume with 0·01 *N* hydrochloric acid.

To the 25 ml. of solution (or an aliquot diluted to 25 ml. with 0·01 *N* hydrochloric acid) add 5 ml. of acid potassium phthalate solution and 1 ml. of sodium bisulphite solution. From a burette add dithizone solution (15 mg. per litre) with intermittent vigorous shaking until an excess is present, as shown by a purple or greyish-purple colour. Add enough carbon tetrachloride to make exactly 15 ml. and shake vigorously for 1 min. If more than 12 ml. of dithizone solution are required make up to 30 ml. and double the amounts of the other reagents and also double the amount of copper obtained from the calibration curve. Draw off the carbon tetrachloride solution through a cotton wool plug into a test tube or small flask and determine the optical density at 520 mμ. and at 625 mμ. against carbon tetrachloride as reference liquid.

To construct the calibration curve place in a series of separators 25-ml. portions of 0·01 *N* hydrochloric acid containing respectively 0, 3, 6, 9, and 12 μg. of copper. Add the acid potassium phthalate and sodium bisulphite solutions. To the first separator add a small excess of dithizone solution as described (*supra*) and carbon tetrachloride to 15 ml. To the others add increasing amounts of dithizone solution up to the maximum amount that can be read accurately in the spectrophotometer (8 ml. at zero copper and 14 ml. at 12 μg.). Extract and determine the optical densities of the extracts at 520 mμ. and 625 mμ. as before. It is preferable to make two or three determinations of each concentration of copper by using several series of separators. From the results derive the calibration equation, preferably by the method of least squares, in the form $C = AD_{520} - BD_{625} - C_0$, where C is the concentration of copper (μg. per 15 ml.), D is the optical density at the indicated wavelength, and A, B, and C_0 are arbitrary constants. In developing the method a Coleman Universal photo-electric spectrophotometer was used with square cells of ½-in. depth, No. ST–10–S, and the calibration equation took the form

$$C = 21{\cdot}8D_{520} - 6D_{625} - 0{\cdot}6$$

To avoid interference by the yellow oxidation product of dithizone this reagent should be prepared and stored in the manner described. Interference by bismuth, silver, and mercury is prevented by the use of potassium iodide and, although zinc and cadmium would be extracted in the preliminary stages, the acidified potassium iodide removes them entirely. A. O. Jones

Cerimetric Determination of Small Quantities of Arsenic in Drugs after Reduction with Hypophosphite. P. Paulssen (*Pharm. Weekblad*, 1949, **84**, 33–38)—The method, as applied to arsenic pills (containing liquorice and sugar), is as follows.

Procedure—Rub down two pills (containing about 1·5 mg. of arsenic) with 10 ml. of 25 per cent. hydrochloric acid, and add 50 mg. of potassium chlorate. After 5 min., heat on the water-bath for 10 min., cool, and repeat the operation with a

further 50 mg. of potassium chlorate. Filter through cotton wool, wash the filter with 20 ml. of 6 *N* hydrochloric acid, and add 10 ml. of 10 per cent. calcium hypophosphite solution, and a small amount of finely divided asbestos. Heat for 30 min. on the water-bath, boil for 5 min., and allow to stand until cold. Collect the precipitate on a perforated platinum plate or sintered-glass filter (1 cm. diameter) covered with a layer of asbestos about 7·5 mm. thick. Wash the precipitate with water and alcohol, and finally with water, remove the filter disc from the tube, transfer it to a 100-ml. flask, and add 25 ml. of 0·008 *N* ceric sulphate solution.

After the arsenic has dissolved (in about 15 min.), add a few drops of 0·25 per cent. osmic acid solution in dilute sulphuric acid and 1 drop of 0·025 *M* *o*-phenanthroline - ferrous solution, and titrate the excess of ceric sulphate with 0·005 *N* arsenious acid solution to the first change to red. One ml. of the ceric sulphate solution corresponds to 0·120 mg. of arsenic. The method can also be used for atoxyl and neo-salvarsan. G. MIDDLETON

Biochemical

Colorimetric Determination of Blood-Chloride by the Iodimetric Method. H. A. Stiff, jun. (*J. Biol. Chem.*, 1948, **172**, 695–698)—A colorimetric method that uses the same reagents as the volumetric method of Van Slyke and Hiller (*Ibid.*, 1947, **167**, 107) is given.

Procedure—Place 5 ml. of the phosphoric - tungstic acid in a 10-ml. centrifuge tube and add 0·2 ml. of serum or plasma from an accurate wash-out pipette. In another tube place 5 ml. of a mixture of 10 ml. of 0·1 *M* sodium chloride with 250 ml. of phosphoric - tungstic acid. Add to each tube about 60 mg. of silver iodate with a glass spoon. Stopper with a rubber stopper and shake vigorously for 40 sec. Centrifuge for 1 min. at 2500 r.p.m. To each of two Evelyn colorimeter tubes add about 200 mg. of sodium iodide and 5 ml. of water. Transfer 0·5 ml. of the clear supernatant layer from each centrifuge tube to its respective colorimeter tube. Add to each tube 10 ml. of a solution of 7·5 ml. of 0·02303 *N* sodium thiosulphate diluted to 200 ml. Read the absorption at 420 mμ. against a water blank.

Maximum absorption is at 400 mμ., and there is no change in the colour intensity up to a period of 5 hr. The intensity varies slightly with temperature and so a standard is necessary for each set of determinations. W. S. WISE

Colorimetric Method for the Determination of Citric Acid in Blood and Plasma. G. H. Wolcott and P. D. Boyer (*J. Biol. Chem.*, 1948, **172**, 729–736)—The method, sensitive to from 5 to 50 μg. of citric acid, is based on the well-known formation from citric acid of a brominated acetone derivative (presumably principally pentabromo-acetone), and on a new micro-colorimetric method for the determination of bromide.

Method—Reagents—(1) 1 *N* Sodium hypochlorite in 0·1 *N* sodium hydroxide. Bubble chlorine, with stirring and cooling, through a solution of 88 g. of sodium hydroxide in about 1·5 litres of water until 71 g. of chlorine have dissolved. Dilute the solution to 2 litres. The concentration of sodium hydroxide should be between 0·08 *N* and 0·12 *N*. To 1 ml. of hypochlorite reagent add 5 ml. of water and about 5 drops of 30 per cent. hydrogen peroxide. When no more oxygen is evolved on adding more peroxide, titrate the alkali, using phenol red as indicator. (2) 2 *M* Hydrazine. Dissolve 32·5 g. of hydrazine sulphate in 50 ml. of 5 *N* sodium hydroxide and dilute to 500 ml.

Procedure—Add 1·0 ml. of fresh whole blood (oxalated or heparinised) or 1·0 ml. of plasma to 4·0 ml. of 10 per cent. trichloroacetic acid contained in a centrifuge tube. Shake well and then set aside for 10 min. Centrifuge and then place 4·0 ml. of the clear supernatant layer in a 125-ml. separating funnel. Add to this 5·0 ml. of 20 *N* sulphuric acid and 3·0 ml. of water. Cool to room temperature.

