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Public Analysts of the Past

An Address by the retiring President, Miss Joan Peden, presented at the Annual General Meeting of the Association of Public Analysts in Taunton, Somerset, on 3 May 1975.

Much of the material for this talk comes from a book called 'Fifty Years of the Society of Public Analysts', published in 1932, by Dr. Bernard Dyer, one of our elder statesmen, who died in 1948, aged 92. Actually, part of the background research was then carried out by a young assistant named Hamence. Another source was the 1974 Society for Analytical Chemistry publication, 'The Practising Chemists', written jointly by Dr. Hamence and Dr. Chirnside. Both are extremely readable books, which any chemist will find to be of interest whether he is a Public Analyst or not.

Last July the Society for Analytical Chemistry had a splendid week of Centenary Celebrations, which I enjoyed as a guest. I said then, in my greetings, that Public Analysts were the true progenitors of the occasion, since they founded the Society and it carried their name for 79 of those 100 years. So I should like to tell you a little of the first 25 years of the Society of Public Analysts, which covers almost exactly the last quarter of the 19th century. We also have a Centenary of sorts this very year, for the first provincial meeting was held at Bristol in August 1875, when the new Food and Drugs Act (Mark III) was discussed.

All Public Analysts know of our official origin in the 1860 Act, the first of its kind in the world, 'for Preventing the Adulteration of Articles of Food or Drink', which merely permitted the appointment of Analysts by various bodies, such as the Courts of Quarter Sessions in the provinces, and the District Boards or Vestries in Outer London.

Such an optional start produced few results and so the improved Act of 1872 was passed, which led to actual appointments. In Somerset, for instance, we have the minutes of an Epiphany Sessions, in beautiful copperplate handwriting, containing an order for the appointment of 'A First Class Analyst' and on 21 February 1874 the Local Government Board approved Mr William Walter Stoddart, who was 'a person professing competent Medical Chemical and Microscopical Knowledge', at a salary of £100 a year, plus £50 for 'making and sustaining a suitable Laboratory'. He was also Public Analyst for the City of Bristol. He died in 1880 and was succeeded by his son.

In their book, Drs. Hamence and Chirnside make a clear distinction between the two types of chemists operational at that time. On the one hand there were the down-to-earth workers who needed to make a living from the job; these

are the 'practising chemists' of their title. The others were the theoretical chemists of the academic world, talented amateurs who treated science as a hobby, dons, doctors of medicine and even some 'interested clergymen'.

They must have been the last of an age when an educated man could dabble in many differing disciplines. The Elizabethan statesman Francis Bacon was also an eminent lawyer and essayist—and, apparently, the first man to prepare frozen chicken. The story goes that he died in 1626 of a chill caught while trying to preserve a dead fowl by stuffing it with snow. Then we have Robert Boyle (of Boyle's Law), who wrote 'The Sceptical Chymist' (a good title) in 1661, and was described by his publisher as the 'Father of Chemistry and Uncle of the Earl of Cork'.

In 1874, many chemical analyses were carried out by professors in University laboratories or by lecturers in colleges. There were a few private consultants, but commercial analysts were regarded as 'outside the pale'. When an appointment had to be made by a Local Authority, the matter was usually put into the hands of the Medical Officer, who occasionally took over the job himself on the basis of his Public Health qualifications, which covered a very little chemistry. There were exceptions like Dr. Letheby, who was Medical Officer and Public Analyst for the City of London and Lecturer on Medical Jurisprudence to the London Hospital.

Six professors from hospitals in London were among the 25 Public Analysts who met at the City Terminus Hotel, Cannon Street, London, on 7 August 1874, a historic date in our story. This meeting was set up by six men: Charles Heisch, A. H. Allen, G. W. Wigner, August Dupré, Theophilus Redwood and Thomas Stevenson. They had invited 77 men, who held 110 appointments between them, and 57 replied, but only 25 were able to attend.

Dr. Redwood (whose portrait is on the S.A.C. President's badge) took the chair; he was Public Analyst for Middlesex and Professor of Chemistry to the Pharmaceutical Society. Members were almost evenly divided between London and other parts of England:—Bucks., Bradford, Cheltenham, Devon, Huddersfield, Ipswich, Leeds, Manchester, Salford, Sheffield, Staffs. and Wolverhampton. It was resolved that an Association of Public Analysts be formed 'for the purposes of mutual assistance and co-operation', and it was meant to be much more practical than academic. Two other objectives of the newly-formed body may sound a little odd at first. They were:—

(*a*) to refute unjust imputations, and

(*b*) to repudiate proposed measures of interference with our professional position.

Both of these arose from the report of the Select Parliamentary Committee set up to study the workings of the 1872 Act. One witness had stated that 'there are not a dozen competent analysts in the Kingdom', and the Committee had proposed that tests of competency should be imposed on all Public Analysts, with 'Somerset House' as the official arbiter for all disputes arising from reports. At that time 'Somerset House' meant the Inland Revenue Laboratory and there was considerable resentment at the suggestion.

They finally decided to call themselves the *Society* of Public Analysts (hereinafter known as the S.P.A.) and, once off the ground, the group sorted out its main tasks, as follows—

1. To define 'adulteration',
2. To agree on food standards,
3. To consider the 1875 Act, then imminent,
4. To educate members in the legal side of their work,
5. To establish the normal composition of foods,
6. To develop agreed methods of analysis.

After great discussion, the members decided on a long, detailed definition of the word 'adulteration', upon which previous Acts had foundered. It starts:—
'An article shall be deemed to be Adulterated in the case of a Food or Drink

(1) if it contains any ingredient which may render such article injurious to the health of the consumer,
(2) if it contains any substance that sensibly increases its weight, bulk or strength, unless the presence of such substance is due to circumstances necessarily appertaining to its collection or manufacture, or be necessary for its preservation, or be acknowledged at the time of sale,
(3) if any important constituent has been wholly or in part abstracted, without acknowledgement being made at the time of sale,
(4) if it be a colourable imitation of, or be sold under the name of another article.'

There were similar provisions for drugs and a few examples of compositional limits proposed for milk, butter, tea, cocoa and vinegar. Milk minima, for instance, were 9·0 per cent. solids-not-fat and 2·5 per cent. fat, but these were later found to be based on an erroneous method of fat determination, which gave results about 0·5 per cent. lower than the true figure.

After all these efforts, it was ironic that the word 'adulteration' was dropped from the 1875 Act, which was entitled the *Sale* of Food and Drugs Act. There was a strong suspicion that the definition of adulteration had been quite beyond the powers of the Parliamentary draughtsman. Nevertheless, the work was not wasted, for it established principles still in use today.

Once associated, our forerunners moved fast. At an S.P.A. meeting a century ago, in May 1875, the subjects were—

Melting Point of Fats (Tripe, Angell and Heisch),
Minimum Solids in Milk (Campbell-Brown, of Liverpool),
Milk Ash (Wanklyn, P.A. for Bucks.),
Tartaric and Citric Acids (Allen of Sheffield) and, most interestingly,
Relation of Public Analysts to their Local Authorities (Wigner), which also covered the division and sealing of samples and forms of report.

These papers and the S.P.A. Proceedings were at first published in 'Chemical News', owned by Mr (later Sir) William Crookes, but 'a little friction arose' and the Council resolved that the Society should publish its own Journal, which would be free to members and cost 3/6 a year to non-members, or 6d a single

copy. The first issue came out in March 1876 and was entitled simply 'The Analyst'. At those prices, it is perhaps not surprising that the loss in the first year was £80, and the publication was taken over for a time by two registered proprietors. The Council of the S.P.A. was rather unhappy about losing a measure of control, but eventually (in 1891) total control was regained. One of the first papers, incidentally, was by R. R. Tatlock, on the danger of metals in foods prepared in enamelled cooking-vessels.

In 1877, the S.P.A. President was Dr Dupré, who was the official Analyst of Explosives to the Home Office. During the Sinn Fein bombing outrages of 1882 he examined many so-called 'infernal machines', especially those of the notorious Whitehead, of Birmingham, who was eventually arrested in the middle of his unstable manufacturing process, with 170 lb. of nitroglycerin in the next room.

We are due for yet another Centenary in 1977, for the Institute of Chemistry (not 'Royal' until later) was formed in 1877. The oldest established body in our science is the Chemical Society, founded in 1841, but the terms of its Charter were then thought to prevent it from acting as the organising and qualifying body for chemists which began to be needed at this time.

It was proposed to call this new association the 'Institute of Professional Chemists' but Government did not approve of this title. The Privy Council seemed to regard professional chemists merely as failed pharmacists and the Board of Trade hardly recognised the existence of chemists at all; they insisted that it be called the 'Institute of Chemistry'.

'It appears to be insufficiently recognised that, in some of the large provincial cities and towns, the local Public Analyst, in addition to this public duties, undertakes such matters as water analysis and toxic investigations, while his Laboratory is the natural centre of commercial analytical work for local industries'. This quotation comes from 'The Analyst' of 1877, and shows that the Public Analysts of those days were able and willing, as they are today, to exercise their analytical skills in many fields other than food analysis.

There is evidence of this in the type of papers then being read to the S.P.A., which show a wide variety of subjects. We have—

1. 'Copper as a normal constituent of Plant and Animal Tissue' by Dupré,
2. 'The Poison of the Cobra' and 'The Albuminoids of Cheese' by Winter-Blyth,
3. 'Composition of Commercial White Lead' by Wigner and Harland,
4. 'Elimination of Potassium Chlorate in Urine' by Hehner,
5. 'Assay of Carbolic Powders' by Allen,
6. 'Nitrogen Compounds of Cereals and Coke' by Wigner,
7. 'The Analysis of Cleopatra's Needle' by Wigner. (This Egyptian monolith had been purchased for £10,000 by Sir Erasmus Wilson, a Victorian dermatologist, who gave it to the nation. It had been proposed to treat it with water-glass before it was erected on the Embankment, where it still stands, but Wigner advised wax.)

You may think that there are few foodstuffs in that list, but the cruder forms of food adulteration were still very much present. In the second half of Dyer's book, Ainsworth Mitchell (then Editor of 'The Analyst') records many of the more interesting cases of those first five decades.

In 1878, rice was adulterated with added wax, muffins with plaster of Paris, marmalade with saltpetre and skim milk with chalk. There were prosecutions for tea coloured with Prussian blue and 'filled' with sand and china clay. One tea contained 6 per cent. of small stones carefully wrapped in leaves—the sort of craftsmanship one does not get nowadays. In this year, also, a publican at Stafford was fined £50 for selling beer containing 'grains of paradise' and a retailer at Manchester prosecuted for sweets coloured with 0·4 grains per ounce of lead chromate.

