THE MICROBIOLOGICAL ASSAY OF THIAMINE

By

E. C. Barton-Wright
(Galloway and Barton-Wright, Haldane Place, London, S. W. 18)

An investigation has been made of the microbiological assay of thiamine using the lactic organism, Lactobacillus viridescens 38A (ATCC No. 12706). L. viridescens is less susceptible to inhibitory or stimulatory substances than L. fermenti 36, the organism previously used for the microbiological assay of thiamine. Thiamine is a specific nutrilite for L. viridescens, and on this account the microbiological assay of thiamine is to be preferred to the thiochrome (chemical) method of estimation. Alternative assay procedures are described.

The microbiological assay of thiamine with Lactobacillus fermenti 36 has so many serious disadvantages that it has been almost completely displaced by the thiochrome (chemical) method of estimation. The microbiological assay of thiamine with the organism Lactobacillus viridescens 38A, however, has none of the disadvantages attached to L. fermenti. For example, incubation time is not critical, since the response of the organism is specific to thiamine and it does not react to either the thiazole or pyrimidine moieties of the thiamine molecule. It is also less affected than L. fermenti by either inhibitory or stimulatory substances in the test solutions. For these reasons the microbiological assay of thiamine with L. viridescens is much to be preferred to any chemical method of estimation based on fluorescence.

The author at first used the basal medium described by Deibel et al as prepared in the dehydrated state by Difco Laboratories (No. 0808). Assays with this basal medium proved to be highly successful at the start, but later serious difficulties were encountered. These difficulties were traced to the presence of "Tween 80" (sorbitol mono-oleate) in relatively high concentration in the basal medium. "Tween 80" sets up a number of side reactions in the dehydrated medium leading to rapid deterioration, darkening and a very short shelf-life. In view of this short shelf-life manufacturers

would be well advised to omit the "Tween 80" from the dehydrated product with instructions for its separate addition whenever an assay is to be carried out. These criticisms also apply to the Difco dehydrated medium (No. 0665) for the assay of vitamin B_{12} with Ochromonas malhamensis.

Preparation of Basal Medium: The basal medium devised by the author is rather more elaborate than that of Deibel et al,[1] but is not difficult to prepare, and contains the following:-

Reagents.

Thiamine-free Tryptone Solution: Dissolve 10 g of Oxoid tryptone in 100 ml water, add conc. HCl until the pH of soln is 2.8 to 3.0 and then add 2 g of a good quality activated charcoal, stir mechanically for 30 minutes and filter. Repeat the operation twice more. Preserve under S-free toluene at a temperature not exceeding 4°C. and use within one month of preparation.

Thiamine-free Yeast Supplement: Dissolve 10 g of Difco yeast extract in 50 ml of water. Slowly bubble in SO_2 from a siphon until the solution smells strongly of the gas. Stopper the flask and stand overnight at room temperature; autoclave at 15 p.s.i. for 15 minutes, and cool. Dissolve 5 g of anhydrous $FeCl_3$ (or 8.4 g of hydrated salt) in 50 ml of water and add to the yeast extract. Raise the pH to 8-9 with 30-40 % NaOH and add 2 g of K_2HPO_4. Steam for 30 minutes, cool, and filter on a pump using filter aid. Adjust the volume to 100 ml with water and add 1 g of K_2HPO_4; boil, cool and filter. Adjust the pH to 6.0, autoclave at 10 p.s.i. for 10 minutes, cool and filter. Preserve under S-free toluene at a temperature not exceeding 4° C. and use within one month of preparation.

Cystine-Tryptophan Solution: Boil 2 g of L-cystine and 2 g of dl-tryptophan in approximately 25 ml of water and add gradually 5 ml of conc. HCl to boiling solution. Cool and make up volume to 500 ml with water. Preserve under S-free toluene at a temperature not exceeding 4°C. and use within 3 months of preparation.

Adenine-Guanine-Uracil Solution: Warm 0.1 g of adenine, 0.1 g of guanine and 0.1 g of uracil in a little water and add conc. HCl dropwise until the mixture has dissolved; cool, dilute to 100 ml with water, store under S-free toluene at a temperature not exceeding 4°C. and use within one month of preparation.

Xanthine Solution: Dissolve 0.1 g of xanthine in a small quantity of strong ammonia solution, dilute to 100 ml with water and preserve under S-free toluene at a temperature not exceeding 4°C. Use within one month of preparation.

Niacin Solution: Dissolve 0.1 g of niacin in 100 ml of water, preserve under S-free toluene at a temperature not exceeding 4°C. and use within 7 days of preparation.

Calcium-d-Pantothenate Solution: Dissolve 0.1 g of Ca-d-pantothenate in 100 ml of water and preserve under S-free toluene at a temperature not exceeding 4°C. Use within 7 days of preparation.

Pyridoxine HCl Solution: Dissolve 0.1 g of pyridoxine HCl in 100 ml of water. Preserve under S-free toluene at a temperature not exceeding 4°C. and use within 7 days of preparation.

Riboflavin Solution: Dissolve 50 mg of riboflavin in glacial acetic acid and make up to 1000 ml with water. Protect from light and store at a temperature not exceeding 4°C. Use within one month of preparation.

Biotin Solution: Dissolve 20 mg of biotin in a small quantity of water and make up to 1000 ml with 20 per cent ethanol solution (v/v). Preserve at a temperature not exceeding 4°C. and use within one month of preparation.

Inorganic Salt Solution: Dissolve $MgSO_4, 7H_2O$, (16 g), $MnCl_2$ (2.8 g) and $FeSO_4$, $7H_2O$ (0.8 g) in a little water. Add five drops of conc. HCl and make up to 100 ml with water. This solution keeps indefinitely at room temperature.

"Tween 80" Solution: Dissolve 10 g of "Tween 80" in 50 ml of water warmed to 45°C. and make up to 100 ml with water. Store at a temperature not exceeding 4°C. and use within one month of preparation.

Thiamine Stock Solution: Dissolve 0.1 g of thiamine, accurately weighed, in 100 ml of N/200 HCl. Store at a temperature not exceeding 4°C. and use within one month of preparation.

Basal media for assays with lactic organisms are invariably made up at double strength, and tubed in 5 ml

aliquots. Test tubes measuring 18-20 x 160-180 mm are the most suitable size for carrying out microbiological assays.

Preparation of Thiamine-free Basal Medium (Sufficient for 100 tubes)

Tryptone soln.	100 ml
Yeast Supplement	50 ml
Cystine-Tryptophan soln	25 ml
Adenine-guanine-uracil soln	10 ml
Xanthine soln	10 ml
Glucose (monohydrate)	10 g
L-Asparagine	1 g
K_2HPO_4	5 g
NaCl	5 g
Riboflavin soln	10 ml
Niacin soln	2 ml
Ca-d-pantothenate soln	1 ml
Pyridoxine HCl soln	1 ml
Biotin soln	0.1 ml
"Tween 80" soln	10 ml
Inorganic salt soln	5 ml
Water to	500 ml

Note :- Glass distilled water must be used for the preparation of the basal medium, special reagents and all operations of microbiological assay.

After mixing, adjust the pH of the medium to 6.0 with NaO solution using bromothymol blue as external indicator (or electrometrically) and make up to 500 ml with water.