Prepare a series of standards for a calibration curve. Place in a 125-ml. separating funnel 5·0 ml. of 20 *N* sulphuric acid, 4·0 ml. of 8 per cent. trichloroacetic acid solution and 3·0 ml. of a series of standard citric acid solutions containing from 5·0 to 50·0 μg. of citric acid. Prepare a reagent blank with water in place of the citric acid solution.

Add to the contents of the funnel 1 ml. of a mixture of 90 ml. of 1 *M* potassium bromide with 10 ml. of 1 *M* sodium bromate. Add slowly, with vigorous agitation, 2·0 ml. of 0·5 *M* potassium permanganate. Allow to stand for 10 min. and then slowly add the 2 *M* hydrazine solution until the solution is decolorised (about 1 ml.). Wash the sides of the funnel with 1 to 2 ml. of water. Add 15 ml. of light petroleum (b.p. above 60° C.), tightly stopper the funnel with a clean rubber stopper, and shake well for 5 min. (preferably on an automatic shaker). Release the pressure, allow the layers to separate, and then discard the aqueous phase. Wash the petroleum layer and the sides and top of the funnel four times with glass-distilled water. Transfer the extract to another separating funnel and rinse the first funnel three times with light petroleum. Wash the extract twice more with water. Remove the aqueous layer.

Add to the contents of the funnel 15 ml. of a solution, freshly prepared every 10 days, containing 0·05 *M* sodium sulphite and 0·40 *M* sodium di-hydrogen phosphate. Tightly stopper the funnel with a clean rubber stopper and shake well for 5 min. Allow the layers to separate, take a 10-ml. volumetric flask, the neck of which is rinsed with a few drops of water. Heat the flask in boiling water for about 5 min. and then add exactly 2 ml. of the hypochlorite solution. Rinse the neck of the flask twice with less than 1 ml. of water, mix the contents and continue heating for exactly 4 min. from the time of the addition of the hypochlorite. Add 1·0 ml. of 6 *M* sodium formate, remove the flask from the bath, and shake the contents. Rotate the flask so that the hot formate solution comes into contact with the inner wall of the neck and then cool the solution to room temperature. Add 1 drop of a solution of 10 g. of ammonium molybdate per 100 ml. and rinse the sides of the flask with 5·0 ml.

of 3 *M* phosphoric acid. Mix the contents and add 1·0 ml. of potassium iodide solution (5 g. per 100 ml.; the solution must not be coloured yellow). Make up to the 25-ml. mark and transfer to a colorimeter tube. After 10 to 15 min. read the absorption at 400 mμ. against a reagent blank.

The Beer's law curve is not linear when a standard curve is prepared with bromide. A linear curve is obtained with citric acid standards. This is an artefact probably due to a higher yield of bromoacetone derivative with higher citric acid concentrations.

Low results were obtained in the presence of large amounts of glucose, which can be detected by an increased consumption of permanganate. In the presence of large amounts of glucose, use the modification of Goldberg and Bernheim (*Ibid.*, 1944, **156**, 33).

Acetone bodies may interfere. If samples are known to contain acetone or acetoacetic acid, take a 4·0-ml. trichloroacetic acid aliquot and boil with 10·0 ml. of 10 *N* sulphuric acid until the total volume is less than 10 ml. Bring the volume to 10 ml. and transfer to a separating funnel, the rinsing being done with two 1-ml. portions of water. Carry out a preliminary bromination by adding 1 ml. of a mixture of 50 ml. of 1 *M* potassium bromide and 10 ml. of 1 *M* sodium bromate. Allow to stand for 30 min. and then extract with three 10-ml. aliquots of carbon tetrachloride. Discard the carbon tetrachloride layer, which contains any free bromine or ether-soluble brominated compounds. Carry out the determination as above.

W. S. WISE

Turbidimetric Method for the Assay of Hyaluronidase. A. Dorfman and M. L. Ott (*J. Biol. Chem.*, 1948, **172**, 367–375)—Previous methods for estimating hyaluronidase have been either inaccurate or time-consuming. The following method is not open to these objections. A mixture of hyaluronic acid and acidified albumin produces a turbidity that is directly proportional to the hyaluronic acid content up to 2·0 mg. Above this, a clot forms. The turbidity development shows a maximum at pH 3·82, is sensitive to the ionic strength of the solution, and decreases with time. By using standard conditions, the turbidity after treatment of the solution for a fixed time with hyaluronidase is directly proportional to the enzyme concentration.

Materials—(1) *Hyaluronic acid*—Wash human umbilical cords free from blood and store in cold acetone. Prepare an "acetone powder" by grinding in a meat grinder and washing with acetone. Stir vigorously together for 2 hr. 200 g. of dry powder, 1200 ml. of Hayem's solution, and 3000 ml. of water. Centrifuge and then filter through glass wool. Precipitate the hyaluronic acid by pouring the solution into 12 litres of cold acetone. Wash three times with cold acetone, filter, and wash twice with alcohol and anhydrous ether. Dry over phosphorus pentoxide for 24 hr. This procedure gives a yield of 6 per cent. and the product has a relative viscosity of 3·0 at a concentration of 10 mg. per ml. For the determination, use a solution containing 3·0 mg. per ml. in 0·3 *M* phosphate buffer of pH 5·5. The solution so prepared is slightly opalescent, but can be obtained water-clear by filtration through a Seitz filter. Dilute to a standard turbidity—this usually requires a 2 : 3 dilution. (2) *Acidified horse serum albumin*—Prepare crystalline horse serum albumin (Kekwick, *Biochem. J.*, 1938, **32**, 553). Dissolve 1 g. of the purified albumin in 1 litre of 0·1 *M* acetate buffer of pH 4·1 and then adjust the pH to 3·75 with 4 *N* hydrochloric acid. This solution can be stored indefinitely at 4° C.

Procedure—Make up the enzyme solution in 0·5 ml. of 0·2 *M* borate buffer of pH 7·5 mixed with 0·5 ml. of 0·9 per cent. saline. Add 1 ml. of hyaluronic acid solution. Keep at 38° C. for 45 min. in cuvettes. Then add 10 ml. of acidified albumin reagent at room temperature and shake well. After exactly 5 min., read the absorption at 600 mμ.

W. S. WISE

Agricultural

Determination of the Lignin Content of Fresh Plant Tissue without Preliminary Drying. D. MacDougall and W. A. DeLong (*Canadian J. Res.*, 1948 [*B*], **26**, 468–471)—There is some evidence that the drying of succulent plant tissue at high temperatures either causes combination of lignin with nitrogenous substances or, if such complexes are previously formed, renders them more resistant to subsequent decomposition. To avoid errors due to this cause, removal of the interfering matter before drying was attempted; water saturated with ether was found to be the most effective non-acid extractant.