In 'The Analyst' of 1881, C. T. Kingzett stated that he had 'frequently stressed, orally and in print, the desirability of the official extension of the duties of the Public Analysts in many directions, with some additional office, such as Municipal Chemist, to advise on gas and water supplies, sewage treatment and disposal, disinfection, dust-laying and road-surfaces, etc. The Public Analyst should be in the wider field of public utility'. A few years later, Allen was to suggest that we examined such articles as oil and petrol, yeast, disinfectants, carpets, wallpaper and rat-killers, under the control of a Sanitary Analysis Bill.

G. W. Wigner was President of the S.P.A. in 1883, and died in office at the age of 42. By 1884, Dr. Augustus Voelcker was suggesting that the law against adulteration should be extended to cattle-foods, and 8 years later a Bill was introduced in Parliament to prevent "Adulteration of Manures and Feeds", which led to the first Fertilisers and Feeding Stuffs Act, of 1893. In 1885, a chemist called Reichert put forward a distillation method for the examination of butter-fat, which was 'very coldly received'.

The President at this time was Alfred Hill of Birmingham and he was followed by A. H. Allen, already mentioned, who was to become well-known for the many volumes of his 'Organic Analysis', By 1889, it was Matthew Algernon Adams, of Kent, who 'enjoyed an extensive practice as a specialist in ophthalmic surgery, but loved his Laboratory better than his consulting room'—hence the Adams coil, long used in milk analysis.

There has not, as yet, been any real mention of drugs or medicines. This was an age when the remedy for a headache was not a couple of aspirins, but could be a mixture of '2 drams of Rochelle salt, 1 dram infusion of senna, 1 tsp. tincture of cardamom; to be taken in a wineglassful of Eau de Cologne'. It was also the hey-day of patent medicines, such as—

Morrison's Universal Pills—said to prevent insanity and old age
Battley's Sedative —a strong tincture of opium
Norris's Drops —tartar emetic in wine
Parr's Life Pills —stated to cure both constipation and diarrhoea

and Holloway's Famous Ointment, which takes us on to advertising. Now conditioned by ethical codes of practice, we might have been a little surprised

by the popular press of the day. One pictorial advertisement showed good Queen Victoria enjoying a steaming cup of Cadbury's cocoa and another had a heading 'Two Infallible Products' over pictures of the Pope and a bottle of Bovril. However, even they drew the line somewhere and Thomas Holloway was thwarted in his intention to paint a gigantic advertisement for his Ointment on the White Cliffs of Dover—presumably for the benefit of passing mariners. The real maestro in this respect was the original Beecham, who started as a pedlar of herbal remedies and rose to fame and fortune on the Pills, mainly by outrageous advertising. The story goes that in 1889 the Vicar of South Shields appealed to him for some new hymn books. Beecham agreed, provided that a small advertisement could be inserted. When the books came the Vicar scanned all the end-pages anxiously, but could find no such insertion. All was revealed on Christmas Day, however, when the choir burst forth—

Hark! the herald angels sing,
Beecham's Pills are just the thing,
For easing pain and mothers mild,
Two for adult, one for child!

Talking about mother's mild, 'The Analyst' carried some advertising matter at this time, including one for 'Invalid Stout', at 12/6 for 9 gallons, 'suitable for nursing mothers'. All the products were severely scrutinised, of course, which may account for the cautious note in describing Sanitas as 'the latest development in science for the time being'.

Public Analysts were checking the accuracy of prescriptions as far back as 1876. Some prosecutions concerned Castor Oil Pills with no castor oil (plea of 'trade practice', nominal fine), Paregoric with no opium (alleged to have been sold as 'Paregoric Substitute', case dismissed) and Milk of Sulphur containing calcium sulphate (defence successfully cited the B.P. 1724). A number of cases were actually won.

In the year 1890 there was a strong move to amalgamate the S.P.A. with the Institute of Chemistry, which was then about four times as large as the S.P.A., the membership figures being roughly 800 and 200. Those in favour said that all the original objectives of the Society had now been achieved and all the work now fell within the functions of the Institute; they wanted to have a Standing Committee to represent chemists of all Government Departments and Local Authorites. There was some opposition from both sides, however, and the proposal failed. Dr Dyer notes that 'history has proved the wisdom of a separate but inter-dependent existence'.

Three years later, the Institute set up a qualifying examination system, and, by 1896 there was the 'Branch E' examination in the 'Analysis of Waters, Food and Drugs . . .'. Later, Therapeutics, Pharmacology and Microscopy were added, and the examination was recognised as a qualification for Public Analysts by the Local Government Board in 1900.

One has to record that there had been some bitter passages and hard feelings during these years between the S.P.A. and the aforementioned 'Somerset House',

but a new era dawned in 1894. In that year the Laboratory of the Government Chemist was formed, by combining the Inland Revenue and Customs Laboratories. Dr Thorpe was appointed head when the Laboratory was established in Clement's Inn Passage, and Dr Bell, the former director of the 'Somerset House' Laboratory, was invited to the first formal Annual Dinner of the Society. He made an historic speech, foretelling much better and more harmonious relations, which is happily true today.

Before this formal affair, Dr Dyer records that 'We had always thoroughly relaxed ourselves in Bohemian joviality', with no serious speeches but much music. His recollections of the Society's monthly meetings also have their social side. From the start, when they left the Chemical Society's Rooms, a number of members would 'repair to a secluded corner of the Criterion Bar to sit and smoke and drink lager beer and talk, usually until midnight. This afforded opportunity for the easy exchange of personal experiences and the discussion of professional matters, far more intimately than would have been possible in open meeting'.

Obviously there were no ladies present, since there were no women members until the present century, in spite of a notable champion called Mrs Dr Hoggan. She was asking, as early as 1879, 'There are female pharmacists and why not female analysts?'. The Editor of 'The Analyst', quoting this, must have thought it quite safe to reply 'We would welcome to our ranks any lady who had the courage to brave several years' training in a Laboratory'.

This story ends just when the Annual Dinner had to be cancelled because of the death of Queen Victoria, although one small trip into the twentieth century is irresistible. It was in 1909 that the President and Mr Chapman were sent to Paris, to attend an International Congress. They returned full of British indignation to report that 'they could not acquiesce in the formulation of any international standards'. All the French suggestions had been accepted; the French considered that borates were indispensable in butter, for instance, and Congress was to raise the water content to 18 per cent. Our men said it was 'at least questionable whether such standards would become legally binding here'.

I have to conclude the S.P.A. story before we added all those Other Analytical Chemists in 1906 and long before the tail wagged the original dog so hard that the dog fell off, in 1953, and became the Association of Public Analysts. With all the differences from those early days of the first Public Analysts, it is nevertheless possible to see, with respect and affection, that they were really *very* like ourselves. In 1975, I am glad to say 'All our own work and 101 years of it!'

An Automated Digestion Procedure for Food Samples

by H. EGAN

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This paper was presented to the Annual Conference of the Association of Public Analysts at Taunton, Somerset, on 3 May 1975.

I would like to say something in the context of trace metal analysis about a practical problem which has not received a great deal of attention, namely the automation of sample preparation.

Analysis and Specificity

In a recent lecture to the Society of Chemical Industry[1] on 'Chemical Analysis and Environmental Quality' I set out to describe the role of the analyst in the examination of the environment. Pollution and contamination are matters of opinion, although occasionally the law takes away our discretion in the matter, and *says* for example that, subject to certain defences, it is an offence for most foods to contain more than 2 parts per million of lead. A pollutant can be defined as something which is present in the wrong place at the wrong time in the wrong amount. Analytical chemistry is perhaps the most important tool, which, when coupled with toxicology, gives us the main scientific approach to safety aspects of the environment, and indeed to amenity aspects also. Environment and health are closely related concepts; environmental quality and environmental health are cost-benefit situations.

Most environmental situations which are encountered by the analyst involve trace analysis. The simplest case, perhaps, is fresh water analysis. Although the levels of contaminants may be minute in water, the clean-up problems are often minimal. This is in contrast with the examination of food or other biological material, where the initial removal, by solvent extraction, acid digestion etc., of the great bulk of the organic material, must be accomplished without loss of the trace of contaminant which is to be measured. Indeed, the objective here is to finish up with a simple, clean, aqueous solution of the contaminant, which can then be examined by an appropriate end-method of analysis.

With water, this often is, in fact, the starting point of the analysis. This is true for trace metals such as copper, lead and zinc, which can be measured spectrophotometrically on the water sample itself or after only a simple concentration stage. The advantages and limitations of modern analytical techniques for trace metal pollutants—atomic absorption, atomic fluorescence, atomic emission and mass spectrometry, neutron activation analysis, X-ray fluorescence and anodic stripping voltammetry—have recently been reviewed by Coleman[2]. These methods enable the analyst to deal, in water, with quantities down to about one nanogramme per millilitre (in some cases even less) and of course they do not relate exclusively to the aquatic environment. Selective

ion electrodes are also well established for investigating many inorganic pollutants. A large range is available, the lower limits of detection in water being normally of the order of 0·1 to 0·01 mg/litre, but these electrodes are subject to interference by other ions (the nitrate electrode is a good example) and electrodes should be regarded as 'ion selective' rather than 'ion specific'. The possibility of extending their use to organic compounds by means of immobilised enzyme systems has also been discussed. Such systems cannot, however, be more selective than the enzyme systems they involve.

Specificity is not always a dominant consideration for the trace analyst, but it must be taken into account. This is particularly true for organic pollutants. Where the nature of the contaminant is not known in advance, the specificity of methods of examination cannot be taken for granted. A classic example was the occasion when aldrin appeared to be present in soil samples which had been kept in sealed jars since before 1940, i.e., before the advent of chlorinated insecticides[3]. Gas chromatographic responses, which 23 years later could be properly interpreted, thus subsequently were regarded as being due to naturally occurring interferences.

The situation has been dramatically if somewhat theoretically described by Widmark, in what he has called the *tracer cosmos*[4]. By this he means the vast increase in the number of different trace compounds which become theoretically possible as one passes into the higher realms of analytical sensitivity. As an example, consider a substrate which is 99 per cent. pure. In the simplest case, the impurity is 1 per cent. of one other substance. Alternatively, however, there could be 0·1 per cent. each of 10 different impurities, or 10,000 different compounds each at the 1 part per million level. A number of analyses today are of interest at the parts per thousand million level, or even lower. If all the impurities were present at this kind of level, one might theoretically have to contend with a million or more different trace compounds. The *cosmos* is represented by the integration of all these levels, and they might be represented simultaneously in a 'real life' situation. The disposal of toxic wastes to land —a subject dealt with by the Royal Commission on the Environment in its Second Report[5]—perhaps conjures up an extreme example of the possibilities.