Preparation of the Inoculum

To 50 ml of double strength basal medium, add thiamine, so that on dilution to 100 ml with water, each ml contains 1 µg

of the vitamin. Distribute in 10 ml amounts in 1 oz. screw-top bottles and sterilize for 10 minutes at 10 p.s.i. Store at a temperature not exceeding 4°C. and use within one month of preparation.

Inoculate a bottle of the broth from an agar stock culture of L. viridescens and incubate overnight at 30°C. Centrifuge and reject the supernatant liquid. Re-suspend the bacterial cells in 10 ml of quarter-strength Ringer solution and re-centrifuge. Reject the supernatant liquid; suspend the bacterial cells in 10 ml quarter-strength Ringer solution and add 1 ml. of this suspension to 100 ml of sterile water. Add one drop of the dilute suspension to each of the assay tubes with a sterile pipette.

Preparation of Samples for Assay

Suspend a suitable quantity (1-5 g), accurately weighed, of the test sample in 50 ml of 0.1N HCl and autoclave for 20 minutes at 15 p.s.i. Cool, add 2 ml of 2.5M sodium acetate solution and adjust the pH to 4.5 with sodium hydroxide solution electrometrically, or by using bromocresol green as external indicator. Make up to a suitable volume with water and filter. Further dilution may be necessary depending upon the thiamine content of the material. The final solution should contain approximately 0.005 µg/ml of thiamine.

Note:- Materials with a high fat content, e.g. wheat germ, oats, meat etc., should be given a preliminary extraction for 16-18 hours with Analar light petroleum (b.p. 40-60°C.), to prevent the formation of oily emulsions which may interfere with the extraction.

1. Assay Procedure

A separate standard curve must be established for every assay. Prepare a solution of thiamine to contain 0.005 µg/ml and add the following amounts plus water to establish a range of standards. Blank, 5.0 ml water; 0.5 ml standard soln + 4.5 ml water; 1.0 ml standard soln + 4 ml water; 2 ml standard solution + 3 ml water; 3 ml standard solution + 2 ml water and 4 ml standard solution + 1 ml water.

The assay of test samples must be carried out at at least three different concentration levels. Set up all the concentration levels of the standard and test solutions in duplicate and after

the level of liquid in each tube has been adjusted to 10 ml with water, cap the tubes with aluminium thimbles and sterilize either by steaming for 30 minutes or for one minute at 10 p.s.i. in the autoclave. Cool and inoculate.

Incubate for 20 to 22 hours at 30°C. and determine the response nephelometrically.

Method of Calculation

The standard curve in invariably curvilinear and Wood's 'log-log' procedure[2] should be used to calculate the results of an assay. Alternatively, direct reading from the standard curve may also be employed. This method has the advantage of being rapid and showing immediately whether an assay is valid or not. Provided that the values found at three or more concentration levels do not differ among themselves by more than ± 10 per cent., the mean value will give the amount of thiamine present in the test sample. A regular upward or downward 'drift' in the values at different concentration levels shows the presence of interfering substances and the assay must be regarded as invalid.

Maintenance of Stock Cultures

Maintain Lactobacillus viridescens in an agar stab culture prepared from the basal medium described above at single strength plus 2 per cent. of agar and 10 µg/ml of thiamine. Subculture at weekly intervals.

2. Cup-Plate Assay

Prepare the basal medium described above to its final strength with the addition of 2 per cent. agar. Steam the medium to melt the agar and transfer 20-ml portions to Pyrex bacteriological test tubes, plug with cotton wool and sterilize at 10 p.s.i. for 10 minutes. The tubes may be stored at a temperature not exceeding 4°C. but must be used within 7 days. Just before use, heat the tubes on a boiling water bath until the medium has melted and then hold in bath at 45°C. until required. Inoculate the tubes with 1 ml of a suspension of the organism, mix thoroughly and pour into Petri dishes. Allow to cool on a flat bench. Cut out five holes in each dish with a sharp 10 mm cork borer. Add 0.1 ml of the standard or test solutions to the appropriate 'cups' from an accurately graded pipette and incubate the plates at 30°C. for 20-22 hours. At the end of the incubation period, measure the diameters of the zones of

growth to the nearest 0.5 mm. A satisfactory diameter for the zones is about 25 to 30 mm. Adequate accuracy is possible using five cups per dish, with random distribution of doses among the cups.

Preparation of Inoculum.

A suspension of Lactobacillus viridescens after centrifuging and re-suspending in quarter-strength Ringer solution is diluted with sterile water (or quarter-strength Ringer solution) so that the opacity corresponds to tube No. 5 of the Wellcome series of standard opacity tubes. One-ml portions of this suspension are added to 20 ml of medium.

Relation between Thiamine Concentration and Zone Diameter.

The diameters of the zones are proportional to the logarithms of the concentrations of thiamine. The range of thiamine to establish a standard curve is: 0.25 µg/ml to 1.0 µg/ml. Dilute the volume of the test solution after filtration to 1 + 1, 1 + 3, 1 + 7 and 1 + 15. Use five plates for the standard solution and five for the test preparation.

By plotting the diameters of the zones of growth against the logarithms of the dose, a straight line will be obtained for the standard solution and a parallel straight line for the test preparation. If the lines are not parallel, the assay is invalid.

Extraction of Test Sample.

Use the same method of extraction as for the tube method of assay, but owing to the insensitivity of the method, the final concentration of the extract must be 10 times as high.

References

1. Deibel, R.H., Evans, J.B. and Niven, C.F. J.Bact., 1957, 74, 818.

2. Wood, E.C. Analyst, 1947, 72, 84.

TOXIC METALS IN SCHOOL MATERIALS AND TOYS

J.H. Barker[θ], W.B. Chapman[θ] and A.J. Harrison*

A review of recent legislation and control of the amount of lead in Paint and Children's Toys is followed by an account of routine methods for the approximate quantitative determination of various toxic metals in these materials.

The presence of toxic metals, particularly of lead, in pencils and other school materials has been the cause of considerable concern over a number of years. More recently, attention has also been directed to the presence of lead in plastic toys which are available to young children. The poisonous nature of lead in paints was legally recognised as early as 1926, when the Lead Paint (Protection against Poisoning) Act was enacted. In this Act and Regulations made under it, lead paint was defined as containing 5 per cent. or more lead (expressed as PbO) in the pigment. This lead was to be determined, after isolation of the pigment by suitable solvents, by shaking with 1000 times its weight of a 0.25 per cent. w/w hydrochloric acid solution for one hour at room temperature followed by standing for a further hour. Similar legislation was later introduced for pottery glazes. These definitions were principally designed to protect workmen using or handling the materials concerned in their daily work. In 1961, however, the British Standards Institution published a "Code of Safety Requirements for Children's Toys and Playthings" (B.S. 3443: 1961), in which a standard for coating materials for toys of not more than 1 per cent. lead was included, in this case, total lead being specified.