Procedure—Weigh out a 20-g. sample and a 75-g. sample and cut these into ¼- to ⅛-in. pieces. Dry the 20-g. sample at 105° C. and use the 75-g. sample for lignin determination. Place the sample in a Waring Blendor with 450 ml. of water, add 50 ml. of ether and, after blending for 30 min., separate the residual material by centrifuging and repeat the extraction twice. Reflux the residue with 1 per cent. hydrochloric acid solution for 3 hr., using 150 ml. for each gram of dry matter in the original sample. Estimate the volume at this point. Filter, air-dry the material at room temperature, and extract it in a Soxhlet extractor with ethanol-benzene (1 + 2) for 30 hr. and use the final residue for lignin determination.

This method was studied with oat plants of various stages of growth and was compared with the standard A.O.A.C. extraction procedure (*Methods of Analysis*, 5th Ed., 1940). Lignin was determined by the method of Manning and DeLong (*Sci. Agr.*, 1941, **22**, 69) and the lignin for nitrogen and methoxyl determinations was isolated by filtering in sintered-glass crucibles with naphthalene as filter aid (Mueller and Herrmann, *Tech.-Wiss., Teil, Papierfabr.*, 1926, **24**, 185; *Chem. Abst.*, 1926, **20**, 2746). Nitrogen was determined by the micro-Kjeldahl method with the digestion mixture of Campbell and Hanna (*J. Biol. Chem.*, 1937, **1**, 119). For methoxyl determinations by the Viebock and Schwappach modification of the Zeisel method, as

described by Clark (*J. Assoc. Off. Agric. Chem.*, **1932, 15, 136**; *Analyst*, **1932, 57, 402**), samples were weighed in gelatin capsules and a suspension of red phosphorus was used in the scrubber, as recommended by Samsel and McHard (*Ind. Eng. Chem., Anal. Ed.*, **1942, 14, 750**; *Analyst*, **1943, 68, 27**). The hydriodic acid used was prepared as described by Clark (*loc. cit.*).

The results obtained showed that the modified procedure gives a lower apparent lignin content for young succulent tissues than the standard method. The difference decreases as the age of the tissue from which the lignin is extracted increases. Lignin isolated by the modified procedure always contains less nitrogen than that isolated by the standard procedure, and it is thus apparent that interference by nitrogenous bodies has been lessened. The absolute amount of methoxyl isolated by the standard method is greater than that isolated by the modified procedure, except with mature tissue, and thus there is apparently less interference by methoxy-containing carbohydrates in the modified procedure.

Results with field-grown oat plants show that about 50 per cent. of the difference in the results obtained by the two methods is due to modification of the pre-treatment of the sample and not to the drying of the material for the standard method, the main difference being that the acid treatment is preceded in the modified method by cold ether - water extraction and in the other by hot water extraction. With young tissue, results obtained by the modified procedure are more trustworthy than those obtained by the standard method, but with mature tissue, in which the amount of interfering matter is comparatively low, the standard method appears to give trustworthy results.

A. O. JONES

Organic

Colorimetric Determination of Small Quantities of Formaldehyde by means of Chromotropic Acid. T. Kleinert and E. Srepel (*Mikrochem.*, 1948, **33**, 328–332)—Formaldehyde yields a violet-red colour when warmed with chromotropic acid in 72 per cent. sulphuric acid. The reaction is selective for formaldehyde in the presence of many other aldehydes, *e.g.*, acetaldehyde, propionaldehyde, *n*- and *iso*-butyraldehyde, *iso*-valeraldehyde, oenanthol, crotonaldehyde, chloral hydrate, glyoxal, aromatic aldehydes, and furfural. The foreign aldehyde may be in considerable excess. The qualitative reaction described by Eegrive (*Z. anal. Chem.*, 1937, **110**, 22) shows considerable variations in colour intensity, and various factors are investigated to render the method suitable for quantitative work. The resulting procedure is suitable for 0·002 to 0·05 mg. of formaldehyde, and the error varies from 2 to 5 per cent. when the Lange colorimeter is used.

Procedure—Heat 90 ml. of 76 per cent. sulphuric acid to 60° C. in a tall 150-ml. beaker. Add 5 ml. of a freshly prepared 1 per cent. solution of chromotropic acid in 72 per cent. sulphuric acid and then, with stirring, 5 ml. of the solution for test. Keep at 60° C. in a water-bath for 20 min. with frequent stirring, then cool quickly in cold water and allow any air bubbles to disperse. Determine the formaldehyde content from the colour intensity, a colorimeter with comparison standards containing known amounts of formaldehyde being used.

W. C. JOHNSON

Practical Test for Estimating Storage Stability of Gasolines. E. L. Walters, D. L. Yabroff, H. B. Minor, and H. E. Sipple (*Anal. Chem.*, **1947, 19, 987–991**)—A modification of the extrapolated gum-time method (Walters *et al.*, *Ind. Eng. Chem.*, to be published) is presented, in which the accelerating effect of oxygen pressure is eliminated and the effect of temperature is moderated to the extent that a single gum-time or ageing gum determination gives a trustworthy prediction of storage stability, which is as accurate as the longer method and yet is insensitive to the effect of experimental error.

The effect of temperature on gasoline stability at constant oxygen pressure can be expressed by the equation $\log(t_1/t_2) = B(1/T_1 - 1/T_2)$, where t_1 and t_2 are the gum-times at the respective absolute temperatures T_1 and T_2, and B is the temperature coefficient. The oxygen pressure effect at constant temperature is expressed by $\log(t_1/t_2) = D\log(P_1/P_2)$, where t_1 and t_2 are the gum-times at the respective absolute oxygen pressures P_1 and P_2 (lb. per sq. in.), and D is the oxygen pressure coefficient. The value of B for different gasolines ranges from about 5000 to about 6000, averaging about 5500. Temperature extrapolations are normally made from 100° C. (test temperature) to, say, 90° F. (storage temperature). Assuming a B value of 5500, extrapolations for this temperature range could be in error by a factor of about 2 if B were actually 500 units different from the assumed value. This possible uncertainty is greater than desired, but could be reduced appreciably by reduction of test temperature, although at the expense of increased test time. Another uncertainty is the effect of oxygen pressure, which is small for some gasolines and rather large for others. Oxygen pressure can be eliminated as a test accelerant, especially at lower temperatures (Yabroff and Walters, *Ind. Eng. Chem.*, 1940, **32**, 83), by conducting the ageing test at atmospheric pressure, provided a sufficiently high air-to-gasoline volume ratio is maintained to avoid oxygen depletion effects (Walters *et al.*, *loc. cit.*).

