# THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Extraordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, October 5th, the President, Mr. F. W. F. Arnaud, being in the chair. Special Resolutions were passed for the alteration of certain of the Articles of Association of the Society.*

This was followed by an Ordinary Meeting, at which Certificates were read for the first time in favour of Lionel James Dent, B.Sc., and Leonard Arthur Haddock, B.Sc., A.I.C.

Certificates were read for the second time in favour of Arthur Littlewood, M.A., A.I.C., and John Henry Weber, B.Sc., A.I.C.

The following were elected Members of the Society:—Charles Carr Marginson, B.Sc., A.I.C., Ph.C., Wilfred Mather, A.M.C.T., F.I.C., Alec Duncan Mitchell, D.Sc., F.I.C., and M. Niyogi, M.Sc.

Mr. E. Hinks, B.Sc., F.I.C., gave an account of the work embodied in the Third Report of the Milk Products Sub-Committee, on The Analysis of Condensed Milk in which the Sucrose has altered during Storage, and the following papers were read and discussed:—"A New Copper Reagent for Sugar Determinations," by E. B. Hughes, M.Sc., F.I.C.; and "A Colorimetric Method for the Determination of Chloroform," by W. G. Moffitt, Ph.D., A.I.C.

* That Article 6 of the Articles of Association of the Society be deleted and the following substituted therefor:

6. Every candidate for membership of the Society shall be not less than 21 years of age and shall be or have been engaged in analytical, consulting or professional chemistry. Each candidate for election shall be proposed by three members of the Society who shall provide written testimony of their personal knowledge as to his scientific and professional fitness. If the Council in their discretion think fit such testimony may be dispensed with in the case of a candidate not residing in the United Kingdom. Every application shall be placed before the Council and the Council shall have the power in their absolute discretion to suspend or reject any application. Each application passed by the Council shall be read at the next Ordinary Meeting of the Society and shall be circulated to members prior to the next following meeting so that the voting which shall be by ballot may take place at that meeting. It shall be competent for members who are unable to be present to send their ballot papers through the post. The candidate shall be elected if not less than three-fourths of the votes recorded are in favour provided also that the total number of votes recorded be not less than 20. If less than 20 votes are recorded the candidate shall come up again for election at the next Ordinary Meeting of the Society.

6a. Any member may at any time by notice in writing to the Secretaries resign his membership but such resignation shall not free him from liability to pay any annual subscription or money which may be due from him to the Society.

That in Article 34, line 1, "Article 39" be deleted and "Article 36(b)" be substituted therefor.

That in Article 36, line 1, "(a)" be inserted after "36," and that at the end of the Article the following be added: (b) Notwithstanding the provisions of Articles 34, 35 and 36(a) the Officers and Council shall include the Chairman for the time being of the North of England Section of the Society as an additional Vice-President and the Secretary for the time being of that Section as an additional Member of Council.

## NORTH OF ENGLAND SECTION

A MEETING of the Section was held on October 15th, in Manchester. The Chairman (Mr. J. Evans) presided over an attendance of thirty-four.

The following papers were read and discussed:—"The Quantitative Separation of Aluminium from Iron," by J. Haslam, M.Sc., F.I.C.; "An Improved Micro-apparatus for the Determination of Molecular Weights," by A. F. Colson, B.Sc., A.I.C.; "The Spontaneous Combustion of Hay," by F. Robertson Dodd, F.I.C.; "The Examination of a Proposed New Method for the Identification and Estimation of Oils and Fats," by J. R. Stubbs, M.Sc., F.I.C., and Arnold Lees, F.I.C.; and "The Freezing-point of Pasteurised and Sterilised Milks," by G. D. Elsdon, B.Sc., F.I.C., and J. R. Stubbs, M.Sc., F.I.C.

# Deaths

THE Society has suffered a great loss by the death, on October 17th, of Mr. A. Chaston Chapman, Past-President. An obituary notice will be published later.

We also regret to have to record the death of Mr. W. Hepworth-Collins, who had been a member of the Society since 1897.

# The Estimation of Hormones

BY K. CULHANE, B.Sc., A.I.C., AND S. W. F. UNDERHILL, M.A., B.M., B.CH.