For some years prior to this, the use in London County Council Schools of pencils coated with lead based paints or varnishes and crayons in which a large proportion of the pigments was lead compounds was considered undesirable, since chewing of such materials by small children could occur. In the Annual Report of the Scientific Adviser to the London County Council for 1960, it was recommended that not more than 10 mg of the following toxic metals should be present in a pencil for school use:- arsenic, lead, acid soluble cadmium, acid soluble chromium, acid soluble barium and acid soluble antimony. This limit was derived from a consideration of the toxicity of the metals and a maximum allowance of consumption of the coating or core of the pencils by children. Subsequently,

θ Scientific Branch, County Hall, London, S.E. 1.
* City Analyst's Dept., Trafalgar Place, Clive Road, Portsmouth.

in consultation with both the Ministry of Education and trade representatives, a suggested maximum limit was agreed of 250 p.p.m. of lead in paint films and cores of pencils and crayons and also a variety of other school materials. The trade representatives indicated that this lower limit was technically feasible and is therefore acceptable as more readily approaching the ideal of complete absence of lead.

A similar limit for lead has now been applied by the Home Office to plastic toys, recent samples of which have been found to contain 4000 p.p.m. or more of lead. Such a limit is tentative however, since any metal can only exhibit toxicity if it is absorbed by the body. The extraction of lead from plastics will vary considerably depending upon the composition of the lead compounds present and also the composition of the plastic material. Extraction tests are, therefore, desirable to indicate whether a particular plastic which contains lead is likely to give rise to toxic effects. Such extraction techniques have been suggested by the British Plastics Federation, in their Second Report of the Toxicity Sub-Committee. However, these techniques are designed to simulate conditions when food is in contact with the plastics but are not designed to provide assessments of the extractability of lead when the plastics is chewed or actually ingested. Some modification of these tests will, therefore, be necessary before they can be applied to this particular problem and a possible technique, which has been used by the authors, is suggested below. The analytical methods used in assessment of samples in connection with such standards were the subject of some investigation involving both methods of preparation of solvents and actual estimates of the metals present.

Preparation of sample solution

For paint films and cores of pencils and crayons, direct ashing followed by dissolution of the residue in acid was used. However, possible loss of lead by volatilisation and/or formation of insoluble lead compounds by ashing could be avoided by treating the material with alcoholic KOH followed by dissolution of the residue on evaporation in acid. Where the lead pigments contain or are likely to contain red lead, lead dioxide or calcium plumbate, treatment with aqua regia is necessary to convert all the lead to soluble form. Powder colours, plasticine, chalks and similar materials containing large amounts of calcium carbonate were considered to be satisfactorily treated

by boiling with acid alone, ensuring that excess remained after the neutralising power of the carbonate had been overcome.

The testing of plastics is more difficult since the total lead obtained by ashing may bear no relationship to the lead which can be extracted by gastric juices and only such lead will have a toxic effect. After several testing techniques had been tried, treatment successively with alcoholic potash and nitric acid was used. It is thought (though no experimental work has been carried out in this aspect) that this treatment is at least as rigorous as that likely to be produced by chewing or ingestion of plastics. If no significant amount of lead or other toxic material is extracted from the plastics by these conditions it is thought reasonable to consider the plastics as safe material for use in preparation of toys.

It may be that the presence of red lead, lead peroxide or similar pigments in plastics would necessitate different treatment as mentioned above in the case of paints.

Determination of metals

The aim in the following tests was to provide a rapid method which could be subsequently confirmed by more accurate work if required:-

Arsenic - The well known Gutzeit method provided adequate sensitivity for these materials.

Antimony - After trials using other methods, the colour reaction with iodide was used, although this is not as sensitive as would be desirable.

Barium - Great difficulty was experienced with tests for this element and no really satisfactory test was devised. The method suggested, based on turbidity produced by barium sulphate, is neither sensitive nor accurate but is relatively specific and simple in use.

Cadmium - Dithizone in the presence of caustic alkali and tartrate is reasonably specific and is sufficiently sensitive for these materials. Tests using $\alpha\alpha'$-dipyridyl were not very successful.

Chromium - Diphenyl carbazide reaction after oxidation with hypobromite was found satisfactory.

Lead - Ammoniacal dithzone in the presence of cyanide was considered sufficiently specific for routine use.

EXPERIMENTAL

All apparatus should be of borosilicate glass and rinsed with hot acid before use. Glass stopped test tubes 6" x $\frac{3}{4}$" are used for colour developments. Reagents must be similarly free from or low in lead and appropriate blanks should, of course, be carried out.

Preparation of Sample Solution

Reagents

All reagents to be of Analar quality where available.

1.	Alcoholic N KOH	10 N KOH (aqueous) (10 ml), plus Industrial Methylated Spirits (74° O.P.) (90 ml). I.M.S. Redistilled over NaOH may be used.
2.	Aqua Regia	3 volumes of concentrated HCl + 1 volume of concentrated HNO_3.
3.	3N HCl	260 ml concentrated HCl made up to 1000 ml.
4.	4.5N HCl	390 ml. concentrated HCl made up to 1000 ml.
5.	3N HNO_3	190 ml concentrated HNO_3 made up to 1000 ml.

1. Paint films and cores of crayons

Two alternative methods have been used as follows :-

(a) Transfer a weighed quantity (preferably 1 g) of paint film or core to a porcelain dish, add 5 ml of alcoholic N KOH, cover with a watch glass and heat on a steam bath for 15-20 min. Remove the cover and continue heating until evaporation to dryness is complete. To the dry residue, add 5 ml aqua regia, replace the cover and heat on a steam bath for 5-10 minutes. Remove the cover and complete the evaporation to dryness. Add

2- Jne water + reevaporate to dryness to get rid of HNO_3

5ml

To the dry residue ass an appropriate volume of 3N HCl, such that the final concentration of HCl is 0.6N and boil gently for 5 minutes. Cool and dilute with water to give a solution containing 1 per cent. of the paint film.

2025 ml.

(b) Transfer a weighed quantity (preferably 1 g) of paint film or core to a silica basin and ignite gently at not more than 550°C. until all organic matter is destroyed. To the residue add an appropriate amount of 3N HCl and boil gently for 5 min. Cool and dilute with water to give a 1 per cent. solution of sample in 0.6N HCl.

NOTE - The second method is not suitable for use with pigments containing lead chromate, due to the possibility of fusion into an insoluble form.

2. Powder Colours and Chalks

Transfer 1 g (accurately weighed) into a clean, 250 ml conical flask, add 100 ml 3N HCl and boil gently for 15-20 min. Cool, dilute to 100 ml if necessary and filter through a dry acid washed filter paper (Whatman No. 41), rejecting the first runnings. If a clear solution is not obtained, filter through a finer acid washed paper (Whatman No. 42).

3. Plasticine

Transfer 10 g (accurately weighed) to a 250 ml Quickfit and Quartz extraction flask and add 100 ml 4.5N HCl. Heat under a reflux condenser, maintaining gentle boiling, for 1 hour. Cool slightly and filter through an acid washed filter paper (Whatman No. 41) and dilute the filtrate if necessary to 100 ml. Prepare a 1 in 10 dilution of this 10 per cent. solution giving a 1 per cent. solution of sample.

4. Plastics

Transfer 10 g (accurately weighed) of the plastic cut in small pieces or preferably shredded to a 250 ml Quickfit and Quartz extraction flask and add 50 ml alcoholic N KOH. Boil gently under a reflux condenser for 3 hours and decant the liquid into a 250 ml beaker. Rinse the residual plastic once with about 10-15 ml of water and add to the beaker. Heat the beaker on a steam bath to evaporate the alcoholic KOH extract to dryness.