Man is, of course, exposed to the atmospheric environment, to the aquatic environment and the terrestrial environment—less to the second than the first. As far as trace metals and pesticides are concerned, for the greater part of the population, the main exposure is by way of the terrestrial environment—particularly food. Thus, as regards the intake of lead, it has been estimated that some 85 per cent. of the total intake is by way of food, with perhaps only 10 per cent. from water and 5 per cent. from the air[6]. Indeed, Galley has shown from pharmacodynamic considerations and from the minute levels of organochlorine pesticide compounds present in the atmosphere, that man exhales more dieldrin, derived from the trace amounts in his body fat, than he inhales[7]! As far as trace pesticide residue analyses are concerned, the developments in chromatographic techniques (initially paper chromatography, then gas-liquid chromatography and more recently high performance liquid

chromatography) have given the analyst an order of specificity and sensitivity which a generation ago was undreamed of.

The possibilities of interference in these analytical methods are as important as with other methods. Sample extraction and clean-up (often themselves chromatographic in character) must be properly conducted, and due regard must be paid to the need for confirmatory procedures where the identity of a trace contaminant is unknown. Similarly with trace metals; atomic absorption spectroscopy (coupled where necessary with chelation techniques and solvent extraction methods of separation and concentration) has provided the analyst with a powerfully discriminating and sensitive tool. Atomic spectroscopy methods are applicable to some sixty different elements. The limits of detection vary from about 10 microgrammes to about one-tenth of a nanogramme per litre (depending on the element), with flame or high frequency plasma emission sources being somewhat more sensitive than atomic or fluorescence flame sources[2]. These methods can be highly specific, but due regard must be paid to matrix and other possible interference effects. Standard reference materials are highly desirable but are not always available.

In these days of intense interest in the environment, more and more detailed information is sought on the occurrence and distribution of contaminants, and there is one constraint which, even if financial resources were not a consideration, would limit the analyst's practical ability to undertake comprehensive environmental surveys. That is the *preparation* of the sample, for its eventual examination by chromatography, spectrometry, or whatever other end-method of analysis is to be used.

Automated Analysis

There is already a wide range of automatic analytical equipment, much of it available in the pioneering field of clinical chemistry. Many instrumental systems are discrete in character, in that they relate to individual, unit analytical processes. There is, for example, a large range of commercial sampling equipment and colorimetric analysis equipment, with or without sample separation processing facilities. There are a few continuous analyser systems, the main one being the Technicon AutoAnalyzer. Details of all of these have been published by Foreman and Stockwell[8].

A great deal of effort has recently been devoted to the development of instrumental methods for the direct examination of a wide variety of samples. This has not been the situation, however, with the preparatory aspects of the analysis of complex samples of biological origin, such as food or body tissues. Techniques such as X-ray fluorescence spectrometry can look directly at such samples after they have been freeze-dried, but they have limited sensitivity and are unsuitable for trace analyses. Activation analysis has been used in this area but it cannot cover the full range of elements required, without recourse to chemical separations. Most other techniques require some sample manipulation, usually wet or dry oxidation.

In only a few areas do the practical aspects of the work of the Laboratory of the Government Chemist call for the examination of very large numbers of routine samples. Where these do occur, and in certain other cases where a heavy staffing involvement can thereby be reduced, a study has been made of the automation of the analytical methods concerned. Thus the Laboratory has studied the automatic dosing and monitoring of fluoride in water supplies, and the automation of methods for the extraction, identification and estimation of quinazarin[9] and furfural[10] which are the revenue markers in hydrocarbon fuel oils. Analytical processes based on the estimation of alcohol and reducing sugars[11–13] can be used to assess the original gravity of beers. Specificity does not feature strongly in the latter system, which is used mainly as a screening test. Thus the process for the estimation of original gravity is used only to select the occasional sample, perhaps one in 20, which needs to be examined by the full distillation and gravity procedures. The starting point for the automated methods may be commercial equipment, where suitable items are available; but these are usually modified, often profoundly, and occasionally in co-operation with the manufacturers. Usually, however, it has been necessary to build new, special units.

Because of the practical importance of the preparatory stages in routine survey work, the Laboratory of the Government Chemist has in recent years made a special study of how the 'real life' problem of preparation of samples for food analysis might be automated.

The automation of food sample preparation for the determination of toxic substances (which should be suitable also for pesticide residues analysis) by means of grinding, solvent extraction and centrifugal separation, has recently been described by Coulson[14] and is based on the centrifugal separation of Vallis[15] and the principles developed by Anderson[16].

The automatic preparation and wet digestion of biological samples has been neglected, for the very simple reason that it is extremely difficult. It is necessary to manipulate corrosive reagents at relatively high temperatures, and to transfer awkward samples such as food slurries. However, as a result of several years' work, an automatic digestion system has been developed, which combines the main advantages of the Technicon continuous approach, together with some aspects of the system used by other manufacturers. It is currently being tested for a wide range of food samples and is being compared with manual methods of digestion both for reliability and accuracy. The complete system for the digestion of foods for trace metals examination has due regard for specificity, and it comprises a sample introduction unit, a continuous digester, a digest collection and neutralisation unit, a chelation extraction and phase separation unit, and a chelated extract collection unit. It is illustrated diagrammatically in Fig. 1.

The sequence of operations is as follows. A 5–10 grammes sample is weighed into a plastic bag, and a measured amount of 50 per cent. of sulphuric acid is added. The mixture is heated to 60°C in an oven and homogenised in a 'stomacher'[17]. The mixture is then weighed, and a weighed sub-sample of

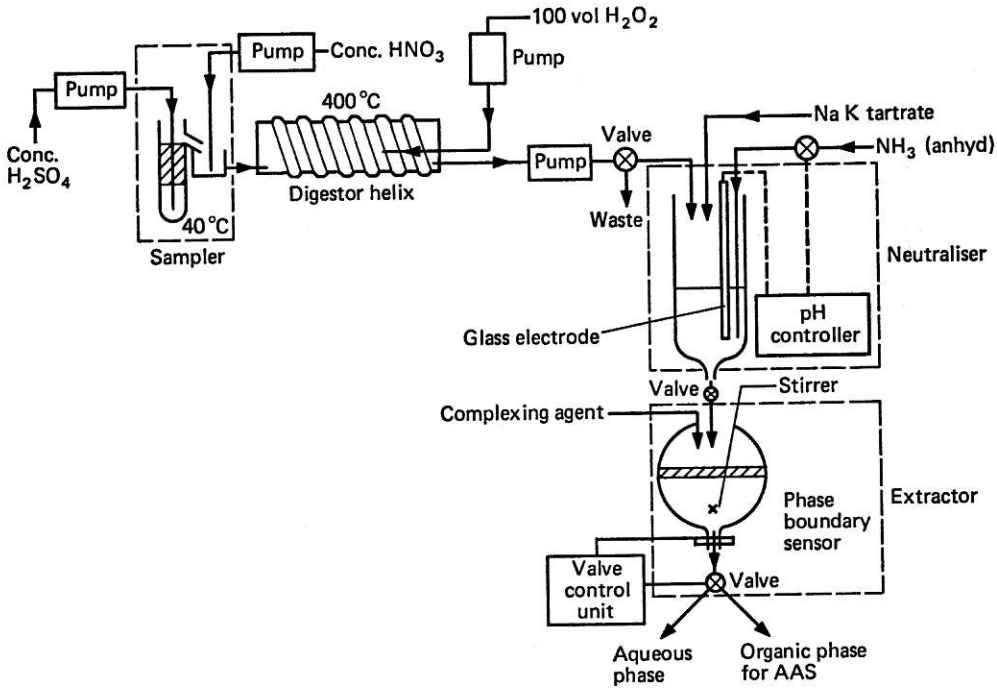

FIG. 1. Automatic Digester System: Flow diagram.

slurry is poured into a glass sampler tube and placed on a turn-table. The processes which follow are completely automatic. A platinum-iridium probe dips into the sample and pumps concentrated sulphuric acid into the bottom of the sampler tube, thus displacing the sample through a side-arm into a rotating helical digester, together with a stream of concentrated nitric acid. Hydrogen peroxide may also be added about half way through the digest process. The sample is digested in the modified Technicon digester, and the strong acid digest resulting from this process is pumped into a water-cooled neutraliser vessel where a glass electrode is used to control a flow of neutralising ammonia gas, in order to bring the final digest to a predetermined pH. The digest is then transferred to an extractor vessel, where a chelating agent is added, and the metals are extracted into a suitable organic solvent. The vessel is emptied through a valve which is controlled by a phase boundary detector so that the aqueous phase goes to waste and the organic phase is retained for analysis, by atomic absorption spectrometry for example.

The modification of the Technicon digester unit, which was necessary to improve the temperature control, has been described by Jackson, Morley and Porter[18], and the phase separation unit, which incorporates a sensor based on change in refractive index, has been described by Porter, Jackson and Bunting[19]. Although the system sounds very simple, a number of important problems had to be overcome during its development. Thus, it was necessary not only

to modify the normal temperature control system of the Technicon digester to give stable high temperatures all the way along the helix but it was also necessary to retain the samples in the turn-table at about 60°C to prevent a separation of fats from the slurry. Careful attention had to be given to the materials of construction. It is necessary to ensure that these can withstand the corrosive action of strong acid and that they do not introduce unacceptably high blanks into the system. Some foods do not break down adequately in the sulphuric acid/nitric acid medium. It is for these that hydrogen peroxide is added about half-way along the digester, thus enabling the subsequent chelation stage for the separation of trace elements from the sample to be achieved more efficiently.

A wide variety of chelating agents was studied in a search for one which extracted the maximum number of elements with virtually 100 per cent. recovery. The most satisfactory was diethylammonium diethylcarbodithioate (DDDC), at pH 3, with heptan-2-one as the extracting solvent. Good recoveries of iron, cobalt, nickel, copper, zinc, cadmium and lead are possible from foods as different as flour and corned beef. The normal ammonium pyrrolidine dithiocarbamate (APDC)[20] chelation system was not found to be suitable for automatic analysis, since the excess nitric acid used in the digestion decomposes the APDC and it is not convenient to boil it off. Moreover, the APDC-metal chelates are less stable. This is a matter of little importance when spectrophotometric measurements are made immediately after extraction, but with an automatic digestion system working at three samples per hour, an expensive on-line measuring system may not be justified if the metal-chelate extracts can instead be collected, stored (perhaps overnight), and examined as a continuous batch. In the search for alternative chelating agents, oxine[21] gave good results, but DDDC was even better. Heptan-2-one is used as an extraction solvent in preference to 4-methylpentan-2-one (MIBK), since the former gives a larger difference in refractive index, and the phase boundary detection system[22] for separation is based on this.