Accordingly, the simplified procedure comprises oxidation of gasoline under 1 atmosphere of air at some temperature below 100° C., and the results are then extrapolated to the desired storage temperature by assuming a value for B of 5500. The uncertainty in the extrapolation arises primarily from the assumption of an average value for B and not from exponential accentuation of experimental errors, as in the complete extrapolated gum-time procedure.

The uncertainty factor (UF), caused by the use of an average value for B, can be defined by $\log(UF) = \Delta B(1/T_{ex} - 1/T_{ac})$, where T_{ex} and T_{ac} are the absolute temperatures of extrapolation and of the accelerated test respectively, and ΔB is the

positive difference between the assumed and actual B values. At a given temperature, the true storage life is equal to the extrapolated storage life multiplied or divided by the uncertainty factor UF, according as the assumed B value is larger or smaller than the true one. The extent of extrapolation or magnification factor (MF) can be expressed as the ratio of the times (in months) at the extrapolated temperatures to those (in days) at the accelerated temperatures. MF can then be defined by $\log(MF) = B(1/T_{ex} - 1/T_{ac}) - 1{\cdot}4829$. The predicted storage times or temperatures can be conveniently related to the accelerated test measurement by means of a nomograph constructed from the equation defining MF (*supra*). Thus a single ageing time of 24 hr. at 69·9° C. would be equivalent to 3 months storage at 90° F. and a 5-mg. gum-time of 3 days at 73° C. would be equivalent to 6·3 months at 100° F.

Method—The bombs should be of low-carbon, cadmium-plated steel, built to withstand a pressure of 50 lb. per sq. in. at test temperatures. No pressure gauges or valves are required because the bombs are opened and closed at atmospheric pressure. The bombs are fitted with pint, quart, or half-gallon bottles according to size. Fill the clean bottle with the desired amount of gasoline, pre-cooled to about 5° C., fit a loose glass cap over the neck, and immediately place and seal the bottle in the bomb, avoiding undue agitation of the liquid. Pre-heat the sealed bomb in boiling water to the temperature of oxidation chosen, *viz.*, for 50° C., 9 min.; 55° C., 11 min.; 60° C., 30 min.; 65° C., 15 min.; 70° C., 18 min.; 75° C., 20 min.; 80° C., 23 min. The time of testing is that between placing the bomb in the pre-heating bath before, and in the ice-bath after, the oxidation. Place the bomb in a thermostat at the prescribed temperature, allow the oxidation to proceed for the prescribed interval, then plunge the bomb into ice-water for at least 15 min. Open the bomb and remove the sample. If only a portion of the sample is to be used, chill the remainder, gently purge it with air, replace it in the re-cleaned bomb, and, repeat the heating, operating, and cooling procedure as many times as will permit the determination of gum-formation as a function of the time.

Comparative complete accelerated stability tests were made with two full-range cracked gasolines by the modified gum-time and the extrapolated gum-time methods. Agreement between the measured and predicted figures by the two methods is good.

The air-to-gasoline volume ratio in the bomb need not be controlled closely provided it is between about 3 and 25. Bomb size is not an important factor. Specific points requiring the closest attention are the use of clean, dry apparatus and bomb equipment free from leaks. If leaks occur, the apparent stability of the gasoline will increase owing to oxygen depletion effects. For this reason the use of a small positive air pressure instead of atmospheric pressure during the ageing test is advantageous. The small accelerating effect of this can be neglected in stability predictions.

A. O. JONES

Micro-analytical Detection of Glycerol with 2:7-Dihydroxynaphthalene. K. Fürst (*Mikrochem.*, 1948, **34**, 25–29)—In the direct method sulphuric acid converts the glycerol to acrolein, which then reacts with 2 : 7-dihydroxynaphthalene in the same solution to give an intensely coloured fluorescent product.

METHOD—*Reagent solution*—A solution of 0·01 g. of 2 : 7-dihydroxynaphthalene in 100 ml. of concentrated sulphuric acid. The solution, when freshly prepared, has a deep yellow colour and a green fluorescence, both of which disappear on standing overnight or on warming for a short time. Red and violet colours are produced by oxidising agents and by atmospheric oxidation. Pale red solutions may be used if a blank test is used for comparison.

Direct procedure—Add 1 or 2 drops of the solution under test to 2 ml. of the reagent solution, and heat in boiling water for 20 to 25 min. A yellow or reddish-yellow colour is obtained, according to the amount of glycerol present, and a deep green fluorescence. The colour and fluorescence are stable to long-continued heating. Sensitivity 1·5 μg.; dilution limit 1 in 33,000.

Normal monohydric alcohols, free from aldehydes, do not interfere. Glycol gives a green colour and a green fluorescence, both becoming less distinct with continued heating in the water-bath (sensitivity about 400 μg.). Sorbitol, mannitol, and dulcitol give a brownish-yellow colour with a green fluorescence (sensitivity about 200 μg.). Most mono- and di-saccharides give darkening due to carbonisation. Formic, acetic, succinic, citric, benzoic, and salicylic acids give no reaction. Lactic and malic acids give yellow colours with pale fluorescence. Some aldehydes also give colour reactions accompanied sometimes by fluorescence. When interfering substances are present the following modification of the direct procedure is prescribed—Evaporate the sample solution to dryness and evaporate the residue to dryness several times on the water-bath with 2 g. of 40 per cent. lime cream. Extract with a mixture of equal parts of alcohol and ether. Evaporate the extract on the water-bath, take up the residue in the same mixture of (anhydrous) alcohol and ether, and filter. Evaporate the filtrate to dryness in a test tube, add 2 ml. of reagent solution to the residue, and heat in boiling water for 20 min. A yellow colour with green fluorescence shows the presence of glycerol. The use of phosphoric acid in place of sulphuric acid, to avoid carbonisation of certain impurities, is not successful.

2 : 7-Dihydroxynaphthalene gives colour reactions with certain aldehydes, and this affords a means of detecting glycerol through its oxidation products.

Indirect procedure—(i) Heat 0·1 ml. of the solution for test with 0·1 ml. of 0·1 *N* potassium permanganate in boiling water. After 10 min. decolorise with 0·1 ml. of oxalic - sulphuric acid solution (5 g. of oxalic acid dissolved in 50 ml. of water and mixed, while cooling, with 50 ml. of concentrated sulphuric acid). Cool, and mix a few drops with 2 ml. of the reagent solution. After prolonged heating in boiling water, a red-violet to

red colour indicates the presence of glycerol. Sensitivity 10 μg.; dilution limit 1 in 10,000. (ii) Mix 0·1 ml. of the solution with 0·9 ml. of fresh 3 per cent. bromine water and heat for 10 min. in boiling water. After boiling off the excess of bromine, mix 0·1 ml. of the solution with 2 ml. of the reagent solution and heat for 20 min. in the water-bath. A red-violet colour indicates glycerol.