Meanwhile to the residual plastic in the 250 ml flask, add 100 ml of 3N HNO_3 and again boil gently under reflux for a further 3 hours. Add the acid extract to the dry residue in the 250 ml beaker, rinse the residual plastic in the flask with two lots of 15 ml of water and add to the beaker. Evaporate the extracts to dryness on a steam bath. To the dry residue add 20 ml of 3N HCl, cover the beaker with a watch glass and boil gently for 10 minutes. Cool, dilute with water to 100 ml to give a solution containing 10 per cent. of sample. Prepare a 1 in 10 dilution of this solution so that the final solution contains 1 per cent. of sample.

Estimation of metals

1. Antimony

Reagents
1. 50 per cent. v/v H_2SO_4.
2. 50 grams KI + 10 grams NaH_2PO_2 made up to 100 ml in water.
3. 10 per cent. w/v NaH_2PO_2 in water.
4. Standard Antimony Solution. Dissolve 0.267 g potassium antimonyl tartrate in water and dilute to 100 ml. 1 ml ≡ 1 mg Sb.
5. Dilute Antimony Solution. Dilute 10 ml of reagent 4 to 100 ml with water. 1 ml ≡ 100 µg Sb.

Method

To a clean, dry, stoppered test tube transfer 4 ml of the prepared sample solution, add 2 ml of Reagent (1) and mix. Add 4 ml of mixed KI/NaH_2PO_2 reagent and again mix. Allow to stand for 15 minutes and note colours throughout this time, recording in particular the colour after 15 minutes standing. At the same time carry out the procedure on two further tubes containing 40 µg and 100 µg of antimony respectively in 4 ml of water, (i.e., 0.4 ml of dilute Standard antimony solution + 3.6 ml of water and 1.0 ml of dilute Standard antimony solution + 3.0 ml of water. Record the colour of the sample solution as a proportion of the 40 µg or 100 µg standards and calculate to parts per million.

(On 4 mls. of a 10 per cent. solution, 40 µg antimony is equivalent to 100 p.p.m.).

NOTE - If Fe is present, I_2 is formed immediately on the addition of reagent but is subsequently decolourised by the NaH_2PO_2, some minutes being required before the reduction is complete. In the presence of iron therefore, it is essential to ensure that this reduction has occurred fully. Even 10 per cent. Fe_2O_3 in the original sample would be effectively removed.

2. Arsenic

A suitable aliquot (5 ml) of the sample is transferred to the arsenic flask and the Gutzeit method carried out, according to the method of the British Pharmacopoeia.

1 µg stain of arsenic on 5 ml of a 1 per cent. solution of sample is equivalent to 20 parts per million of As in the sample.

3. Barium

Reagents
1. 3N NH_3 (50 ml of 0.880 NH_3 made up to 300 ml with water).
2. N H_2SO_4 (25 ml of concentrated H_2SO_4 diluted with water, cooled and made up to 900 ml).
3. Standard solution. Dissolve 0.178 g of $BaCl_2.2H_2O$ in water and dilute to 100 ml; 1 ml ≡ 1 mg Ba.

Method

In each of two tubes place 6 ml of sample solution, add 0.6 ml of Ba solution (equivalent to 0.6 mg Ba) to only one test tube and mix. To each tube add 4 ml 3N NH_3 and mix, followed by 0.5 ml of N H_2SO_4 with mixing. Stand for up to half an hour and record the presence or absence of turbidity in both tubes.

NOTE - A turbidity should be produced in the tube containing the sample with added barium, whereas no turbidity will be noticeable in the sample tube alone in the absence of barium. This test is relatively insensitive and will detect only 0.1 per cent. of barium even when using a 10 per cent. sample soln. It does however avoid

precipitation of calcium sulphate, when the sample contains a considerable amount of calcium carbonate.

4. Cadmium

Reagents
1. 20 per cent. w/v sodium potassium tartrate.
2. 40 per cent. w/v sodium hydroxide.
3. 0.002 per cent. w/v dithizone in carbon tetrachloride.
4. Standard cadmium solution. Dissolve 0.228 g of $3CdSO_4.8H_2O$ in water and dilute to 100 ml. 1 ml ≡ 1 mg Cd.
5. Dilute cadmium solution. Dilute 1 ml of reagent 4 to 100 ml. 1 ml ≡ 10 µg Cd.

Method

To a clean, dry, glass-stoppered test tube, transfer 1 ml of sample solution and add 8 ml of water. Add 5 ml of 20 per cent. sodium potassium tartrate soln. and mix. Add 2 ml of 40 per cent. NaOH soln. and mix. Add 5 ml of 0.002 per cent. dithizone, shake vigorously for 60 seconds and allow the layers to separate. Treat a blank and a standard tube, consisting of (a) 9 ml water and (b) 8 ml water + 1 ml of dilute Standard cadmium solution respectively, in a similar manner and compare the colour of the organic layer of the sample solution with that of the blank and standard.

10 µg Cd on 1 ml of a 1 per cent. solution is equivalent to 1000 p. p. m. and a tenth of this amount is readily detectable.

5. Chromium

Reagents
1. Hypobromite solution. 3 ml 10.N. NaOH + 7 ml of saturated bromine water.
2. 3N H_2SO_4. 25 ml of concentrated H_2SO_4, diluted with water, cooled and made up to 300 ml.
3. Phenol-disulphonic acid reagent. Dissolve 18 g of phenol in 10 ml of water and add 110 ml of concentrated H_2SO_4. Mix and heat on a steam bath for 8 hours.

4. 1 per cent. diphenylcarbazide in industrial methylated spirits.
5. Standard chromium solution. Dissolve 0.960 g of chromic potassium sulphate in water. Add 1 ml of 3N H_2SO_4, dilute to 100 ml. (1 ml ≡ 1 mg Cr.).
6. Dilute chromium solution. Dilute 1 ml of reagent 5 to 100 ml with water. (1 ml ≡ 10 µg Cr.).

Method

Transfer 1 ml of sample solution to a clean glass stoppered test tube, add 4 ml of water and mix. Add 1 ml of hypobromite solution, mix and allow to stand for 3-5 minutes. Add 1 ml of 3N H_2SO_4 and mix, when free bromine will be liberated. Add 0.5 ml of phenol-disulphonic acid reagent, mix and ensure that the bromine is completely removed and the solution is colourless. If not, add a further 0.5 ml of phenol-disulphonic acid. Add 2 drops of diphenylcarbazide solution and mix. Treat a blank and a standard consisting of (a) 5 ml water and (b) 4 ml water + 1 ml dilute standard chromium solution respectively, in similar manner and compare the colour of the sample solution with the colours given by the blank and standard. 10 µg of Cr in 1 ml of a 1 per cent. solution is equivalent to 1000 p.p.m. Cr and one-tenth of this amount can readily be detected.