The investigation of a wider range of foods and elements is under consideration, using a sequential chelation system. The process is described in the 1974 Report of the Government Chemist[23].

The digestion unit system which has been described can process about 20 samples per day, and by the combination of units it should become possible to examine a much larger number of samples than has been possible hitherto. This should provide an overall improvement in 'job satisfaction' for the laboratory staff concerned. This also means that it should become possible to increase the number of samples examined and to design trace metal studies in a way which will give ample coverage without their being restricted by staff limitations.

I am grateful to Dr Peter B. Stockwell for discussions on this paper. He and members of his team in the Laboratory have carried out much of the original work upon which the foregoing description is based.

References

1. Egan, H., *Chem. & Ind.*, 1975, 814.
2. Coleman, R. F., *Anal. Chem.*, 1974, **46**, 989A–996A.
3. Bowman, M. C., Young, H. C., and Barthel, W. G., *J. Econ. Entomol.*, 1965, **58**, 896.
4. Widmark, G., *Adv. Chem. Series No.* 104, American Chemical Society, Washington, D.C., 1973.
5. 'Royal Commission on Environmental Pollution, Second Report.' H.M.S.O., London, 1972
6. Patterson, C. C. *Arch. Environ. Health*, 1965, **11**, 344.
7. Galley, R. A. E., *Chem. & Ind.*, 1974, 179.
8. Foreman, J. K. and Stockwell, P. B., 'Automated Chemical Analysis' Wiley, London, 1974.
9. Tucker, K. B. E., Sawyer, R., and Stockwell, P. B., *Analyst*, 1970, **95**, 730.
10. Lidzey, R. G., and Stockwell, P. B., *Analyst*, 1974, **99**, 749.
11. Sawyer, R., and Dixon, E. J., *Analyst*, 1968, **93**, 669; 680.
12. Sawyer, R., Dixon, E. J., and Johnston, E., *Analyst*, 1969, **94**, 1010.
13. Sawyer, R., Dixon, E. J., Lidzey, R. G., and Stockwell, P. B., *Analyst*, 1970, **95**, 957.
14. Coulson, D. M., *J. Assoc. Offic. Anal. Chem.*, 1975, **58**, 174.
15. Vallis, D. G., U.K. Patent Application 14964/67.
16. Anderson, N. G., *Amer. J. Clin. Path.*, 1970, **53**, 778.
17. Sharpe, A. N. and Jackson, A. K., *Appl. Microbiol.*, 1972, **124**, 175.
18. Jackson, C. J., Morley, F., and Porter, D. G., *Lab. Practice*, 1975, **24**, 23.
19. Porter, D. G., Jackson, C. J., and Bunting, W., *Lab. Practice*, 1974, **23**, 111.
20. Mellssa, H., and Gomiscek, S., *Z. Analyt. Chem.*, 1959, **169**, 402.
21. Bode, H., and Neumann, F., *Z. Analyt. Chem.*, 1960, **172**, 1.
22. Stockwell, P. B., *Proc. Soc. Anal. Chem.*, 1975, **12**, 273.
23. 'Report of the Government Chemist, 1974.' H.M.S.O., London, 1975.

The Effect of Somatic Cells on the Reference Method for the Determination of Dirt in Milk

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The Society of Public Analysts' (S.P.A.) analytical reference method for the determination of dirt in milk has been investigated. Quite apart from the recognised variation in the results given by this method, it has been found that it measures not only extraneous dirt but also somatic cells, which consist of leucocytes and epithelial cells. Leucocytes are associated chiefly with udder infection and epithelial cells with physiological change. An earlier technique published by Tankard for the separation of dirt and somatic cells has also been investigated.

Samples of milk having an average somatic cell count of 496,750 were analysed after filtration to remove extraneous matter. The S.P.A. reference method gave an average value of 5 parts by volume of moist dirt in 100,000 parts by volume of milk, but this figure was reduced to less than 0·5 parts per 100,000 after removal of somatic cells by the Tankard technique.

In 1937 a Sub-Committee of the Analytical Methods Committee of the Society of Public Analysts (S.P.A.) published a reference method and made recommendations in relation to limits for dirt in milk[1]. The S.P.A. Council endorsed the limits laid down in the following recommendation of the Sub-Committee: 'The Sub-Committee considers that clean milk, when examined by their recommended method, contains less than 1 part by volume of moist dirt in 100,000 parts by volume of the milk, and that a limit of 2 parts of moist dirt is the maximum which can be conceded, but in stating this opinion they must not be understood to mean that milk containing less than 2 parts of dirt is necessarily a clean milk. In dealing with milk for the purposes of the Food and Drugs (Adulteration) Act, 1928, the Committee suggests, having regard to the degree of variation in the results obtained in their joint experiments, that it would not be desirable to recommend legal action in the case of an isolated sample of dirty milk unless the amount of moist sediment, determined by the recommended method, exceeds 3 parts by volume in 100,000 parts of the sample'.

The S.P.A. reference method involves the precipitation of dirt, from about 200 ml of formalin preserved milk, by gravity over a 5-day period followed by separation of the sedimented dirt by centrifuging. The moist sediment so obtained is collected in a graduated tube about 1 mm in diameter in which it is washed three times with water to remove milk solids. It is then treated at least twice, for half hour periods, with cold normal ammonium hydroxide solution followed by normal hydrochloric acid with water washings in between. The dirt column height is read when the tube is finally taken from the centrifuge,

and when related to the volume of milk taken for test gives a measure of the moist dirt in parts per million by volume.

This reference analytical method and recommended limits have been increasingly applied by Public Analysts in recent years. In one case a Public Analyst, using the S.P.A. method for prosecution for sediment in milk, reported 'the sediment consisted mainly of leucocyte cells, with a little vegetable and mineral debris'. The authors are of the opinion that the S.P.A. method was not intended to measure somatic cells (see Section 3 of this report). Accordingly the relationship between the cell count and the results obtained with the S.P.A. method has been investigated.

Tankard, who chaired the S.P.A. Sub-Committee, previously published a method[2] for the treatment of the sediment in centrifuge tubes to separate somatic cells and insoluble milk solids or curdy material from 'dirt', using saturated brine of specific gravity 1·18 to 1·20 at 15·5°C. The cells and curdy material float in the brine and are decanted from the dirt which is deposited at the bottom of the sediment tube. The Tankard method[2] has therefore been used in conjunction wth the S.P.A. method to determine the effect of cells on the 'dirt' content of milk samples of known somatic cell count.

Millard and Cheeseman[6], describing an improved centrifugal method for the separation of dirt from milk recommended the chemical solubilisation of insoluble calcium caseinate (curdy material) with sodium dodecyl sulphate (0·001 per cent. w/v) and alkaline ethylene diamine tetra-acetic acid (0·1 per cent. w/v). They also used sodium hypochlorite (0·1 per cent. w/v) for the solubilisation of 'cellular debris originating from the mammary tissue'. These methods of removing curdy material and somatic cells have not been used in this work but would seem to offer an alternative to the Tankard technique and are being investigated.

Substances to be determined by the SPA method

The S.P.A. Sub-Committee's publication[1], Section B, defined the substances to be determined by the reference method as follows:

> 'The Sub-Committee recommends that the designation of the substances to be determined by the sedimentation method should be simply "dirt". By dirt is meant all matters insoluble in, and foreign to, milk as it leaves the cow's udder. It may be added that by the recommended method of procedure for determining dirt in milk the bulk of micro-organisms will be removed from the sediment'.

In this definition of 'dirt' there is no specific mention of somatic cells. Somatic cells comprise leucocyte or white blood cells and epithelial cells. The former are associated mainly with udder infection and the latter with physiological change associated with 'wear and tear' of the udder tissue. They are naturally present to varying degrees in all milk as it leaves the cow's udder. The Sub-Committee's definition of 'dirt' being 'foreign to milk as it leaves the cow's udder' must, therefore, exclude somatic cells from the designation of 'dirt'.

This interpretation is supported by earlier publications by Tankard in which he states:

(*a*) 'If after this treatment with alkali the deposit still consists in part of the cells from the milk, showing as a white layer on top of the extraneous sediment, the main part of the liquid is poured out and the treatment with alkali repeated until the cells etc. have been dissolved and washed away'[3] and

(*b*) 'The treatment of the separated sediment in the small centrifuge tube with dilute sodium carbonate solution is fairly satisfactory in most cases; but frequently one examines samples of milk which have a high cell content and occasionally others which give a small quantity of curdy deposit, and these deposits are sometimes difficult to separate completely from true extraneous sediment. The new method to be described appears to be uniformly successful in all cases in giving a complete separation of the white cellular deposit from the extraneous matter, and allows the further examination of the sediment after measurement'[2].

Tankard goes on to describe his method for the separation of cellular material from extraneous matter by flotation of cells etc. using saturated sodium chloride.

It should be noted that neither of the treatments described under (*a*) and (*b*) was adopted for the reference method.

Materials and methods

Samples were examined in two batches as follows:

(1) Seventeen samples of milk from individual cows were collected. Subsamples were tested in duplicate for cell count using the Coulter Electronic Counting method and singly for 'sediment' using the S.P.A. reference method.

(2) Eight further individual-cow samples were collected. Four were tested in duplicate for cell count using the Coulter Electronic Counting method, and in duplicate for sediment, with and without the addition of 2 mg per litre of dry 'standard dirt'[4], using the reference S.P.A. method. The remaining four samples were filtered through a Whatman No. 114 filter-paper to remove any dirt before being tested in duplicate for cell count using the Coulter Electronic Counting method and in duplicate for sediment, with and without the addition of 2 mg per litre of dry 'standard dirt', using the reference S.P.A. method.

In testing for extraneous matter the S.P.A. method[1] was strictly adhered to and the volume of the sediment following acid and alkaline washings was recorded. The sediment was then treated with saturated sodium chloride using the Tankard technique in order to 'float off' undissolved cells and curdy material.

Calculation of moist volume occupied by somatic cells

Assuming formalised somatic cells to be spheres of minimum diameter 4·56 microns (the threshold taken for electronic counting), it is possible to estimate the theoretical minimum volume which would be occupied by cells deposited in the S.P.A. method. The calculated volume of a single cell of radius 2·28 microns, *i.e.* $2{\cdot}28 \times 10^{-4}$ centimetres, is 50×10^{-12} cubic centimetres. Assuming that there is no 'free space' between the compressible packed cells the minimum volume occupied by cells in milks of known cell counts has been calculated and recorded with results. For example, the minimum moist volume attributable to somatic cells in a milk having a cell count of 500,000 per ml is theoretically 25 parts per million, or 2·5 parts by volume per 100,000 parts by volume of milk.