(*Read at the Meeting, April* 6, 1932)

MANY glands of the body secrete into the blood-stream substances which exert a powerful drug-like action and serve to correlate the functions of different organs and tissues. The earliest step in the investigation of the functions of these glands of internal secretion was the observation of the effects caused by removal of the glands from animals, or their grafting into a different position in the body. The study of the phenomena produced by the injection of extracts has been a later development. Berthold, in 1849, found that the implantation of testicular grafts into capons restored the appearance of the birds to that of normal cocks, and concluded that the testes must pass into the blood some substance which affects the whole organism. Some ten years later Claude Bernard formulated the idea of internal secretion, but gave it wider significance than the term connotes to-day. A quarter of a century later the Swiss surgeons, J. L. and A. Reverdin, and Th. Kocher found that removal of the thyroid gland in patients suffering from goitre led to the onset of a condition which had already been recognised as a clinical entity termed myxoedema. A few years later Minkowski and von Mehring showed that removal of the pancreas from animals led to the development of diabetes mellitus, and Brown-Séquard described the effects upon himself of the administration of testicular extracts.

The first demonstration that it was possible to obtain active extracts of the

glands of internal secretion occurred in 1894, when Oliver and Schäfer injected simple extracts of a number of them intravenously into animals, and observed the effects produced upon the blood pressure. They found that extracts of the suprarenal or pituitary glands caused a rise in blood pressure, whilst a fall was produced by extracts of all the others investigated. Ten years later Bayliss and Starling reported the discovery of secretin, and proposed the name "hormone" for the active principle of the glands of internal secretion. Secretin is, perhaps, the simplest example of a hormone. The acid which passes from the stomach into the duodenum with the semi-digested food is absorbed by the cells lining this part of the small intestine, and converts the precursor of secretin in these cells into the active principle itself. The hormone is then absorbed into the blood-stream and carried round the body until it reaches the pancreas, the acinous cells of which are stimulated to pour out their secretion into the ducts of the gland, whence it finds its way into the upper part of the duodenum. Secretin thus has the single function of stimulating the cells of the pancreas, and does not act on other cells in the body (except possibly those of the liver).

Other hormones have more complicated functions, acting upon a variety of tissues. Thus adrenaline, the active principle of the medulla of the suprarenal glands, has the same effects upon the tissues as stimulation of the sympathetic nerves proceeding to them. Although removal of this part of the suprarenal gland has not been shown to have any very striking result, yet stimulation of the secretion of adrenaline or its administration is productive of very profound effects. Adrenaline, in fact, is secreted at times of stress or emotion, and is responsible for the rapid heart beat, the dry mouth, the cold and clammy skin observed at moments of extreme shock. The familiar response of a cat to the approach of a strange dog is conditioned by the outpouring of an extra amount of adrenaline from its own glands.

The powerful effects which the active principles of these glands exert upon the bodily economy is evinced by the observation of the results of their under- or over-production in human beings. Hypo-secretion of insulin, the active principle of the internal secretion of the pancreas, leads to the development of the disease known as diabetes mellitus. Again, hypo-secretion of thyroxine, the active principle of the thyroid gland, is responsible for the condition known as cretinism in the child or myxoedema in the adult. A cretin shows under-development of both mind and body; a myxoedematous patient shows dulling of the mental faculties, lethargy, and a peculiar oedematous condition of the skin. Hyper-secretion of the thyroid gland, on the other hand, is the cause of Graves' disease, in which the patient suffers from palpitation, nervousness, flushing, wasting, and protrusion of the eye-balls; whilst over-production of the secretion of the anterior lobe of the pituitary gland results in gigantism if it occurs before growth has ceased, or in acromegaly in the adult.

Enough has been said to show the profound effects which these compounds exert upon both mind and body. It might be expected, therefore, that extracts of these glands would be powerful therapeutic agents, and it has, in fact, gradually become apparent that the body itself is the best factory for a number of therapeutic substances.

The hormones have been used to replace the natural secretion when that is deficient, and also as drugs to modify the function of the body in some desired manner. The use of insulin in diabetes mellitus, of thyroxine in myxoedema or cretinism, of pituitary posterior lobe extract in diabetes insipidus, and of extracts of the cortex of the suprarenal gland in Addison's disease, are all examples of this replacement or substitution therapy. On the other hand, adrenaline finds its chief clinical value in asthma, and as a constituent of solutions used in local anaesthesia. Pituitary posterior lobe extract is widely used as a uterine stimulant at the end of labour, and to raise the blood pressure in conditions of shock.