6. Lead

Reagents

1. 10 per cent. w/v citric acid
2. 1 per cent. w/v hydroxylamine hydrochloride.
3. 3N NH_3 (50 ml 0.880 NH_3 made up to 300 ml with water.
4. 5 per cent. w/v KCN.
5. Chloroform - redistilled.
6. 0.1 per cent. w/v dithizone in chloroform (store in refrigerator when not in use.
7. 0.02 per cent. w/v dithizone in chloroform. (Prepared from (6) immediately before use).
8. Standard lead solution. Dissolve 0.183 g of lead acetate (A.R.) in water, add 1 ml of 3N HNO_3 and dilute to 100 ml. (1 ml ≡ 1 mg Pb).

9. Dilute lead solution. Dilute 1 ml of reagent (8) to 100 ml with water. (1 ml ≡ 10 µg Pb).

Method

Transfer 1 ml of sample solution into a clean glass-stoppered test tube, add 4 ml of water and mix. Add 2 ml of 10 per cent. citric acid; mix, add 1 ml of 1 per cent. $NH_2OH.HCl$ and mix; add 3 ml of 3N NH_3 and mix. Add 1 ml of 5 $^{w}/_{v}$ KCN and mix, followed by 5 ml of $CHCl_3$. Pipette 0.3 ml of 0.02 per cent. dithizone into the tube and shake vigorously for 60 seconds.

Treat a blank and a standard, consisting of (a) 5 ml of water and (b) 4 ml of water + 1 ml of dilute standard lead solution respectively, in a similar manner and compare the colour of the sample solution with the colours given by the blank and standard.

10 µg Pb in 1 ml of a 1 per cent. solution is equivalent to 1000 p.p.m. Pb and one-tenth of this amount can readily be detected.

The above methods are not designed to give very accurate results but serve to distinguish clearly those samples which require further work.

The sensitivity of the barium method is inadequate to enable 250 p.p.m. soluble Ba to be detected and the sensitivity of the antimony method is only sufficient for 100 p.p.m. if a 10 per cent. solution of the sample is available. With the other metals, however, adequate sensitivity is available.

We wish to thank Dr. S.G. Burgess, Scientific Adviser, London County Council, and Mr. A.L. Williams, City Analyst, Portsmouth, for their continued interest and helpful advice.

THE DETERMINATION OF TIN IN CORNED BEEF

by J.H. Shelton and J.M.T. Gill

(Public Analysts' Laboratory, 7, Tudor Street, London, E.C.4)

A rapid method for the determination of tin in canned meat is described, using toluene - 3 : 4 - dithiol. The colour is developed after preliminary solution of the ashed sample in sodium hydroxide solution.

Existing procedures for the determination of tin in canned meats employ either a lengthy wet oxidation process, or a dry ashing process involving a fusion mixture. The following method entails simple ignition of the sample and the subsequent solution of the tin from the ash using sodium hydroxide solution The determination of the tin in the solution so produced follows the procedure of R. De Giacomi[1] using dithiol. Recovery experiments using the above procedure have been found to be satisfactory, and the method gives comparable results to those obtained using the wet oxidation technique of R. De Giacomi.

Reagents

1. NaOH: 50 per cent.
2. HCl: concentrated.
3. Thioglycollic acid.
4. Strong soln. of tin: Dissolve 0.1 g of pure tin in 10 ml of conc. HCl. Add 0.5 ml of thioglycollic acid and dilute to 100 ml.
5. Dilute soln. of tin: Dilute 1 ml of the concentrated soln., with the addition of two drops of thioglycollic acid, to 100 ml.
6. Toluene-3:4-dithiol: Dissolve 0.1 g of toluene-3:4-dithiol and 0.25 ml of thioglycollic acid in 50 ml of a 1 per cent. aqueous soln. of NaOH.

Method

Ignite 10 g of the sample in a 50 ml capacity silica crucible overnight at 500°C. Add 10 ml of 50 per cent. w/v sodium hydroxide, cover with a Pyrex watch glass and boil gently for 5 minutes. Transfer quantitatively to a 100 ml, calibrated flask with hot water. Cool, acidify to litmus with concentrated hydrochloric acid, add 2 drops of thioglycollic acid and make up to the mark.

Dilute an appropriate aliquot of the solution, containing not more than 0.04 mg of tin, to 5 ml. Add 5 ml of distilled water, 0.5 ml of concentrated hydrochloric acid, followed by 0.5 ml of dithiol reagent. Heat in a boiling water-bath for 30 seconds, allow to stand for 1 minute and compare the depth of colour by reflected light with standards prepared in the same way from 1, 2, 3 and 4 ml of the dilute tin solution. If the colour so obtained exceeds that from the 4-ml standard, a smaller aliquot should be taken and the procedure repeated.

Discussion

After the solution of the ash in sodium hydroxide, the resultant liquid frequently possesses a slight red opalescence. This does not appear to have any effect upon the development of the colour or the recovery of the tin. The reaction between tin and dithiol is specific under the conditions of the above experiment[2].

Results

Comparison of the ignition procedure described with the classical method of wet oxidation showed that the former gave slightly higher results. The following recoveries were obtained from experiments using both methods.

	By ignition	By wet oxidation
Sample A	160 p. p. m.	150 p. p. m.
Sample B	80 p. p. m.	72 p. p. m.