Results

SOMATIC CELL CONTENT AND SEDIMENT CONTENT

Table I details the means of duplicate cell counts, the single reference S.P.A. and Tankard method results for 17 individual cow samples of a wide range of cell counts. In calculating the average results samples 15, 16 and 17 were excluded because they all showed abnormally high cell counts, unlikely to be encountered in bulked milk.

TABLE I

SOMATIC CELL COUNT AND SEDIMENT CONTENT OF MILK BEFORE AND AFTER REMOVAL OF SOMATIC CELLS

		Volume of moist sediment in milk *(parts per million)*			
				Attributable to somatic cells	
Sample	Mean cell count per ml	S.P.A. Official method	S.P.A. results after removal of cells by Tankard method	Removed by flotation (2–3)	Minimum calculated from cell count (1)
	(1)	(2)	(3)	(4)	(5)
1	211,000	20	8	12	11
2	206,000	60	38	22	11
3	236,000	15	5	10	12
4	225,000	10	5	5	12
5	260,000	43	30	13	13
6	292,000	23	5	18	15
7	347,000	30	10	20	18
8	458,000	30	5	25	24
9	484,000	35	25	10	24
10	491,000	13	5	8	25
11	517,000	28	13	15	26
12	583,000	25	10	15	29
13	636,000	30	20	10	32
14	1,095,000	155	23	132	95
Average	431,500	37	14	23	25
15	1,916,000	140	35	105	96
16	1,800,000	100	10	90	89
17	3,107,000	675	45	630	156

Table I shows that milks having an average cell count of 431,500 cells per ml when tested by the S.P.A. reference method had an average sediment content of 37 p.p.m. (column 2), whereas the sediment found after the Tankard saturated

sodium chloride separation method (column 3) was 14 p.p.m. The difference between the two is 23 p.p.m. which is in good agreement with the mean theoretical minimum cell content, 25 p.p.m., in view of the reported poor repeatability of the S.P.A. method[1,5,6] and the assumptions made in calculating the cell volume.

SOMATIC CELL COUNT AND SEDIMENT CONTENT OF MILK TO WHICH 'DIRT' WAS ADDED

Results in Table I do not indicate whether the Tankard method differentiates clearly between 'dirt' and cells, and further examinations were made on samples of known cell count to which 'standard dirt' had been added (Table II). The results are given in Table III for milk samples which were filtered to remove extraneous matter before standard dirt was added.

Tables II and III show that the S.P.A. determinations on unfiltered and filtered milk, with and without the addition of standard dirt, gave significantly higher results prior to the removal of cells by the Tankard method.

It is believed that in the case of the sample in Table II containing over 2 million cells per ml, the three sodium chloride washes did not remove all the cells. Apart from this sample, the samples to which standard dirt was not added contained very little residual dirt after the removal of cells. Those samples to which 2 mg/l of dry standard dirt were added had a moist sediment value in the region of 20 p.p.m. after removal of cells and this is consistent

TABLE II

THE SOMATIC CELL COUNT AND SEDIMENT CONTENT OF MILK WITH AND WITHOUT ADDITIONS OF STANDARD DIRT

Mean cell count per ml	Dry** standard dirt added *mg/l*	Volume of moist sediment in milk *(parts per million)* S.P.A. Official method	S.P.A. results after removal of cells by Tankard method	Attributable to somatic cells: Removed by flotation (2–3)	Attributable to somatic cells: Minimum calculated from cell count (1)
(1)		(2)	(3)	(4)	(5)
241,000	Nil	18	3	15	12
	Nil	20	5	15	
	2	38	23	15	
	2	38	15	23	
492,000	Nil	10	3	7	25
	Nil	18	5	13	
	2	65	23	42	
	2	60	10	50	
990,000	Nil	53	5	48	50
	Nil	50	8	42	
	2	98	20	78	
	2	100	25	75	
2,391,000	Nil	546	28	518	120
	Nil	532	20	512	
	2	592	30	562	
	2	432	35	397	

** Standard dirt (B.S. 4938[4]) was added at a level of 2 mg per litre of milk which equates to 20 p.p.m. wet volume when measured by the S.P.A. method.[6]

TABLE III

THE SOMATIC CELL COUNT AND SEDIMENT CONTENT OF FILTERED MILK WITH AND WITHOUT ADDITIONS OF STANDARD DIRT

Mean cell count per ml	Dry** standard dirt added *mg/l*	Volume of moist sediment in milk *(parts per million)* S.P.A. Official method	S.P.A. results after removal of cells by Tankard method	Attributable to somatic cells: Removed by flotation (2–3)	Attributable to somatic cells: Minimum calculated from cell count (1)
(1)		(2)	(3)	(4)	(5)
361,000	Nil	20	5	15	18
	Nil	33	3	30	
	2	58	20	38	
	2	85*	20	65	
472,000	Nil	43	3	40	24
	Nil	75	5	70	
	2	80	20	60	
	2	88	20	68	
473,000	Nil	43	3	40	24
	Nil	23	5	18	
	2	63	20	43	
	2	66	20	46	
681,000	Nil	65*	5	60	34
	Nil	90*	8	82	
	2	110*	25	85	
	2	73*	23	50	

* Curdy precipitate of insoluble milk solids present even after alkaline and acid washing.
** Standard dirt (B.S. 4938[4]) was added at a level of 2 mg per litre of milk which equates to 20 p.p.m. wet volume when measured by the S.P.A. method[6].

with the tenfold relationship between dry standard dirt and moist sediment by volume[6]. In general, the S.P.A. results increase with cell count, whereas the results after removal of cells are consistent with the known amounts of extraneous matter in the sample. The samples in Table III which were filtered to remove extraneous matter and to which dirt was not added, *i.e.* the 'clean' samples had a mean cell count of 497,000 per ml, a mean S.P.A. value of 50 p.p.m. (*i.e.* 5 parts by volume of moist dirt in 100,000 parts by volume of milk) which was reduced to 5 p.p.m. after removal of cells by the Tankard method. Consequently the measured mean value for extraneous matter was only 0·5 parts by volume of moist sediment per 100,000 parts of milk.

EFFECTS OF THE ALKALINE AND ACID WASHES ON THE S.P.A. METHOD

In the S.P.A. method the sediment is collected in the 1 mm diameter tubes and washed twice with normal ammonium hydroxide solution, water, acid and water again. The mean volume of the sediment after these washings is recorded in Table IV.

TABLE IV

THE EFFECT OF THE ALKALINE WASH RECOMMENDED IN THE S.P.A. METHOD ON THE VOLUME OF SEDIMENT

Mean cell count/ml	Volume of sediment *(parts per million)* Before washing	After first ammonia wash	First acid wash	Second ammonia wash	Final
691,500	32	80	52	63	29

The above results are the average of sixteen determinations.

It is seen that the ammonia causes significant swelling of the deposit and this would undoubtedly result in incorrect high readings being obtained if intimate mixing of acid and swelled cells were not achieved. Washing is extremely difficult in the 1 mm diameter tube. Table IV also shows that the final average volume is not significantly different from the original sediment volume, indicating that cells and curdy material present have not been removed by this treatment.

EFFECT OF TREATMENT OF THE S.P.A. SEDIMENT WITH SATURATED BRINE

The effect of Tankard's sodium chloride flotation has been examined in order to ascertain the number of washes required.

TABLE V

THE EFFECT OF THE SATURATED SODIUM CHLORIDE WASH RECOMMENDED IN THE TANKARD METHOD ON THE VOLUME OF SEDIMENT

Volume of moist Sediment (*parts per million*)					
Before sodium chloride wash	After first wash	After second wash	After third wash	After fourth wash	Somatic cells etc. removed (by difference)
63	32	26	13	15	50

Table V shows that on a further sixteen samples of milk the Tankard flotation method produced a significant reduction in volume of sediment, and in this case at least three washes were required to remove the cells and curdy material.

Discussion

It is generally acknowledged that the results of the S.P.A. reference method for the determination of moist dirt tend to be variable,[1,5,6] so much so that 'it would not be desirable to recommend legal action in the case of an isolated sample of dirty milk unless the amount of sediment determined by the recommended method, exceeds 3 parts by volume in 100,000 parts of the sample'. It has perhaps not been fully appreciated that a milk which is free from foreign matter as it leaves the cow's udder can give a significant volume of moist sediment determined by the S.P.A. method. This is because of the presence of naturally occurring somatic cells, consisting of leucocytes associated mainly with udder infection and epithelial cells associated with 'wear and tear' of the udder due to physiological change, which are present in milk as it leaves the cow's udder.

It is desirable that a method of assessing sediment in milk should be developed which would distinguish quantitatively between somatic cells, present in milk as it is discharged from the cow's udder, and extraneous dirt entering the milk after it has left the udder.

Conclusions

1. The S.P.A. reference method for the determination of the moist volume of extraneous matter in milk measures somatic cells, which are present in cow's milk as it leaves the udder as well as extraneous dirt which enters the milk after it has left the udder.
2. The washing of the sediment with alkali and acid could, if subsequent washing is not thorough, give erroneously high results.
3. Somatic cells in milk may make a substantial contribution to the volume of moist sediment as determined by the S.P.A. method, and it is therefore desirable that a method be developed which distinguishes quantitatively between somatic cells and extraneous dirt.

Acknowledgments

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References

1. Society of Public Analysts, *Analyst*, 1937, **62**, 733.
2. Tankard, A. R., *Analyst*, 1923, **48**, 444.
3. Tankard, A. R., *British Food Journal*, 1922, **24**, 51.
4. 'Method for the rapid determination of sediment in milk by filtration', B.S. 4938, 1973, Appendix B. British Standards Institution, 2 Park Street, London W1A 2BS, 1973.
5. Harding and Hoyle, *Dairy Industries*, 1972, **37**, 79.
6. Millard and Cheeseman, *Journal of the Society of Dairy Technology*, Vol. 27, No. 2, April 1974.

A Note on the Calculation of the Lean Meat Content of Sausages

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A simplified formula is given to enable the lean meat content of sausage (lean meat free of visible fat, as defined in the Sausage and Other Meat Product Regulations, 1967) to be calculated from the defatted meat content calculated by the Stubbs and More[1] calculation using the traditional factors of 3·45 for pork and 3·55 for beef.

The assumptions by Pearson[2] that the allowable limit of fat in added lean meat (LM) is 10 per cent. and that 90 per cent. of fat is an average fat content for outside fatty material are used as a basis for the calculation. Although this assumption may be over-generous to the manufacturers, references to it occur in other formulae for calculating the lean meat content of sausages[3,4,5].