As with all drugs, there is a limiting dose which cannot be safely exceeded. It is, therefore, essential for the physician to employ an accurate dosage, and this necessitates special methods of test when the compound used cannot be obtained in a chemically pure condition. An over-dosage of insulin, for instance, may produce most alarming symptoms. As the blood sugar falls, the patient shows signs of uneasiness with palpitation and sweating; mental distress and disorientation are common, and may be followed by coma which can be distinguished from diabetic coma only by determining the sugar in the blood. Convulsions, as a result of this over-dosage, are rare in human beings, although they are a frequent symptom of the toxic action of insulin in animals.

Although active extracts have been obtained from the majority of glands possessing an internal secretion, in only three or four cases have pure crystalline compounds been isolated, and in only two, namely, those of adrenaline and thyroxine, has the isolation been followed by a determination of the chemical constitution. Little is as yet known of the chemical constitution of insulin, although it can now be crystallised, or of the active principles of the pituitary and the parathyroid glands. For advances in this field it is necessary to have available suitable physiological and chemical methods of assay. Insulin would never have reached its present position in therapeutics without a simple method of physiological assay, and a simple method of estimating the sugar in the blood, nor would oestrin, one of the active principles of the secretion of the ovary, have been isolated in a pure crystalline condition, if a simple, accurate, and rapid method of physiological assay had not been available.

As long ago as 1908 Zuelzer obtained extracts of pancreatic glands which were active in diabetic subjects. Knowlton and Starling, a few years later, obtained an extract which restored to normal the decreased rate of disappearance of sugar in the heart of a diabetic animal. Other workers obtained extracts which were frequently potent, but, without a suitable method of assay, found it impossible to purify the hormone and separate it from other toxic compounds. Soon after Banting and Best commenced their work in 1921 they found that injections of their extracts resulted in the lowering of the blood-sugar of normal rabbits. Thus they had at their disposal a simple test, since the rabbit is a convenient laboratory animal from which small amounts of blood can rapidly be withdrawn without harm, and methods of determining the sugar in the blood had by then been so far developed that the assay could be carried out with the highest accuracy on very small quantities.

In 1923 Allen and Doisy found that the rat showed a vaginal reaction similar

to that described by Stockard and Papanicolaou in the guinea-pig: the cells obtained by rubbing a spatula on the vaginal walls varied according to the oestrous condition of the animal. The isolation of oestrin was made possible by this discovery, since the reaction, as employed with the rat or mouse, is capable of a quantitative expression when a number of animals are used, and has enabled investigators to follow the activity in all the processes of purification and isolation. It is of interest to note that the clinical indications of oestrin are still obscure. The same is true of the hormone of the testis which A. Butenandt (*Z. angew. Chem.*, **1931, 44**, 905) has recently claimed to have isolated in pure crystalline form. The great differences in the reproductive processes of different species, their cyclic character, and the fact that the same gland may secrete a succession of hormones, are at the base of our lack of knowledge of the clinical value of these preparations.

Although extracts of the parathyroid glands have a considerable therapeutic value, little is known of the chemical properties of the hormone itself. It may be surmised that this is due to the fact that a simple method of physiological assay has not yet been found, nor is there available a satisfactory micro-method for the estimation of calcium in blood. Small laboratory rodents are resistant to parathyroid gland extracts, and it is necessary to carry out the tests upon dogs.

Thyroxine is more stable than the other hormones, and is characterised by its content of iodine. It was, therefore, possible to follow its isolation from the thyroid gland by chemical means, the physiological test being used only to confirm the activity of the isolated product.

Extracts of the glands of internal secretion are of great and increasing importance in therapeutics, and their commercial production has, so far, preceded the definition of their chemical properties, so that they present great difficulties to the analyst; yet, in view of their potent action and the important uses to which they are put, it is essential that adequate tests should be carried out before their issue for use by physicians.

At present, insulin, extract of the posterior lobe of the pituitary gland, adrenaline, and thyroxine, find widest use in medicine. No chemical methods of estimation or even identification of insulin and pituitary posterior lobe extract are yet available. They can be identified and estimated only by physiological tests. Both are scheduled preparations in the Regulations made under the Therapeutic Substances Act, **1925** (Statutory Rules and Orders, **1931**, No. **633**), and physiological methods of assay have been specified in the British Pharmacopoeia, **1932**.