References

1. R. De Giacomi, Analyst, 1940, 65, 216.
2. Robert E. D. Clark, Analyst, 1936, 61, 242.

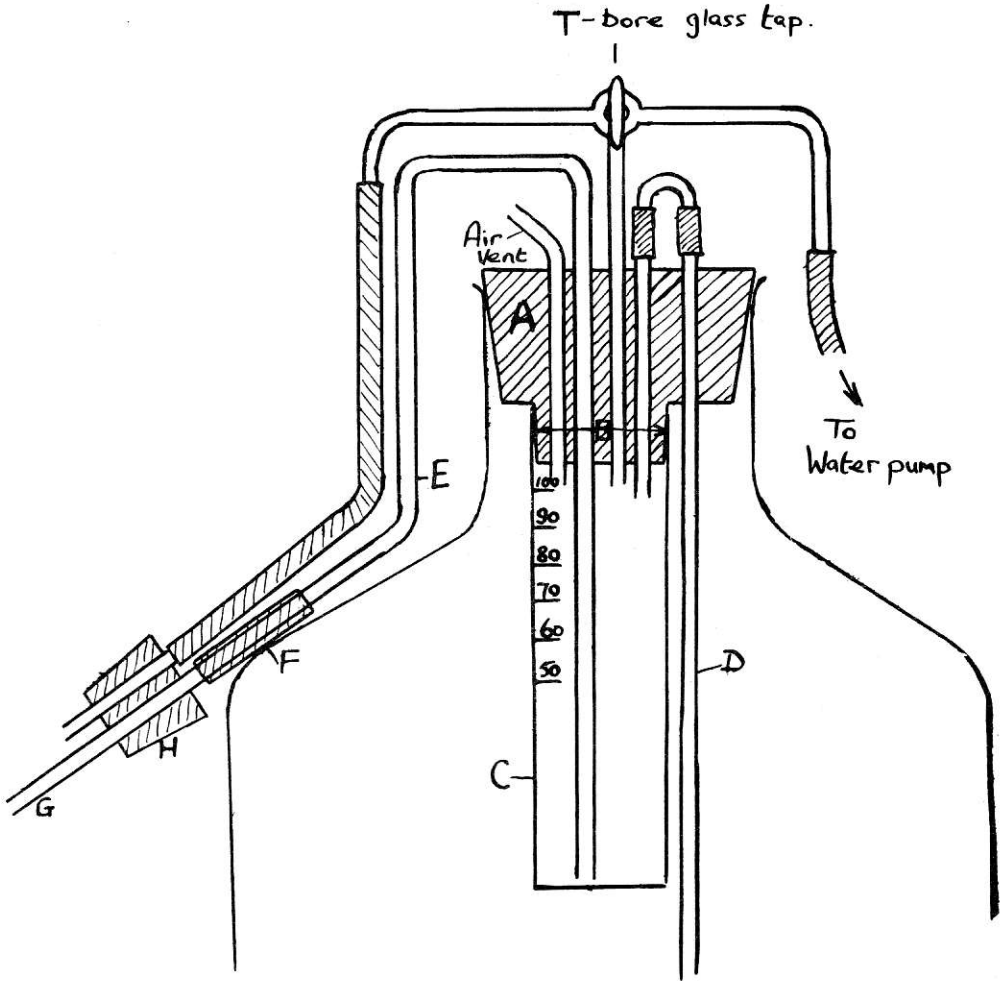
A DISPENSER FOR STRONG CAUSTIC SOLUTIONS

by G. S. Meadows

(City Laboratory, Salford, Lancs.)

A semi-automatic device is described which effectively delivers a suitable volume of fifty per cent sodium hydroxide directly into the distillation flask in the Kjeldahl method for nitrogen determination.

Fifty per cent. sodium hydroxide solution is not an easy liquid to 'handle' and is inconvenient and troublesome to measure by the usual methods. The device shown in the diagram is easy to use and reduces 'handling' of the solution to a minimum. It is fairly simple to assemble and fits neatly in the neck of a 10 litre bottle. In operation, the caustic is gently delivered down the neck of the flask to form a layer below the acid digest.



A dispenser for strong caustic solutions

Assembly (See Figure)

A large rubber bung, A, is shaped as in the diagram, the diameter at B being adjusted to fit tightly into a stout-walled, 100-ml Nessler cylinder (this can be done quite easily with a hacksaw and a file). Tube D extends to the bottom of the 10-litre bottle. The delivery arm, E, is made of thick-walled glass tubing and bent as shown so that the protective rubber tubing F rests on the shoulder of the bottle. The rubber bung, H, is of a size which fits the distillation flask neck and the tip, G, should project about 2 inches so that the caustic is delivered well down the neck of the flask.

Method of Use

Turn on the water pump, attach the distillation flask to H and, with the T-bore tap positioned to apply suction to the measure, place a finger over the air vent. Remove the finger when the liquid reaches the required level (care should be taken not to overfill or the caustic will pass up the suction tube and affect the tap). Finally, turn the tap to apply suction to the distillation flask and the measured amount of caustic will then be transferred to the distillation flask.

THE EXAMINATION OF SAMPLES SUBMITTED UNDER THE AGRICULTURAL LIME SCHEME

by Eric C. Wood[+] and J.H. Hamence[*]

No methods of analysis are prescribed under the Agricultural Lime Scheme, 1964. The necessity for uniformity among analysts is emphasised and a suggested analytical scheme is put forward.

Introduction

The Agricultural Lime Scheme, (S.I. 1964 No.903), which became operative on the 1st August 1964, provides for the payment of contributions to farmers towards the cost of lime or chalk used on their land. The rate of contribution depends on the category into which the material falls in terms of first, the Neutralising Value and secondly, the percentage passing a

+ Official Agricultural Analyst, 6, Clarence Road, Norwich, NOR29T.
* O.A.A., 20, Eastcheap, London, E.C.3.

prescribed sieve. The First Schedule to the Scheme contains a list of kinds of lime and chalk, each defined in this way, together with the contribution per ton for each kind. Inspectors appointed by the Ministry of Agriculture, Fisheries and Food are now taking regular samples from each approved producer of these materials to ensure that his product is of the kind specified in his certificates of approval. These samples are normally divided into parts, one being retained by the Inspector and passed on to one of the analysts appointed by the Ministry, and another being handed to the producer, who may submit it to be analysed by anyone he chooses. In consequence, one of the authors (E.C.W.), whose practice is in an area producing large amounts of lime and chalk, has been receiving such samples for some months.

Unlike the Fertilisers and Feeding Stuffs Regulations[1], however, the Scheme makes no mention whatever of methods of sampling or of dividing samples, methods of analysis or limits of variation. We are not primarily concerned with sampling, although we believe that samples submitted for analysis should be at least 500 g or 1 lb. On the other hand we are much concerned with matters of analysis, because in the absence of any prescribed methods there is more than one way in which even qualified and experienced agricultural analysts can obtain materially different results on counterparts of the same sample by adopting different techniques for the analysis. It is clearly desirable that all the analysts examining samples under this Scheme should adopt the same methods, in order to reduce to a minimum the variance between laboratories. This paper embodies suggestions for the methods to be employed.

Sieving Test

The only test of this kind prescribed in the Fertilisers and Feeding Stuffs Regulations is in Para. 9 of the Seventh Schedule[1] for (inter alia) ground limestone, where the analyst is instructed to carry out the sieving on a 20-g sub-sample. We recommend that the procedure described in this paragraph be followed for samples taken under the Scheme for which a sieving test on a 100 mesh sieve is required. The moisture is determined, if necessary, on part of the original well-mixed sample (see para. 2 of the Seventh Schedule) and the rest of the sample is dried and a 20-g aliquot taken for sieving.

The Scheme requires the use, for some kinds of material, of relatively coarse sieves from 1/8 inch up to 1 inch aperture. The use of a 20-g sub-sample with these sieves would clearly lead to ridiculously large sampling errors. The samples reaching the analysts from the Inspectors appear to average about 500 g, and to take the whole of the sample for the sieving test on a coarse sieve is both practicable and eminently desirable.

A further point is whether or not the sample should be dried before this test is carried out. In the case of an obviously dry material, no useful purpose would be served in drying the sample when sieves of 1/4 inch and larger apertures are involved; the whole sample may be sieved directly.

If the sample appears to be damp, and in all cases where the 1/8 inch sieve is to be used, it is considered that the test should be carried out on the whole sample after it has been dried at 100°C. A separate moisture test on a small portion of a coarse sample would involve too large a sub-sampling error, and it is therefore recommended that the whole of a sample, as received, should be dried to constant weight at 100°C. and the loss in weight determined. It is pointed out that to determine the loss in weight of a 500-g sample on a balance sensitive to 0.1 g is as precise as weighing a 5-g sample to the nearest mg.

The passage of material through these coarse sieves usually comes to an abrupt end as the sieving proceeds, and we recommend that the shaking be continued until no further material passes the sieve after a further 30 seconds' shaking. No attempt should be made to rub through the sieve anything remaining on it, but soft lumps may be crumbled with a soft brush as described in para. 9 of the Seventh Schedule.

Neutralising Value

The method prescribed in para. 7 of the Seventh Schedule[1] is certainly applicable to all the materials covered by the Scheme, provided that the sample and sub-sample are properly prepared.