The original formula given by Pearson for pork sausages, where LM = Lean meat (per cent.), N_T = Total nitrogen in pork sausage (per cent.), C = Dry carbohydrate + cellulose (per cent.), F_{EXT} = Extracted fat (per cent.), was

$$\text{LM (per cent.)} = \frac{N_T - \left[\left(F_{EXT} - \frac{LM}{10}\right) \times \frac{0{\cdot}345}{90}\right] - 0{\cdot}02C}{3{\cdot}45} \times 100 \times \frac{100}{90},$$

which can be rearranged to give

$$LM = \frac{\left[N_T - 0{\cdot}02C\right] \times 100}{3{\cdot}45} \times \frac{100}{90} - \frac{\left(F_{EXT} - \frac{LM}{10}\right) \times \frac{0{\cdot}345}{90}}{3{\cdot}45} \times 100 \times \frac{100}{90}.$$

This can be split up in terms of defatted meat or traditional 'lean meat' (DM) normally calculated from the Stubbs and More correction:

$$LM = DM \times \frac{100}{90} - \frac{\left(F_{EXT} - \frac{LM}{10}\right) \times \frac{0{\cdot}345}{90}}{3{\cdot}45} \times 100 \times \frac{100}{90}$$

Further rearrangement gives

$$LM = \frac{DM \times 100}{90} - \frac{\left(F_{EXT} - \frac{LM}{10}\right)}{10} \times \frac{100}{90} \times \frac{100}{90} = \frac{9}{8}\,DM - \frac{F_{EXT}}{8},$$

$$\text{or } LM = DM + \frac{DM - F_{EXT}}{8}$$

$LM = \frac{100DM}{90} - \frac{(10F_{ext} - LM)}{81}$ $+90LM$

$= \frac{8100xDM - 900F_{ext} + 90LM}{90x81}$ $(90x81)LM = 8100DM - 900F_{ext}$.

$\therefore \div$ by 90, rearrange $\div$ 90.

It follows that the added fat, F_A, can be calculated from

$$F_A = \text{Total Meat} - \text{LM},$$

total 'meat' content being calculated by the classical calculation of Stubbs and More.

By applying the same assumption to beef, the same formula can be deduced.

Discussion

Out of 78 samples of pork sausage examined the average defatted meat content was found to be 39·5 per cent. and the average extracted fat content was found to be 29·0 per cent. Application of the above correction would bring the calculated lean meat to 40·8 per cent. In 11 samples examined the defatted meat content was less than the extracted fat content and the above correction would have given a lower calculated lean meat content.

Out of 64 samples of beef sausage examined the average defatted meat content was found to be 34·0 per cent. and the average fat content was found to be 26·0 per cent. Application of the above correction would bring the calculated lean meat to 35·0 per cent. In 12 samples examined the defatted meat content was less than the extracted fat content and the above correction would have given a lower calculated lean meat content.

Conclusions

The above simplified formula can be used for estimating the lean meat content of sausage but the errors involved when skimmed milk powder or soya protein is present may invalidate the corrections. Pearson,[4] however, gives a formula which can be used when the only sources of protein are meat, cereal filler and dried milk.

If the Food Standards Committee's Review of the Meat Product Regulations recommends suitable limits for the fat content of added lean meat and added outside fatty material, it will be possible to derive an accepted correction to be applied.

References

1. Stubbs, G., and More, A., *Analyst*, 1919, **44**, 125.
2. Pearson, D., *J. Assoc. Publ. Analysts*, 1968, **6**, 129.
3. Pearson, D., *Ibid.*, 1970, **8**, 22.
4. Pearson, D., *Ibid.*, 1971, **9**, 102.
5. Coomaraswamy, M., *Ibid.*, 1972, **10**, 33.

Food Microscopy

(An Annotated Bibliography)

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Part IIC. Major Ingredients: eggs and egg products

In addition to the use of the hen's egg as a food item in itself, eggs and egg products are used as ingredients in many other foods such as bakery products (*e.g.* biscuits, cakes, dry cake mixes), some beverages, confectionery (*e.g.* icing and sweets), dairy products (*e.g.* ice cream), fruit products (*e.g.* fruit curds) and general kitchen products (*e.g.* custards, macaroni, mayonnaise, noodles, salad dressings).

A considerable amount of work has been done on the microscopy of egg and egg products, and useful studies will be found in such journals as *British Poultry Science*, *Poultry Science* and the *U.S. Egg and Poultry Magazine*. Many other papers are widely scattered in the literature.

The microscopical literature on eggs covers a wide range of interests. Extensive studies have been made of the structure of the hen's egg, including the germ cell or blastodisc, the yolk, the vitelline membrane, the albumen, the inner and outer membranes, the air cell and the shell and cuticle. The yolk is essentially an emulsion containing globules of 25–100 μm, having a refractive index (n_D^{25}) of 1·418 and a coagulation temperature of 65°C. The structure of the yolk has been studied by heat fixation and sectioning and has been found to consist of two components—white yolk and yellow yolk. The albumen consists of three main layers—the inner white, the middle white and the outside white. The inner and outer layers are thin liquid fractions but the middle layer is a thick layer of albumen containing many mucin fibres suspended in a liquid fraction. The refractive index of the albumen (n_D^{25}) is 1·356 and it has a coagulation temperature of 61°C. In recent years the ultrastructure of the egg components has been increasingly studied by means of the electron microscope. This technique has proved useful in assessing the effect of various physical and chemical treatments on the ultrastructure of egg components.

The foaming properties of egg white are of great interest and studies have been carried out on the microscopical structure, foam volume and stability of egg white foams as a function of the age of the egg and the presence of additives such as milk, oils and fats, salt, sugar, water, yolk and other chemical compounds, and as a function of pH, temperature, beating time, mixing equipment, etc. The viscoelastic properties of egg white have been investigated by a micro-rheological method, involving microscopic observation of the displacement in a magnetic field of minute nickel particles introduced into the egg white.

Microscopical studies have been made on the ageing and preservation of whole eggs and the structure and porosity of the shell has received much attention in relation to bacterial invasion. The principal means of preservation are by (*a*) use of immersion methods, (*b*) cold storage or freezing and (*c*) drying of whole egg, yolk and albumen. The crystals that sometimes develop in the albumen and the yolk on cold storage have been investigated microscopically. Studies have also been made of the structure of dried whole egg, albumen and yolk. Some work has been done on the relationship between egg structure and properties and baking performance in various products, but much still remains to be investigated. The following references provide an introduction to the complex field of egg microstructure and properties.

References

1. Moran, T., 'Effect of Low Temperature on Hen's Eggs', *Proc. Roy. Soc.* (*London*), 1925, **B98**, 436.
2. Winton, A. L., and Winton, K. B., 'The Structure and Composition of Foods', Vol. III, Wiley, New York, 1937, Part II, p. 215.
3. Barmore, M. A., 'Influence of Chemical and Physical Factors on Egg-White Foams', *Colorado Agr. Expt. Stat. Tech. Bull.*, 1934, **9**, 1; *C.A.*, 1935, **29**, 240.
4. Stewart, G. F., 'The Structure of the Hen's Egg Shell', *Poultry Sci.*, 1935, **14**, 24.
5. Moran, T., and Hale, H. P., 'Physics of the Hen's Egg. I. Membranes in the Egg', *J. Exp. Biol.*, 1936, **13**, 35.
6. Haines, R. B., 'The Preservation of Eggs', *Chem. and Ind.*, 1940, 391.
7. Haines, R. B., and Moran, T., 'Porosity of, and Bacterial Invasion through, the Shell of the Hen's Egg', *J. Hyg.*, 1940, **40**, 453.
8. Grodzinski, Z., 'Influence of the Increase in the Osmotic Pressure upon the White Yolk Spheres of the Hen's Egg', *Bull. Int. Acad. Cracovic*, 1946, No. 87.
9. Shaw, T. M., Vorkoeper, A. R., and Dyche, J. K., 'Determination of Surface Area of Dehydrated Egg Powder', *Food Res.*, 1946, **11**, 187.
10. Schaible, P. J., and Bandemer, S. L., 'Crystals Observed in Fresh and Storage Eggs', *Poultry Sci.*, 1947, **26**, 207.
11. Bell, R. N., 'Polarising Microscope—A Quality Control Instrument', *Food Industries*, 1948, **20**, 1775. *Ibid.*, 1949, **21**, 83.
12. Romanoff, A. L., and Romanoff, A. J., 'The Avian Egg', Wiley, New York, 1949, Ch. III, p. 113–173.
13. Sharp, D. G., Lanni, F., and Beard, J. W., 'The Egg White Inhibitor of Influenza Virus Hæmagglutination. II. Electron Microscopy of the Inhibitor', *J. Biol. Chem.*, 1950, **185**, 681.
14. Forsythe, R. H., and Bergquist, D. H., 'The Effect of Physical Treatments on Some Properties of Egg Whites', *Poultry Sci.*, 1951, **30**, 302.
15. Bergquist, D. H., and Stewart, G. F., 'Atomisation as a Factor Affecting Quality in Spray-Dried Albumen', *Food Technol.*, 1952, **6**, 201.
16. Brooks, J., and Taylor, D. J., 'Eggs and Egg Products', D.S.I.R. Food Investigation Special Report, 1955, No. 60, Part I.
17. Tyler, C., 'Studies on Egg Shells. VII. Some Aspects of Structure as Shown by Plastic Models', *J. Sci. Food Agric.*, 1956, **7**, 483.
18. Forsythe, R. H., 'Some Factors Affecting the Use of Eggs in the Food Industry', *Cereal Sci. Today*, 1957, **2**, 211.
19. Simkiss, K., and Tyler, C., 'A Histochemical Study of the Organic Matrix of Hen Eggshells', *Quart. J. Microscop. Sci.*, 1957, **98**, 19.
20. Brooks, J., and Hale, H. P., 'Mechanical Properties of the Thick White of the Hen's Egg', *Biochim. Biophys. Acta*, 1959, **32**, 237.
21. Brooks, J., "Strength in the Egg', S.C.I. Monograph, 1960, No. 7.
22. Bellairs, R., 'The Structure of the Yolk of the Hen's Egg as Studied by Electron Microscopy. I. The Yolk of the Unincubated Egg', *J. Biophysical and Biochemical Cytology*, 1961, **11**, 207.
23. Doran, B. M., and Mueller, W. J., 'The Development and Structure of the Vitelline Membrane and their Relationship to Yolk Mottling', *Poultry Sci.*, 1961, **40**, 474.
24. Masshoff, W., and Stolpmann, H. J., 'Investigation of the Shell Membrane and Shell of Hen's Eggs, by Light and Electron Microscopy', [In German], *Z. Zellforsch.*, 1961, **55**, 818.
25. Heyn, A. N. J., 'Electron Microscope Observations on the Structure of Calcite in Avian Egg Shell', *J. Appl. Phys.*, 1962, **33**, 2658.