Adrenaline is a well-defined chemical compound which may be prepared either from suprarenal glands or synthetically. Synthetic adrenaline may be contaminated with small amounts of impurities which have not so far been detected chemically, although they have a considerable influence upon the physiological activity. The physiological test, which is simple and accurate, is therefore more reliable than chemical or physical methods of estimation.

In the case of thyroxine, determinations of the percentage of iodine and of the melting point offer sufficient indications of its purity, and are much simpler than physiological tests. In therapeutics, however, thyroxine is most commonly administered in the form of dried thyroid gland. In this preparation it is present as laevo-thyroxine in peptide combination. The laevo compound is more active

physiologically than the synthetic racemic substance, and the activity is still further modified when it is present as a peptide, partly owing to its greater solubility. Determination of thyroxine iodine, therefore, may not prove a completely reliable index of the potency of such preparations, but physiological tests are difficult to carry out, and do not show a high degree of accuracy, so that, for practical purposes, the chemical method is the more satisfactory.

Extracts of the male and female reproductive glands, and of the anterior lobe of the pituitary gland, have as yet no definite clinical indications. Extracts of the parathyroid glands and of the cortex of the suprarenals are of definite clinical value, but no active principle has yet been isolated; except in the case of oestrin the methods of physiological assay of all these hormones are only very roughly quantitative.

In the following account we confine ourselves to a description of the assay of insulin, pituitary posterior lobe extract, and adrenaline.

PRINCIPLES OF BIOLOGICAL TESTS.—When the same dose of a drug is injected into a series of animals of the same species, it is usually found that the effects produced on the different animals differ in degree. Thus a dose can be found which will kill some of the animals, but not others, or which will have a greater physiological effect in some animals than in others, for example, in reducing the blood sugar or raising the blood pressure. At one time physiologists were inclined to overlook these individual variations, and, by the use of a few animals given rather widely spaced doses, attempted to determine the minimum dose of the compound under examination which would have an effect in the particular group of animals used. Hence arose the term "minimum lethal dose" or M.L.D. The figure obtained for the M.L.D., however, may vary according to the variations in the response of different animals under the same conditions. Even if the average lethal dose is taken instead of the minimum lethal dose, there is no guarantee that this figure will be the same when determined on different stocks of animals by different investigators. Owing to the many factors affecting animal reactions it is possible to obtain comparable results in different laboratories and in different countries only when the sample under examination is assayed in terms of a suitable standard. The results of the assay are then expressed in terms of this standard instead of in terms of the animal reaction. An example of the use of the standard will perhaps make this clear. If a given dose of insulin is injected subcutaneously into rabbits, the blood sugar may fall after a couple of hours to a level of 45–80 mgrms. per 100 c.c. If 45 mgrms. per 100 c.c. is taken as the unit of the reaction, it is clear that a larger dose of insulin will be required in the case of the more resistant animals than in the case of the more sensitive to reduce the blood sugar to this level. As it is not possible to obtain animals of uniform sensitiveness, a dose which will produce an average fall to 45 mgrms. per 100 c.c. on one occasion or on one stock may on another occasion on another stock only reduce the blood sugar to 80 mgrms. per 100 c.c., in which case the potency of the preparation will be considered to be only one-half its previous value, although actually it is of exactly the same activity. If, instead of the actual level of the blood sugar in a series of animals being taken as the unit of reaction, the effect of the

test solution is compared with the effect produced by the standard injected at the same time into a similar series, the activity of the former can be expressed as a percentage of the activity of the standard, since the variation in the sensitiveness of the different animals under controlled conditions is likely to be the same. The effect of the variability of the animals can be reduced to a minimum only by using fairly large groups of a uniform stock.

The preparation of suitable standards of reference is a matter of international importance, and has been undertaken by the Health Organisation of the League of Nations. Standards have been issued for insulin, pituitary posterior lobe extract, and adrenaline, as well as for a number of other biological products and drugs which cannot yet be submitted to a chemical or physical test. Other standards will be adopted and issued from time to time by the Permanent Commission on Standardisation of Sera, Serological Reactions and Biological Products, as our knowledge of the physiology and clinical value of this group of substances warrants. The same standard of reference is now used in all countries to control the potency of these preparations so that their activity wherever obtained, when made to a definite potency, should be exactly the same. In Great Britain the Medical Research Council maintains and distributes suitable standards for these biological products (*cf.* ANALYST, 1932, 173). The international adoption of these standards marks a great advance when it is remembered that most of these substances were unknown a quarter of a century ago.