W. C. JOHNSON

Potentiometric Titrations with Potassium Iodate. Part VII. Potentiometric Determination of 2-Ascorbic Acid. G. Spacu and P. Spacu (*Z. anal. Chem.*, 1948, **128**, 233–238)—The method depends on the oxidation of ascorbic acid to dehydroascorbic acid with an excess of iodine and titration of the residual iodine with sodium thiosulphate.

Procedure—To a solution containing approximately 50 mg. of ascorbic acid add a known volume of 0·1 *M* potassium iodate, 1 to 2 g. of potassium odide, and 1 to 5 ml. of 2 *N* sulphuric acid. Titrate the excess of iodine with 0·1 *M* sodium thiosulphate, a potentiometric end-point being used as above.

The results obtained with pure ascorbic acid and with vitamin C tablets are satisfactory.

J. G. WALLER

Potentiometric Titration in Two-phase Systems. I. Hedlund and M. Steninger (*Acta Chem. Scandinavica*, 1948, **2**, 583–591)—The potentiometric titrations of two weak acids in the presence of each other is possible if the pK values for the two acids are sufficiently separated; if this is not so, only one end-point will be detected. If the titration is carried out in the presence of an organic solvent that is immiscible with water, then if the distribution coefficients of the acids between water and the organic solvent are significantly different, two inflections in the titration curve will appear and the two acids can be determined.

Two-phase titrations of this sort have been carried out on humulon and lupulon, two acids present in hops, light petroleum being used as the organic solvent, and the method has been employed for the separation of these acids. J. G. WALLER

Inorganic

Spectrochemical Determination of Beryllium. J. Cholak and D. M. Hubbard (*Anal. Chem.*, 1948, **20**, 970–972)—The paper describes a method, for determining beryllium in biological materials, which has been developed from that described previously by the same authors (*Ibid.*, 1948, **20**, 73; *Analyst*, 1949, **74**, 269). In this application the solution is placed in the crater of a graphite rod and dried before being made the cathode of a 10-amp. D.C. arc. The optical system is adjusted so that the light from the sensitive "cathode layer" of the arc enters the spectrograph slit and the high sensitivity provided by this method of working enables quantitative determinations to be made down to 0·001 μg. of beryllium in the arc. In the quantitative method, a stepped sector is employed, but if this is dispensed with as little as 0·00025 μg. of beryllium can be detected. Working graphs may be prepared in two ways: (1) by the convential method of "log I ratios," in which case a background correction is applied, and (2) by using the separation of the blackening curves. For satisfactory results the operating conditions must be strictly standardised, for example, the same portion of the cathode layer must be photographed each time, and therefore the arc-gap width and the optical alignment are critical. Details are given of the buffer solutions employed and their use is described at some length. H. R. CLAYTON

Buffer for the pH range 5 to 7. G. Smits (*Biochim. Biophys. Acta*, 1947, **1**, 280)—Mixtures of di- and mono-sodium maleates give buffer solutions having the following advantages: no precipitation with magnesium, manganese, copper, cobalt or zinc ions, no action on aneurine or its pyrophosphate, and no effect on the carboxylase activity of yeast. The following solutions are prepared. Solution I, mono-sodium maleate: 1·160 g. of maleic acid in 0·100 *N* sodium hydroxide to 100 ml. Solution II, di-sodium maleate: 1·160 g. of maleic acid in 0·200 *N* sodium hydroxide to 100 ml.

Ml of soln. I	Ml. of soln. II	pH
0	10·0	7·76
1·0	9·0	6·82
2·0	8·0	6·49
3·0	7·0	6·26
4·0	6·0	6·08
5·0	5·0	5·93
6·0	4·0	5·79
7·0	3·0	5·63
8·0	2·0	5·36
9·0	1·0	4·99
10·0	0	3·81

G. MIDDLETON

Effect of Melting Conditions on the Spectrographic Determination of Copper in Lead Alloys. L. C. Bannister and R. H. Price (*J. Inst. Metals*, 1948, **75**, 151–162)—In the method described for preparing lead - copper alloy standards for spectrographic analysis, the constituent metals are accurately weighed, and are then melted under conditions that reduce the formation of dross. The lead is melted in an iron ladle and is then heated from the surface by a Bunsen flame; when a two-cone, non-luminous flame is used the effect of this treatment is to reduce the dross formed in the preliminary melting. When all the dross is reduced, a weighed amount of copper is added, and heating is continued until dissolution is complete. After stirring, the alloy is cast into samples for spectrographic analysis.

In describing tests on standards prepared in this way, the authors give calibration graphs showing the effect of antimony on the calibration for lead - tin - copper alloys. Experiments carried out to determine the optimum method of sampling indicate that surface heating is desirable in re-melting lead alloys, and that there is no difference in the behaviour of samples cast as slabs, and those cast as sticks and subsequently rolled into slabs.

H. R. CLAYTON

Polarographic Analysis of Hydrogen Peroxide. P. A. Giguère and J. B. Jaillet (*Canad. J. Res.*, 1948 [*B*], **26**, 767–772)—The polarographic determination of hydrogen peroxide can be carried out by making use of the reduction step at −1·0 v. *versus* the saturated calomel electrode. The method is limited to peroxide concentrations of less than 0·15 per cent., owing to the oxidation of mercury by hydrogen peroxide at high concentrations. If a stationary platinum micro-electrode is used in place of the dropping mercury electrode, current-voltage curves in the range from 0 to −0·6 v. *versus* the saturated calomel electrode show a reduction step whose height is proportional to the hydrogen peroxide concentration over a much wider range, and determinations can be carried out simply by measuring the limiting current at −0·6 v. The upper limit of peroxide concentration that can be measured depends on the concentration of the supporting electrolyte, and if saturated potassium chloride solution is used, concentrations up to 0·9 per cent. can be determined.

J. G. WALLER

Analytical Investigation of the Univalent Thallium Ion. R. Ripan and E. Popper (*Z. anal. Chem.*, 1948, **128**, 239–241)—The method described depends on the fact that thallous ions, in presence of calcium and ferricyanide ions, form a compound, $CaTl_2[Fe(CN)_6]$, on addition of ferrocyanide ions. If a solution containing thallous, calcium, and ferricyanide ions is titrated with a solution of potassium ferrocyanide, the end-point can be detected by the usual potentiometric method.

Procedure—To 20 ml. of a 0·05 to 0·10 *M* solution of a thallous salt add an equal volume of an approximately 0·1 *M* solution of calcium nitrate and 1 ml. of a 1 per cent. solution of potassium ferricyanide. Place a platinum indicator electrode and a reference electrode in the solution and titrate with 0·1 *M* potassium ferrocyanide.

The results are in fair agreement with known values.

J. G. WALLER

Thioacetamide in Place of Gaseous Hydrogen Sulphide for Precipitation of Insoluble Sulphides. H. H. Barber and E. Grzeskowiak (*Anal. Chem.*, 1949, **21**, 192)—The reagent is easily obtained, and its odour is not offensive. Its aqueous solution keeps well. Only a small excess is necessary to cause complete precipitation and, as hydrolysis gives a relatively low sulphide ion concentration, coagulation and filtration are more rapid than when gaseous hydrogen sulphide is used. Cost per unit precipitation is also less.