26. Bellairs, R. M., Harkness, M., and Harkness, R. D., 'The Vitelline Membrane of the Hen's Egg; a Chemical and Electron Microscopic Study', *J. Ultrastructure Research*, 1963, **8**, 339.
27. Simons, P. C. M., and Wiertz, G., 'Notes on the Structure of Membranes and Shell in Hen's Egg. An Electron Microscopical Study', *Z. Zellforsch. Mikrosk. Anat.*, 1963, **59**, 555.
28. Fromm, D., 'Strength Distribution, Weight and Some Histological Aspects of the Vitelline Membrane of the Hen's Egg Yolk', *Poultry Sci.*, 1964, **43**, 1240.
29. Howes, J. R., 'The Structure of Avian Egg Shell as Revealed by X-ray Diffraction and Electron Microscopy', *Poultry Sci.*, 1964, **43**, 1329 (Abstr.).
30. Simons, P. C. M., and Wiertz, G., 'Differences in Measurement of Membrane Thickness in Hen's Egg Shells', *Brit. Poultry Sci.*, 1965, **8**, 283.
31. Drachenfels, H. J. von, 'The Problem of Dehydrated Products in Regard to Their Reconstitution', *BFMIRA Abstr.*, 1966, **19**, 32, No. 154.
32. Simons, P. C. M., and Wiertz, G., 'The Ultrastructure of the Surface of the Cuticle of the Hen's Egg in Relation to Egg Cleaning', *Poultry Sci.*, 1966, **45**, 1153.
33. Fromm, D., 'Some Physical and Chemical Changes in the Vitelline Membrane of the Hen's Egg During Storage', *J. Food Sci.*, 1967, **32**, 52.
34. Iordanov, Z., Georgiev, I., and Boyadzhieva-Mikhailova, A., 'Some Structural, Ultrastructural and Physicochemical Properties of the Vitelline Membrane of the Chicken Egg', *C.A.*, 1967, **66**, 72402t.
35. Knight, R. A., Mears, K., Parkinson, T. L., and Robb, J., 'The Baking Properties of Pasteurised Whole Egg', *J. Food Tech.*, 1967, **2**, 143.
36. Schultz, J. R., and Forsythe, R. H., 'The Influence of Egg Yolk Lipoprotein-Carbohydrate Interactions on Baking Performance', *Baker's Digest*, 1967, **41**, 56.
37. El-Boushy, A. R., Simons, P. C. M., and Wiertz, G., 'Structure and Ultrastructure of the Hen's Egg Shell as Influenced by Environmental Temperature, Humidity and Vitamin C Additions', *Poultry Sci.*, 1968, **47**, 456.
38. Hautefort, M., Vasseur, M., and Boitreux, P., 'Morphology and Structure of the Egg', *BFMIRA Abstr.*, 1968, **21**, 425.
39. Powrie, W. D., 'Gelation of Egg Yolk upon Freezing and Thawing', *Recent Advances in Food Science*, 1968, **4**, 319.
40. Shenstone, F. S., 'The Gross Composition, Chemistry and Physico-Chemical Basis of Organisation of the Yolk and the White', in 'Egg Quality. A Study of the Hen's Egg', Carter, T. C. (Ed.), Oliver and Boyd, Edinburgh, 1968, Part I, pp. 26–58.
41. Simkiss, K., 'The Structure and Formation of the Shell and Shell Membranes' in 'Egg Quality. A study of the Hen's Egg', Carter, T. C. (Ed.), Oliver and Boyd, Edinburgh, 1968, Part I, pp. 3–25.
42. Nichols, A. V., Forte, G. M., and Coggiola, E. L., 'Electron Microscopy of Very Low Density Lipoproteins from Egg Yolk Using Negative Staining', *Biochim. Biophys. Acta*, 1969, **175**, 451.
43. Chang, C. M., 'Studies on Egg Yolk', *Dissertation Abstracts International*, Section B, 1970, **31** (2), 740.
44. Donovan, J. W., Davis, J. G., and White, L. M., 'Chemical and Physical Characterisation of Ovomucin, a Sulphated Glycoprotein Complex from Chicken Eggs', *Biochim. Biophys. Acta*, 1970, **207**, 190.
45. Henstra, S., Simons, P. C. M., and Wiertz, G., 'The Scanning Electron Microscope as a Tool in Studies of the Structure of the Birds' Egg Shell', *Jeol. News*, 1970, **8B** (2), 4.
46. Simons, P. C. M., and Wiertz, G., 'Notes on the Structure of Shell and Membranes of the Hen's Egg; A Study with the Scanning Electron Microscope, *Ann. Biol. Anim. Bioch. Biophys.*, 1970, **10** (2), 31.
47. Simons, P. C. M., 'Ultrastructure of the Hen Eggshell and its Physiological Interpretation', Centre for Agricultural Publication and Documentation, Wageningen, The Netherlands, 1971.
48. Bellairs, R., Backhouse, M., and Evans, R. J., 'A Correlated Chemical and Morphological Study of Egg Yolk and Its Constituents', *Micron*, 1972, **3**, 328.
49. Hasiak, R. J., Vadehra, D. V., Baker, R. C., and Hood, L., 'Effect of Certain Physical and Chemical Treatments on the Microstructure of Egg Yolk', *J. Food Sci.*, 1972, **37**, 913.
50. Holdsworth, G., and Finean, J. B., 'A Structural Study of the Low-Density Lipoprotein Fraction from Hen's Egg and of an Isolated Surface Component', *Chem. Phys. Lipids*, 1972, **9**, 217.
51. Kamat, V. B., Lawrence, G. A., Barratt, M. D., Darke, A., Leslie, R. B., Shipley, G. G., and Stubbs, J. M., 'Physical Studies of Egg Yolk Low Density Lipoprotein', *Chem. Phys. Lipids*, 1972, **9**, 1.
52. Tung, M. A., and Richards, J. F., 'Ultrastructure of the Hen's Egg Shell Membranes by Electron Microscopy', *J. Food Sci.*, 1972, **37**, 277.
53. Fujii, S., Tamura, T., and Okamoto, T., 'Studies on Yolk Formation in Hen's Eggs. I. Light and Scanning Electron Microscopy of the Structure of Yolk Spheres', *Food Sci. Tech. Abstr.*, 1973, **5**, 12Q, 180.

54. Kaplan, S., and Siegesmund, K. A., 'The Structure of the Chicken Egg Shell and Shell Membranes as Studied with the Scanning Electron Microscope and Energy Dispersive X-Ray Microanalysis', *Poultry Sci.*, 1973, **52**, 1793.
55. Meyer, R., Baker, R. C., and Scott, M. L., 'Effect of Hen Egg Shell and Other Calcium Sources upon Egg Shell Strength and Ultrastructure', *Poultry Sci.*, 1973, **52**, 949.
56. Meyer, R., and Hood, L. F., 'The Effect of pH and Heat on the Ultrastructure of Thick and Thin Hen's Egg Albumen', *Poultry Sci.*, 1973, **52**, 1814.
57. Meyer, R., Potter, N. N., and Baker, R. C., 'Changes in Ultrastructure of Egg Albumen Pasteurised with Additives to Minimise Heat Denaturation, *Poultry Sci.*, 1973, **52**, 2064.
58. Stadelman, W. J., and Cotterill, O. J., 'Egg Structure and Technology', Avi. Westport, Connecticut, 1973, pp. 26–27.
59. Vadehra, D. V., and Nath, K. R., 'Eggs as a Source of Protein', *C.R.C. Critical Reviews in Food Technology*, 1973, **4** (2), 193.
60. Bayer, R. C., Harris, P. C., and Muir, F. V., 'Egg Inclusions from Brown and White Shell Eggs as Observed with the Scanning Electron Microscope', *Poultry Sci.*, 1974, **53**, 1768.
61. Evans, R. J., Bauer, D. H., and Flegal, C. J., 'The Egg Yolk. Very Low Density Lipoproteins of Fresh and Stored Shell Eggs', *Poultry Sci.*, 1974, **53**, 645.
62. Garland, T. D., 'Studies on Egg Yolk Myelin Figures and Granule Low Density Lipoproteins', *Dissertation Abstracts International*, Section B, 1974, **34** (12), 6047.
63. Kamat, V. B., Patrick, B., Yoell, R., and Stubbs, J. M., 'Baking Performance of Hen's Egg Yolk Plasma Before and After Induced Aggregation', *J. Food Tech.*, 1974, **9**, 79.
64. Meyer, R., and Potter, N. N., 'Changes in Ultrastructure of Egg Albumen Pasteurised with Additives used to Minimise Heat Denaturation, *Poultry Sci.*, 1974, **53**, 761.

Book Review

THE ANALYSIS OF DETERGENTS AND DETERGENT PRODUCTS. By G. F. LONGMAN. Pp. 587 + 42. Chichester: John Wiley & Sons Ltd. 1975. Price: £11.50.

Once upon a time, when cleanliness and soap were virtually synonymous (although by no means universal), foam was shy and evanescent, washing was either clean or dirty and analysts slept easy in their beds. This happy state of affairs was brought to an end by the detergent revolution, conceived in Germany during World War I and subsequently bestowed, a lusty infant, on the wider world outside by the commercial exigencies of peace. Indeed, the process of beating swords into ploughshares could scarcely be better exemplified than by I.G. Farbenindustrie which, as Mr Longman recalls, utilised not only the plant but also the raw materials from mustard gas production to launch the Igepon detergents—an interesting snippet of information which provokes the thought that cleanliness, perhaps, comes after godlessness!

The technological impetus imparted by World War II enabled a massive post-war expansion of the detergent industry to take place and a bewildering profusion of surface-active agents appeared on the market. Analytical papers appeared in increasing numbers as analysts strove to keep pace with the ingenuity of organic chemists and the increasing complexity of formulation. It is noteworthy that an early method for the determination of anionic detergents by titration with a surface-active agent of opposite sign has survived, in modified form, to the present day. First suggested by Hartley and Runnicles in 1938, it was modified ten years later by the introduction of a second phase and, later still, by the use of a dual indicator. The application of the dual indicator, dimidium bromide/disulphine blue, to quantitative work is attributed by the author, no doubt correctly, to Herring (1962), but it is worth noting that the use of this indicator as a qualitative test was recommended five years earlier in a paper in *The Analyst* by Holness and Stone, who selected this combination as the most useful of a number of dye mixtures investigated.