The portions into which the sample has been divided by the sieving test are re-combined and the whole sample is then ground to pass a B.S. 16 sieve. The sample is then quartered down until a portion of approximately 50 g is obtained, and this is reground to pass a B.S. 30 sieve. The 500-mg sample

referred to in para. 7.3 is then weighed out and the rest of the procedure of this paragraph followed. The result is calculated and reported in terms of the sample as received, correcting for moisture content if preliminary drying has been necessary.

It is appreciated that some samples of lime may possibly absorb CO_2 during the drying and grinding processes, and thereby give erroneous results. This possibility should be investigated, but we think it unlikely that absorption could occur to such a degree as to cause significant errors in the analysis.

Summary of Analytical Recommendations

It is recommended that the fineness test and neutralising value should be carried out on samples received under the Scheme as follows:-

1. Fineness Test

   (a) With samples for which a 100-mesh sieve is to be used, follow the procedure laid down in para. 9 of the Seventh Schedule of the Fertilisers and Feeding Stuffs Regulations[1]. Determine the moisture if necessary on part of the original well-mixed sample as in para. 2 of the Regulations.

   (b) With dry samples and a sieve of 1/4 inch aperture or larger, sieve the whole of the sample as received.

   (c) If the sample appears moist or damp, and in any event when a 1/8 inch sieve is to be used, weigh the sample as received, dry to constant weight at 100°C. and calculate the moisture content from the loss in weight. Sieve the whole of the dried sample.

2. Neutralising Value

   Re-combine the portions of the sample and grind if necessary to pass a B.S. 16 sieve. Mix thoroughly and quarter down to obtain a portion of approximately 50 g. Re-grind this portion to pass a B.S. 30 sieve and determine the neutralising value as in para. 7.3 of the Seventh Schedule of the Regulations.

If a preliminary drying has been employed, calculate the neutralising value on the basis of the original moist material.

References

1. The Fertilisers and Feeding Stuffs Regulations, 1960, No. 1165. H.M.S.O., Kingsway, London, W.C. 2.

LETTERS TO THE EDITOR

DETERMINATION OF RESIDUAL CHLORINE IN SWIMMING BATH WATER

Sir –

It would be rather unfortunate if the suggestions made by Dr. H. A. Williams[1] received any wide acceptance in the field of modern swimming bath treatment, although his report on the differences that arise in determining residual chlorine by various methods is, of course, to be welcomed as a further contribution to the vast amount of published evidence already pointing to the deficiencies, now universally recognised, of the ortho-tolidine test.

Any discussion of residual chlorine in swimming bath water, however, first requires an appreciation of the aim of present-day chlorination practice. It is to produce and maintain in the water, whatever the bathing load, an adequate level of free-chlorine. Here Dr. Williams finds ortho-tolidine gives lower results than the DPD method. The experience of most analysts is exactly the opposite which clearly undermines the whole basis of the proposal that the ortho-tolidine figure, being lower, is therefore the safer one to take. But in any case erratic results from ortho-tolidine are to be expected.

So far as combined-chlorine is concerned, Dr. Williams has confirmed what is already well known, namely that ortho-tolidine gives very low results compared with other methods. His suggestion that this lack of response is not important, however, because combined-chlorine has questionable bactericidal value is rather like "making a virtue of necessity". The simple fact is that the question never arises in practice since no-one conversant with the requirements of modern swimming bath treatment would advocate placing even the

slightest reliance upon it as a bactericide. It is the rapid rate of bacterial kill by free-chlorine that is relied upon.

The significance of combined-chlorine and its determination actually lies in its possible connection with those conditions which, if treatment control is inefficient, can arise to cause severe discomfort to swimmers. The successful introduction of breakpoint chlorination to public swimming baths some 15 years ago, was made possible only by the development of improved and reliable control tests, so ensuring the highest possible bacterial quality with an absolute minimum of eye irritation and similar troubles. The experience of public swimming bath managers throughout the country is that the DPD test, together with tests for pH and alkalinity, provides the necessary control. A return to ortho-tolidine for chlorine testing would certainly be resisted at all levels.

As already noted, the general experience, unlike that of Dr. Williams, is for the ortho-tolidine test to read high rather than low for chlorine. Lack of space precludes any attempt to make copious reference to the literature but perhaps attention may be drawn to a recent paper by J. F. Malpas[2] who stated that "the free-chlorine fraction, obtained by the ortho-tolidine 'flash' test was decidedly suspect" and advocated the use of better methods now available. N. J. Nicholson of the Water Research Association, in discussing the above paper, also referred to the high free-chlorine figures obtained with ortho-tolidine, whereas in the case of combined-chlorine he pointed out that it was well known for the ortho-tolidine method to give low results "especially in water containing organic matter". In reply Malpas agreed that when compared against the DPD method or amperometric titration "the o-tolidine method tended to give slightly lower total-chlorine residuals as opposed to higher free chlorine residuals".

Enough has probably been said, however, to indicate the dangers of following any suggestion that "the ortho-tolidine figures should from consideration of safety be preferred". Nor should it be overlooked that the ortho-tolidine reagent as still used in this country was discarded by the American Standard Methods Committee nearly 20 years ago, and further, the British Joint Committee to which Dr. Williams refers was quite aware in the first edition of "Approved Methods", published 15 years ago, that the ortho-tolidine test was "not altogether satisfactory". It is understood that wholesale revision of recommended residual chlorine methods may be

expected from this Committee in the near future. For the present, it is believed that the titrimetric version of the DPD method[3] provides the best available laboratory procedure.

A final point to bear in mind in connection with these various tests is that the DPD test measures the concentration of residual chlorine at a pH not so far removed from that of the water. In the ortho-tolidine test the concentration is measured at the very low pH induced by addition of reagent to sample and further, it is known that a relationship appears to exist between the degree of acidity in the final solution and the chlorine lost or consumed by organic matter. When the pH is so lowered the organic matter and the reagent can compete in these changed conditions for the chlorine liberated from the combined chlorine compounds and conceivably in some cases for free-chlorine initially present as such. To what extent this has a bearing on Dr. Williams' results cannot of course be judged on the basis of the limited data supplied, but in any event, it is strongly recommended that a final decision on residual chlorine testing should await the comprehensive report now being prepared by the Water Research Association on behalf of the Joint Committee composed of representatives of the Society for Analytical Chemistry and the other participating bodies.

A. T. Palin

Newcastle and Gateshead Water Company
Throckley,
Newcastle-upon-Tyne.

References

1. Williams, H. A., "Determination of Residual Chlorine in Swimming Bath Waters." J. Ass. Publ. Analysts 1964, 2, 34.

2. Malpas, J. F., "The Chemical and Bacteriological Purification of Swimming Bath Water". Proc. S. W. T. E., 1964, 13, 94.

3. Palin, A. T., "Simplified Methods for Determining Residual Chlorine Compounds in Water Using Diethyl-p-Phenylene Diamine (DPD)". Proc. S. W. T. E., 1957, 6, 133.

VIETH'S RATIO

Sir–

Although the ratio of the constituents of milk (Vieth's Ratio) is used frequently for the calculation of the amount of milk in compounded foods, there appears to be some confusion in the literature as to the exact nature of this Ratio.