M. E. DALZIEL

Conductometric Titration for the Rapid Determination of Sulphur in Organic and Inorganic Compounds. A. Schöberl (*Z. anal Chem.*, 1948, **128**, 210–215)—The method involves the conversion of the sulphur to sulphate and the subsequent conductometric titration of the sulphate with barium acetate solution.

Procedure for organic compounds—Heat the sample to 700° C. in a quartz tube in a stream of oxygen, and absorb the oxides of sulphur in a solution of hydrogen peroxide. When the combustion is complete, neutralise the solution with 2 *N* aqueous ammonia, add a small amount of manganese dioxide, and heat the mixture on a water-bath to destroy any hydrogen peroxide still present. Transfer the solution to a conductivity cell and titrate with 0·05 *N* barium acetate.

Sulphur determinations on a number of organic compounds give results that are in good agreement with the theoretical values. The method can be used for many naturally occurring materials.

J. G. WALLER

Fractional Titration of Amalgams as a Method of Analysis of Easily Fusible Metals. V. A. Tsimmergakl and R. S. Khaymovich (*Zavod. Lab.*, 1948, **14**, 1289–1300)—If a mixed amalgam of a number of metals can be prepared, *e.g.*, by dissolving an alloy directly in mercury, or by electrolysis of an aqueous solution of the metals with a mercury cathode, the metals can be individually extracted by shaking the amalgam with mercuric chloride solution or other oxidising agent, preferably at 100° C., the completion of extraction of each metal being shown by a sudden change in the potential of the amalgam. The individual extracts may then be used for the determinations of the single metals. Alternatively, if an accuracy to within about 1 per cent. is sufficient the volume of mercuric chloride solution expended in each extraction may be used as a measure of the content of the metal.

The process of extraction has been considered theoretically and the mathematical basis is worked out. Even with very small amounts of one metal in presence of very large amounts of another the separation can theoretically be more complete than is attained in practice by normal chemical means provided that the amalgam potentials differ sufficiently. With similar initial concentrations 0·1 per cent. accuracy should be possible with bivalent metals if the difference is 0·22 v. or more.

In practice, direct titration is less accurate than this because of the ease of oxidation of the amalgams by air, and the slowness of attainment of equilibrium. These errors are reduced and other advantages appear if the extraction-titration is carried out at 100° C. The vapour of the boiling solution displaces the air, the concentration of metals in the amalgam is increased at the higher temperature and hence the volume of the amalgam may be reduced and the boiling produces agitation of the solution and of the amalgam and thus reduces the time for the titration.

At 100° C. the solubilities of the metals in mercury are zinc 7 per cent., cadmium 21 per cent., tin 19 per cent., lead 16 per cent., and bismuth 26 per cent. The mean values of the potentials at 100° C., *versus* the normal calomel electrode have been found for the first time.

	Potential (volts)				
Medium	Zn	Cd	Sn	Pb	Bi
N KCl	1·02	0·65	0·59	0·48	0·13
N KOH	1·42	0·88	1·14	0·81	0·56

METHOD—*Apparatus*—A test tube, 4 cm. wide and narrower at the bottom, is fitted with a ground-glass stopper carrying a number of tubes (1) to a vertical water condenser with internal glass stirrer passing into the test tube, (2) electrolytic bridge tube to a calomel electrode, (3) tube containing platinum wire contact to the amalgam, (4) burette jet, (5) tube for insertion of solutions or of metal, and (6) tube used for platinum wire anode when the amalgam is to be formed by electrolysis in the test tube. The capacity of the test tube is sufficient for 100 ml. of solution to be electrolysed therein when necessary. Heating is carried out by immersion of the tube in a heated calcium chloride bath.

Titration is carried out with 0·1 *N* or 0·5 *N* mercuric chloride in neutral solution, 0·4 *N* mercuric acetate in *N* acetic acid, or, most often, with 0·1 *N* or 0·5 *N* mercuric salt in *N* potassium hydroxide. This solution is prepared by dissolving 2·715 g. of mercuric chloride in a solution of 7 g. of potassium iodide in 20 ml. of water and adding a solution of 11·22 g. of potassium hydroxide, followed by dilution to 200 ml.

Direct titration—The amount of mercury taken is dependent on the solubilities of the metals present in the alloy to be analysed, and the amount of sample taken depends on the composition. Place 1 to 3 ml. of mercury in the test tube, add either 5 ml. of *N* potassium chloride or 5 ml. of *N* potassium hydroxide, heat until boiling starts, introduce 0·5 to 10 g. of the alloy, stir, and continue heating until the alloy is dissolved; then connect the tube with the other apparatus, first ensuring that the burette jet is filled with the titrating solution. Set the stirrer in motion, and connect the amalgam and the calomel electrode to a galvanometer, for measuring the E.M.F., through a resistance in series. With the galvanometer needle stable, start adding small amounts of the titrant, at intervals of not less than 1 min., to the energetically boiled and stirred solution. Stop when the needle no longer tends to a stationary position after one of these additions.

With the single metals, zinc, cadmium, lead, bismuth, and tin, the results calculated on the theoretical titre of the mercury solution were accurate to 1 part in 100. With great care and 3-min. intervals between additions, the error was reduced to 0·3 parts in 100. Similar results were obtained for the determination of tin in solder, zinc and tin in zinc - tin alloys containing small amounts of aluminium, and lead in lead - bismuth alloy. Antimony interfered in the determination of tin in Babbitt metal, and aluminium in the analysis of zinc alloys. In these cases, however, the titration may be used simply for extraction purposes, the metal extracted being determined by a suitable chemical method. G. S. SMITH

Colorimetric Determination of Small Amounts of Antimony in Copper and Tin Bronzes. E. I. Nikitina (*Zavod. Lab.*, 1948, **14**, 933–935)—Small amounts of antimony in copper and bronze can be determined colorimetrically as the complex $KSbI_4$ in sulphuric acid medium in the presence of ascorbic acid, which acts as a stabiliser of the colour and also reacts with any traces of oxidising impurities that might otherwise interfere. The ascorbic acid is introduced in the potassium iodide reagent, which contains 12 g. of potassium iodide and 1 g. of ascorbic acid in 100 ml. of water. In the presence of the ascorbic acid, decomposition to free iodine does not occur.

The colour is stable for 24 hr. The acid concentration must be at least 14 to 16 per cent. in sulphuric acid.