There must, by now, be some hundreds of papers dealing with the various aspects of surfactant analysis, but these are distributed throughout the chemical literature and there have been few attempts to gather a selection of them into book form. The present work fills a notable gap with some distinction. The author has provided an immense amount of useful information with a wealth of experimental detail and draws on his long experience as a research analyst in this field for helpful suggestions and critical comment. There is scarcely any facet of detergent analysis which is not covered, procedures for the analysis of all the main types of compounds, including amphoterics, ampholytics and amine oxides being included. There are chapters on the identification of unknowns, the separation of mixtures and on the determination of both organic and inorganic non-detergent components. An appendix lists the various types of surfactants, with notes on their structure. Public Analysts will find the

chapter on the determination of surfactants and detergent additives in sewage and effluents of particular interest.

The book is clearly printed, in double spacing, by some form of typescript reproduction (there are some curious symbols for 'litre' which appear to be longhand interpolations) and the writing is both lucid and interesting. Doubtless, considerations of economy prompted the decision to bind it in soft covers, but the review copy did not escape entirely unscathed from the vagaries of the postal service, which does not augur well for its survival in the laboratory.

One or two minor misprints were noted in passing. On page 30, the first line of the second paragraph reads, 'Soap and alkoyl sarcosinates, although non-ionic, behave as non-ionic detergents under the above conditions'. The first 'non-ionic' should, of course, read 'anionic'. On page 53, the second sentence of the second paragraph contains the verb 'vary' where the sense demands the adjective 'varying', and there are two instances of words being duplicated at the foot of one page and the beginning of the succeeding page. A more serious error occurs in that part of Chapter 10 dealing with the methylene blue method of determining anionics. On page 238, under the heading 'Reagents', two solutions of methylene blue are described; the first, called 'Methylene blue solution', is a simple 0·5 per cent. solution of the dye in water and the second, characterised as 'acid methylene blue solution', consists of 6 ml of the previous solution diluted to 1 litre, with the addition of sodium sulphate and 2N sulphuric acid. On the following page, under the heading 'Procedure', the second sentence contains the instruction '. . . add 25 ml of methylene blue solution . . .'; this should, of course, read '. . . add 25 ml of acid methylene blue solution . . .', the first solution being far too concentrated for matching and lacking demulsifying additives.

These are, however, minor faults and the book can be recommended to anyone interested in detergent analysis. It contains far more than most Public Analysts are likely to need and should prove invaluable to those analysts whose principal concern is with surfactants. For those who require further information, references to the original literature are collected at the end of each chapter.

S. J. BUSH.

Letters to the Editor

Toxic Metal Residue in Soils

Sir,

As agricultural consultants with involvement in this subject, we should like to comment on the paper by E. R. Pike *et al.* (*J.A.P.A.*, 1975, **13**, 19).

Firstly, we are surprised at the failure to report pH levels in the analysed samples. It is well-known that pH has a dominant effect on toxicity, and a knowledge of pH is essential for the interpretation of availability data in respect of toxicity. For example, Patterson reports toxicity from 200 p.p.m. (total?) copper in barley at pH 5·2, but apparently no damage resulting from 1250 p.p.m. at 'high pH'; and with 20 p.p.m. (total?) nickel, symptoms were seen on wheat at pH 5·1, but no effect was recorded from 1280 p.p.m. (total?) nickel at pH 7·5, and no visible effect on growth was seen from 2560 p.p.m. at the same pH, though grain yield was reduced and ripening delayed (M.A.A.F. Technical Bulletin 21, 'Trace elements in soils and crops', H.M.S.O., 1971).

It is therefore essential to determine and report pH whenever analyses are made in connection with toxic metal residues.

Secondly, the paper reflects the potential confusion caused by M.A.F.F. using the level of 250 p.p.m. 'zinc equivalent' as a reasonable limit, but using this for both *available* and *total* contents at the same time. Advisory Paper No. 10 (quoted by the authors) refers at par. 3 to the safety of adding a *total* of 250 p.p.m. 'zinc equivalent' to the soil. At par. 4, however, it is suggested that, for soils previously contaminated, the *available* 'zinc equivalent' should be determined, and only sufficient *total* 'zinc equivalent' should be subsequently added as will bring the existing available content up to total 250 p.p.m. Thus, one starts with a limit on *total* content, subtracts existing *available* and tops up with *total* to produce a figure which is a combination of available and total.

Despite this confusion, it is reasonably clear that the 250 p.p.m. suggestion should in practice be applied to *available* 'zinc equivalent'.

Thirdly, in the context of this recommendation, it is somewhat surprising to note that the authors accept the 250 p.p.m. value despite the occurrence of values as high as 1381 p.p.m. This acceptance is on the basis of possible future variations in soil factors.

It is clear that the 250 p.p.m. recommendation is a fairly arbitrary one, based on a combination of rather adverse soil and crop situations, and that there are very many soil-crop situations where far higher levels are presently satisfactory. The great need is for considerable further survey and research work into the problem (supported by the full data, including pH!) so that the circumstances which define satisfactory limits can be more accurately determined than is presently possible. In the interim, it is as well for those directly concerned to note that a level greater than 250 p.p.m. does not mean immediate disaster.

T. R. Worthington,
Agricultural Consultant, Reading.

Toxic Metal Residue in Soils

Sir,

I have read Mr Worthington's letter with interest and agree that a knowledge of the pH value in the analysed samples is essential for the interpretation of availability data in respect of toxicity to plant life. However, Mr Worthington seems to have missed the real aim of this paper, which was to assess the distribution of toxic metals over the whole site of the sewage works with relation to possible toxic affects upon people living in the proposed residential developments. The paper was not intended to be a work on 'Toxic Metal Residues in Soils' as Mr Worthington's letter is headed but 'An Appraisal of Toxic Metal Residues in the Soils of a Disused Sewage Farm'. Nevertheless, I agree that pH measurements would have provided further knowledge of possible toxicity towards plant life, as would indeed the amount of organic matter in the soil etc., but this aspect was not within the terms of reference laid down when we were instructed to carry out this work. Although interesting and indeed valuable these side issues might have been, we had a time limit (9 months) in which to carry out this investigation. This precluded a thorough investigation of other aspects which we might have wished to pursue.

I regret that Mr Worthington regards the paper as reflecting confusion between the zinc equivalents based upon 'total and available' metal contents. This discussion was merely included in the paper in an attempt to underline the ambiguity of these arbitrary factors and indicate that other factors do influence soil-crop situations. If the article shows that further work is necessary then at least this can be regarded as a positive contribution towards solution of this problem. In the knowledge that many other factors are involved, it would indeed be less than cautious to accept a higher value of 250 p.p.m. for the 'available zinc equivalent' of a soil until all other aspects of this problem have been investigated.

E. R. Pike,
County Analyst, Leicestershire.

Stability of Vitamin C in Ribena

Sir,

Figures relating to the stability of vitamin C in Ribena reported by Pearson[1] in your June issue imply that Ribena is an unreliable dietary source of this nutrient. We believe that any such implication is without foundation.

We have for many years monitored the changes in the vitamin C content of Ribena that occur during storage. Our figures show that in unopened bottles there is on average a loss of 4 per cent. after two months at room temperature (19–21°C) and rather less than 2 per cent. under warehouse conditions. In a refrigerator (4°C) the average loss is only about 1·0 per cent. These figures are fairly consistent for any two-month period during the course of a year.

Our measurements are therefore very different from the 52 per cent. loss in two months at room temperature, and 29 per cent. loss at 5°C reported by Pearson. According to Pearson the level after two months at room temperature was below that declared on the label (206 mg/100 g). If this were so, we would surely have had numerous complaints from enforcement authorities over the past decades. The fact that our files extending back for more than twenty years record not one such complaint is evidence that our experience of the stability of vitamin C more truly reflects the real situation. Our experience is also consistent with the many reports in the literature[2–7] that testify to the exceptional stability of vitamin C in blackcurrant juice and blackcurrant syrups, and which have stimulated other workers to identify the stabilising factors that are involved[8–15].

The analytical method used by Pearson[16] to determine vitamin C (titration with N-bromosuccinimide) is known to be subject to interference from free sulphur dioxide,[17] and it is possible that his determinations were so affected. For example, his figure for initial vitamin C of 385 mg/100g is very much higher than that at which Ribena is controlled at time of bottling. Our method is based on that of Liebmann & Ayres[18] and uses acetone to eliminate interference from free sulphur dioxide[19].

Pearson has also wrongly assumed that Ribena is made with the maximum level of sulphur dioxide of 350 mg/kg permitted by the Preservatives in Food Regulations 1974[20]. In fact, less than half this amount is present at the time of bottling.

We bring these facts to the attention of your readers because the prominence given to our brand name in the title of the paper,[1] and in the abstract immediately underneath, is likely to have a damaging and prejudicial effect on the status of Ribena which, for the reasons set out above, we believe to be unjustified.

References

1. Pearson, D., *J. Assoc. Publ. Analysts*, 1975, **13**, 76.
2. Sills, V. E., 'Ann. Rep.', Long Ashton Res. Stn., 1939, 127.
3. Pollard, A., Kieser, M. E., and Steedman, J., *Chem. and Ind.*, 1944, **63**, 215.
4. Wokes, F., and Organ, J. G., *Quart. J. Pharm.*, 1944, **17**, (3), 188.
5. Pollard, A., Kieser, M. E., and Bryan, J., *Chem. and Ind.*, 1946, 402.

6. 'Recent Advances in Fruit Juice Production', Charley, V. L. S. (Ed.), Commonwealth Bureau of Horticulture and Plantation Crops, 1950.
7. Kyzlink, V., *Oddil potravinářské technol.*, 1957, 37 (Ref. *C.A.*, **53**, 2502–2503, 1959).
8. Hooper, F. C., and Ayres, A. D., *J. Sci. Food Agric.*, 1950, **1**, 5.
9. Timberlake, C. F., *ibid.*, 1960, **11**, 258.
10. Timberlake, C. F., *ibid.*, 1960, **11**, 268.
11. Kuusi, T., State Institute for Technical Research, Helsinki, 1961, UDC 577.16 c: 634.72.
12. Morton, A. D., *J. Food Technol.*, 1968, **3**, 269.
13. Clegg, K. M., and Morton, A. D., *ibid.*, 1968, **3**, 277.
14. Harper, K. A., Morton, A. D., and Rolfe, E. J., *ibid.*, 1969, **4**, 255.
15. Shrikhande, A. J., and Francis, F. J., *J. Food Sci.*, 1974, **39**, 904.
16. Evered, D. F., *Analyst*, 1960, **85**, 515.
17. Mohammad, N., Ikramul Haq, M. Y., and Ehteshamuddin, A. F. M., *Pakistan J. Sci. Ind. Res.*, 1968, **11** (4), 381.
18. Liebmann, H., and Ayres, A. D., *Analyst*, 1945, **70**, 411.
19. Mapson, L. W., *Biochem. J.*, 1942, **36**, 196.
20. 'The Preservatives in Food Regulations'. S.I. 1974, No. 1119, H.M.S.O., London, 1974.

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