In an early paper by Vieth[1] the Ratio was given as

Ash : Proteid : Sugar = 1: 5: 6

and was amended subsequently to

Ash : Proteid : Sugar = 2: 9:13

The present confusion appears to arise from the interpretation of the terms "Proteid" and "Sugar". Thus it appears to have been assumed that "Proteid" is synonymous with the more modern term "Protein", i. e. the product of 6.38 and N as determined by the Kjeldahl procedure - but reference to another of Vieth's papers[3] shows that the "Proteid" content of a milk was determined by precipitating with copper sulphate and weighing the precipitate, after extracting the fat, and allowing for the mineral matter.

It is clear also from Vieth's paper[3] that the "Sugar" in his ratio is anhydrous lactose, but it is often quoted as "Lactose" without defining the state of hydration. Thus Pearson states both "lactose"[4] and "anhydrous lactose"[5]; Davis and MacDonald[6] and also Nicholls[7], state "lactose"; but the latter defines "lactose" as the monohydrate[8].

Richmond concluded that the average composition of milk[9] is

Water	87.34%
Fat	3.75
Milk Sugar	4.70
Casein	3.00
Albumin	0.40
Ash	0.75
Other constituents	0.06

but the ratio calculated from Richmond's figures on the basis of Ash + Protein + Milk Sugar = 24, is 2.03: 9.22: 12.75, and it is suggested that the discrepancy between this and Vieth's ratio of 2: 9:13 is due to the expression of the nitrogenous constituents as protein instead of the empirical "proteid" of Vieth.

If, however, the ratio is calculated on the basis of hydrated instead of anhydrous lactose (i. e. 4.95 per cent), while retaining the use of protein, then the ratio becomes 1.98: 8.97: 13.05.

Thus it would appear that with modern analytical procedure Vieth's Ratio should be re-stated as

Ash: Protein (N x 6.38): Lactose Hydrate = 2: 9:13.

The approximation to whole numbers is justified on the grounds that any error thus introduced would be very small and of no significance when compared with the natural variation in the composition of milk.

R. S. Hatfull

County Laboratory,
County Hall,
Maidstone, Kent.

References

1. Vieth, P. Analyst, 1888, 13, 49.
2. Vieth, P. Analyst, 1891, 16, 203.
3. Vieth, P. Analyst, 1887, 12, 61.
4. Pearson, D. The Chemical Analysis of Foods, 5th Ed. J. & A. Churchill, London, 1962, p. 322.
5. Ibid p. 335.
6. Davis, J.G. and MacDonald, F. J. Richmond's Dairy Chemistry, 5th Ed. Charles Griffin and Co., London, p. 16.
7. Nicholls, J.R. Aids to the Analysis of Food and Drugs, 7th Ed. Ballière, Tindall and Cox, London, 1952, p 71.
8. Nicholls, J.R. ibid., p. 62.
9. Davis, J.G. and MacDonald, F.J., ibid, p. 4.

TINNED STEEL FRYING PANS

Sir–

Following representations made to the Government as a result of our findings on tinned steel frying pans (J. Ass. Publ. Analysts, 1963, 1, 26), the Home Office has stated that, rather than make regulations for this type of article, it is their policy to seek voluntary co-operation of manufacturers in eliminating any danger, and have accordingly written to the appropriate Trade Associations expressing the view that the tinning on tinned steel frying pans should conform to the same standard as tinned steel baking dishes, B.S. 3393, 1961, i.e. not less than 99.75 per cent. tin. A draft British Standard has also recently been brought out for all culinary utensils, which will include tinned steel frying pans.

G. S. Meadows

City Laboratory,
Crescent,
Salford, 5.

THE R_f VALUES OF CERTAIN SYNTHETIC COLOURING MATTERS

Sir–

With reference to published figures for R_f values of colouring materials[1], Ponceau 6R in Table II is almost certainly Ponceau Crystal 6R as the two dyes have very different R_f values. This is to be expected since Ponceau 6R contains four sulphonic acid groups and Ponceau Crystal 6R only two. The confusion arises because Solmedia have supplied the same dye (Crystal 6R) under the two names. This is unfortunate, as Ponceau 6R is an E.E.C. colour and is sometimes found in Great Britain.

Chrysoine and Chrysoine S should be the same dye, being the sodium salt of 2:4-dihydroxyazobenzene-4'-sulphonic acid, while Chrysoine SGX is the 2'-4'-sulphonic acid. Again the colours supplied by Solmedia under the description Chrysoine and Chrysoine SGX are identical and appear to be in fact extremely impure Chrysoine in each case. With pyridine-ethyl acetate-water (11:5:4), using No. 1 Whatman paper and a 17 cm ascending run, four spots are obtained with R_f values 0.00, 0.09, 0.23, and 0.56. The spot at 0.56 represents Chrysoine, although the spot at 0.23 is of about the same intensity. These facts may account for the rather confusing R_f values quoted in Table II.

Alkali Blue is stated to produce a prolonged streak in the Table. This may be due to the fact that this dye is again very impure. Using pyridine-ethyl acetate-water (11:5:4) as above, the dye gave six distinct spots, (0.04, 0.15, 0.35, 0.43, 0.64 and 1.00). With less resolution it would be possible to imagine a streak.

It is also noted that when using phenol/water as solvent[2], a different technique from that used with the other six solvents is adopted.

The chromatogram is run overnight for an unspecified distance. In the circumstances it should be borne in mind that R_f values will not necessarily correspond with those for a shorter run, as comparison with those overleaf indicates:-

TABLE OF R_f VALUES

Dye	Overnight Descending	Ascending 12 cm run
Ponceau MX	0.66	0.39
Ponceau 4R	0.19	0.14
Carmoisine	0.82	0.50
Amaranth	0.12	0.15
Red 10B	0.36	0.30
Erythrosine BS	0.95	0.77
Red 2G	0.46-1.00	0.45
Red 6B	0.48-0.98	0.49
Red FB	0.00-1.00	0.58
Ponceau SX	0.33-1.00	0.47
Ponceau 3R	0.58	0.40
Fast Red E	0.84	0.50
Orange G	0.72	0.50
Orange RN	0.95	0.82
Sunset Yellow FCF	0.46-1.00 (0.08)	0.45
Tartrazine	0.11	0.11
Napthol Yellow S	0.19	0.18
Yellow 2G	0.95	0.50
Yellow RFS	0.51	0.34
Yellow RY	0.10 (0.00-1.00)	0.15
Green S	0.97	0.80
Blue VRS	0.95	0.89
Indigo Carmine	0.00 (0.24, 0.98)	0.31
Violet BNP	0.98	0.90
Brown FK	0.00-1.00	streak
Chocolate Brown FB	0.00	streak
Chocolate Brown HT	0.15 (0.00-1.00)	streak
Black PN	0.16	0.07

References

1. Pearson, D. and Chaudhri, A.B. J. Ass. Publ. Analysts, 1964, 2, 23.
2. Ibid., p. 30.

County Laboratory,
County Hall,
Maidstone, Kent.

R. C. Spalding

Printed and published at the end of each quarter for the Association of Public Analysts by Technical Administration Ltd., 74, South Street, Reading, Berks.