Determination of antimony in copper—Procedure—If tin is absent add 0·02 g. of tin to the sample weight before dissolution. Dissolve 1 or 2 g. of the sample in concentrated nitric acid, evaporate to small bulk, dilute to 30 ml. with water, add 2 ml. of 10 per cent. manganous sulphate solution and 20 drops of 4 per cent. potassium permanganate solution, and boil under cover for 20 to 30 min. Filter, well wash the precipitate on the filter with hot water, then dissolve it completely in hot hydrochloric acid and a few drops of hydrogen peroxide, collecting the filtrate in the original beaker, and washing the filter well. Add sulphuric acid to the filtrate, boil to reduce the bulk by one-half, add aqueous ammonia until the odour persists, then 10 drops of the permanganate solution, boil for 20 min. and filter. Wash the precipitate with hot water containing ammonia, and dissolve it in hydrochloric acid with hydrogen peroxide. Evaporate this solution to dryness with 5 ml. of 16 per cent. sulphuric acid solution. To the residue add 5 ml. of 16 per cent. sulphuric acid solution and, if the solution is yellow, 5 ml. of nitric acid followed by evaporation to sulphur trioxide fumes. Repeat the evaporation with nitric acid if the solution does not become colourless. Transfer the solution to a colorimeter cylinder, using 16 per cent. sulphuric acid for washing and arranging that the total volume does not exceed 15 to 20 ml. Add 4 to 5 ml. of the potassium iodide reagent containing ascorbic acid, mix, and compare the colour with a standard prepared as follows. In another similar cylinder place the same volumes of acid and reagent and add to the solution from a micro-burette a standard solution of antimony in sulphuric acid until the colour matches that of the sample.

Determination in tin bronze—Procedure—Dissolve not more than 0·1 g. of sample in 5 ml. of concentrated nitric acid, dilute to 30 ml. with water, heat for 30 min., filter through a double filter, wash first with 1 per cent. nitric acid solution, then four times with cold water, once with aqueous ammonia and finally with water again. Dissolve in hydrochloric acid with hydrogen peroxide and proceed as described above.

The method is suitable for amounts of the order of 0·001 per cent. in pure copper, and of amounts up to 0·25 per cent. in tin bronze. With 0·25 per cent., the results varied between 0·23 and 0·28 per cent., and with 0·056 per cent. between 0·05 and 0·057 per cent. G. S. SMITH

Potentiometric Titration of Quadri- and Sexa-Valent Selenium and Tellurium with Chromous Ion. J. J. Lingane and L. Niedrach (*J. Amer. Chem. Soc.*, 1948, **70**, 1997–2000)—Quadrivalent selenium and tellurium can be titrated

potentiometrically with chromous sulphate solution. In each reaction the reduction product is the element. The titration curves are well-defined and suitable for analytical purposes. Although the sexavalent ions are also reducible, the reactions involved are too slow for use in analytical work.

Procedure—Place the solution to be titrated in a 250-ml., three-necked, round-bottomed flask, containing a bright platinum indicator electrode, and adjust the volume of the liquid to approximately 150 ml. Connect a saturated calomel reference electrode to the solution by a salt bridge containing dilute sulphuric acid, and pass carbon dioxide or nitrogen through the solution for 10 min. to remove dissolved oxygen. After this, allow the gas to pass slowly through the flask while the solution is titrated with 0·1 *M* chromous sulphate in either 0·1 or 1·0 *N* sulphuric acid. The best results are obtained if the solution to be titrated is about 9·0 *N* with respect to hydrochloric acid and is heated to 60° to 70° C.

Under these conditions, the oxidation - reduction potential of the selenium system is about 0·3 v. more positive than that of the tellurium system, so that both elements can be determined in one titration. Using the same conditions it is possible to determine selenium in the presence of copper.

J. G. WALLER

Potentiometric Titrations with Potassium Iodate. Part II. Potentiometric Determination of Thorium. Part III. Potentiometric Determination of Lanthanum. G. Spacu and P. Spacu (*Z. anal. Chem.*, 1948, **128**, 226–229, 229–231)—The determination is carried out by precipitating the thorium or lanthanum with an excess of potassium iodate and determining the excess of iodate present by a potentiometric method.

Procedure for thorium—Transfer to a 100-ml. volumetric flask 20 ml. of a solution containing approximately 50 mg. of thorium, and add a known volume of 0·02 *M* potassium iodate so that an excess of iodate is present. Allow the precipitate to settle, dilute the solution to the mark with a measured volume of the potassium iodate solution, filter through a dry paper and then transfer 10 ml. of this solution to the titration cell. Add 100 ml. of water, 1 to 2 g. of potassium iodide, and 5 ml. of 2 *N* sulphuric acid. Titrate the solution with 0·1 *M* sodium thiosulphate, using a potentiometric method to determine the end-point (W. Böttger, *Physikalische Methoden der analytischen Chemie*, Part III, 1939, p. 692).

Procedure for lanthanum—Mix 10 ml. of the solution to be examined, containing approximately 7 mg. of lanthanum, with a measured volume (50 to 60 ml.) of 0·01 *M* potassium iodate in a 100-ml. volumetric flask. Dilute the mixture to 100 ml. with ethyl alcohol, and filter. The procedure from this stage is the same as that described for thorium.

The results for standard thorium and lanthanum solutions are in good agreement with the expected values.

J. G. WALLER

Determination of Soluble Molybdates and Tungstates and Analysis of their Mixtures. H. Guiter (*Chimie Analyt.*, 1948, **30**, 180–181)—*Volumetric method*—The potentiometric titration curve of an alkaline molybdate solution with acid shows two pronounced inflexions, one at pH 10·5 to 8, corresponding to the ortho-salt (M_2O,MoO_3), and the other at pH 4·5 to 2·5, corresponding to the meta-salt ($M_2O,4MoO_3$). Similar changes occur with tungstates at pH 10·5 to 8·5 and pH 5 to 2·5. If, therefore, an alkaline solution of a molybdate or a tungstate is titrated with 0·1 *N* sulphuric acid, first to the end-point of phenolphthalein and then to the end-point of methyl orange, the concentration of the solution can be calculated from the equation $x=(T\ V/V_1)(4/3)$, where x is the concentration of the solution in half g.-mol. of MoO_3 or WO_3 per litre, T the normality of the standard acid, V (ml.) the volume of standard acid consumed between the two end-points, and V_1 (ml.) the volume of the molybdate or tungstate solution. The methyl orange must be taken to a full red colour. Although the reaction is reversible, a back titration with alkali is not satisfactory because, in this direction, equilibrium is only slowly established. But the acid titration may be repeated after addition of an excess of alkali and a delay of 15 min. The method is accurate to within ±2 per cent.

Gravimetric method—On addition of a solution of a barium salt to a molybdate solution more alkaline than pH 6·6, barium molybdate of the composition $BaMoO_4$ is precipitated. Similarly, tungstate solutions of pH greater than 6·4 yield $BaWO_4$. The precipitation should be effected at the boiling-point and the filtrates should yield a blue colour with bromophenol blue. The method is accurate to within ±1 per cent.

Analysis of mixtures of molybdates and tungstates—First use the volumetric method to determine the concentration, θ, of the solution in terms of half g.-mols. of (MoO_3 + WO_3) per litre. If x and y represent the respective individual concentrations in the same units, then

$$\theta = x + y \qquad (1)$$

Determine the total weight (m) in g. of mixed barium salts obtained from n ml. by the gravimetric method, then

$$n(0{\cdot}1485x + 0{\cdot}1925y) = m \qquad (2)$$

From (1) and (2), $x = (0{\cdot}1925\theta - m/n)/0{\cdot}044$ and $y = (m/n - 0{\cdot}1485\theta)/0{\cdot}044$.

The precision of this method is of the order of 3 per cent.

W. C. JOHNSON

Useful Stop-cock Lubricant. I. E. Puddington (*Anal. Chem.*, 1949, **21**, 316)—For stop-cocks in exposed positions the lubricant must have a low vapour pressure so that re-greasing is only occasionally needed. The thickened glycerols usually recommended tend to be short-lived in frequently turned taps. A better lubricant is a well stirred dispersion of 20 to 25 per cent. by weight of silica (Santocel C, ignited overnight at 450° C.), in glycerol that has been rendered anhydrous by heating to about 70° C. for 2 hr. in a diffusion-pump vacuum; this dehydration is especially advantageous in preparing a batch for low-pressure systems.

A 6-mm. bore stop-cock so lubricated was unaffected by exposure to 6-cm. pressure of benzene vapour for 6 months.

M. E. DALZIEL

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal. Chem.*, 1949, **21**, 191–192)—The crystallography of pimelic acid is described under the headings given in the original paper of the series (*Ibid.*, 1948, **20**, 275; *cf. Analyst*, 1948, **73**, 579).

M. E. DALZIEL

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal. Chem.*, 1949, **21**, 306–307)—The crystallography of benzalaminophenol is described under the headings given in the first paper of the series (*Ibid.*, 1948, **20**, 275; *cf. Analyst*, 1948, **73**, 579).

M. E. DALZIEL

Reviews

A NEW NOTATION AND ENUMERATION SYSTEM FOR ORGANIC COMPOUNDS. By G. MALCOLM DYSON, M.A., D.Sc., Ph.D., F.R.I.C. Second Edition. Pp. ix + 138. London: Longmans, Green & Co., Ltd. 1949. Price 10s. 6d.

The second edition of Dr. Dyson's book on his system of notation and enumeration follows two years after the first, and in the interval he has consolidated the basis of the system and developed its features, main and subsidiary. He claims that the first edition of the book was deliberately provocative and intended to stimulate discussion and criticism. It is clear that it was most successful in attaining this object. The interest of chemists was apparent as soon as the system was presented to them, and the rapid advances that have been made in the past two years are largely due to the enthusiasm emanating from America and to the collaboration the author has received from American sources.

The major developments that have occurred and that are to be found in the second edition result from the abandonment of the principle of adduction in favour of a more logical treatment of ring systems, and in the re-arrangement of symbols within operations.

When the system first appeared it was possible to give an account of all its salient features on two pages of *The Analyst*. This can no longer be done, and more rules are now needed than formerly. These rules are presented formally in this second edition, and it would appear that some knowledge of the system is assumed. It seems rather a pity that the more elementary mode of presentation of the earlier edition should have been dropped and one would welcome the re-introduction in future editions of some of the beginning of the former Chapter III.

Once the system has been mastered, however, it is seen to be really quite simple, and there is no doubt that the latest developments have added to its elegance and usefulness. The author may well find that it will not long remain merely a "proposal for an international system."

K. A. WILLIAMS

THE ADVERTISING, LABELLING AND COMPOSITION OF FOOD; A REPORT BY THE MINISTRY OF FOOD. An account of four years' administration of the Defence (Sale of Food) Regulations, 1943, by the Food Standards and Labelling Division of the Ministry of Food. Pp. iv + 77. London: H.M. Stationery Office. 1949. Price 1s. 6d. net.

The Report is divided into seven parts, of which the first six, occupying 11 pages, contain a general account of the reasons that led to forming a Labelling Division of the Ministry of Food and describe the state of affairs at its inception, what it set out to do, what it has done and what remains to be done in the future. These parts are of interest, more particularly, to the general public.

For public analysts, manufacturers, lawyers and candidates for the Branch E Examination of the Royal Institute of Chemistry the important section is Part VII, which contains 14 appendixes and occupies 65 pages on the following subjects: the White Paper of 1943 on the labelling and advertising of foods; the Defence (Sale of Food) Regulations, 1943; the Labelling of Food Order, 1946, with its schedules; the Food Substitutes (Control) Order, 1941; Codes of Practice (these are a set of rules that have been mutually agreed upon by the Labelling Division of the Ministry of Food and the manufacturers and packers of food products for the description and composition of 21 classes of food and their varieties); Claims for Vitamin and Mineral Contents; Review of Food Standards and Proposals for Standards; Abstracts of Standards in Commodity Control Orders; Abstracts of Standards Applied by Manufacturing Licences; the Food Standards (General Provisions) Order, 1944; the Dried Egg (Control of Use) Order, 1945; the Fluorine in Food Order, 1947; the Mineral Oil in Food Order, 1949; finally, summarised lists giving the sources of applications for the Minister's consent to proceedings under the Defence (Sale of Food) Regulations, 1943, for the years 1944 to 1948, with the results of the applications and of any proceedings.

These 65 pages of appendixes are fully documented with the numbers and dates of the relative Statutory Rules and Orders and Statutory Instruments. If by frequent revision it should be possible to keep them up to date, this Report will undoubtedly come to be regarded as a textbook on the application of Sections 6 and 8 of the Food and Drugs Act, 1938.

So far as can be seen without an extended working knowledge of the text, the important sections that deal with Statutory Orders and Instruments are accurate and complete; but an omission on page 35 requires attention: there is, on that page, no textual indication to correspond with the footnote marked "(a) S.R. & O., 1946, No. 945." This refers to the Soft Drinks Order, and should, presumably, if the arrangement of the footnotes is intended to be in their order of incidence in the text, be the last and not the first item in a list of six.

All those who are under the necessity of acquiring an accurate and complete knowledge of this particular section of the laws and regulations relating to food, with all their concomitant complications of amendments, revocations and re-enactments, will be grateful to the anonymous compilers of this invaluable compendium.

F. L. OKELL

MINISTRY OF SUPPLY invites applications from CHEMISTS for unestablished posts in Experimental Officer Class in Research and Development Establishments, mainly in S. England.

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N. R. WINWOOD,
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Printed and Published for the Society of Public Analysts and other Analytical Chemists by W. HEFFER & SONS Ltd., Cambridge, England. **Communications to be addressed to the Editor, J. H. Lane, 7–8, Idol Lane, London, E.C.3.** Enquiries about advertisement should be addressed to WALTER JUDD LTD., 47, Gresham Street, London, E.C.2

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