We have used above the term "unit." In the early tests of any of these preparations it was found convenient to select a certain response which was somewhat less than the maximum obtainable as a unit of activity. The next step was the setting-aside of a preparation of the compound as a standard, and its definition in terms of units of activity. Thereafter, it is not necessary, as shown above, for all the animals in a group to show the same response. A given number of units is administered to all the animals of a group, and their average response is determined. The average response of another similar group to a dose of the sample under examination is also found. By adjustment of the dose given it is possible to obtain the same average response in the two groups. The dose of the sample then contains the same number of units as the dose of the standard.

The methods of biological assay to which we refer below illustrate the application of these principles in the differing circumstances of each test. The simplest case is when doses of sample and standard can be administered alternately to the same animal preparation, as in the assay of the oxytocic potency of pituitary posterior lobe extract, or the pressor potency of adrenaline. In the case of insulin, in the rabbit test, the same animal is given at different times a dose of the standard and a dose of the sample; in the mouse test, different animals are injected, some with the standard, others with the sample, at the same time.

## INSULIN

Although insulin can be crystallised and different preparations have the same potency, little is known about its chemical constitution. No chemical or physical tests of identification or estimation are yet available, so that reliance must be

placed entirely upon physiological methods. Chemical and physical methods of estimation have been put forward, but none has been found sufficiently specific. Recently, W. Graubner (*Z. ges. exp. Med.*, 1928, **63**, 527), and W. Kuhn, H. Eyer, and K. Freudenberg (*Z. physiol. Chem.*, 1931, **202**, 97) have found that the ultra-violet spectrum of insulin shows a point of maximum absorption at a wave-length of about 272·5$\mu\mu$. This, however, is also shown by other proteins or mixtures of amino acids such as tyrosine and cystine. F. K. Crews has examined the spectra of a number of samples of insulin of varying potency. Although there is a tendency for those of very low activity to show less absorption, and those of high potency a greater absorption, there is a range between potencies of about 5 to 20 units per mgrm. in which the absorption remains almost constant (unpublished observations).

Methods of physiological assay are based on the reduction of the blood sugar produced by an injection of insulin. Either this reduction is directly measured, or its effect on the animal, namely, the appearance of convulsions, is taken as the criterion. In our experience the method in which rabbits are used, described by Marks (*The Biological Standardisation of Insulin, Publications of the League of Nations*, III, Health, 1926, III, 7 Ch., 398, p. 57), and that in which mice are used, described by Trevan and Boock (*ibid.*, p. 47), are the most suitable. For all accurate assays it is essential to employ a method permitting the direct comparison of the sample with the standard of reference. The present standard is the International Standard adopted by the Health Organisation of the League of Nations (*ibid.*, p. 7), which is defined as containing 8 units per mgrm. For use it is convenient to dissolve it at about 20 units per c.c. in acidified water ($p_H$ 4) containing 0·3 per cent. of cresol. This solution is perfectly stable. It is our practice, however, to renew it every two months. Further dilutions are made from this stock solution as required.

Qualitative Tests.—As a preliminary to a more accurate assay, time and rabbits can be saved if one has some knowledge of the hypoglycaemic potency of the sample to be tested. For this purpose two or three rabbits are starved for 22 hours and their resting blood sugars are then determined. A dose of the sample is injected subcutaneously, and the blood sugar is determined hourly until the minimum is reached. 0·25 unit per kilo. usually gives a minimum at the first hour, 0·5 unit one at the second, and 0·75 unit one at the third hour. By noting the position of the minimum it can thus be seen whether the dose given is of the order of half a unit, or greater or less, information which is invaluable for the selection of a suitable dose for the quantitative test.

## Quantitative Assay

(1) The Rabbit Test.—We have found it essential to use carefully selected animals, and to keep the stock under conditions as uniform as possible. Young healthy rabbits of 1600–3000 grms. in weight, with large ears, are the most suitable. They should be kept at a constant environmental temperature and on a constant diet, *e.g.* oats, hay, and cabbage. As an aid to their selection all animals should receive a subcutaneous injection of 0·5 unit per kilo. of the standard preparation, and any which develop convulsions should be discarded. It is also advisable to discard them after they have been in use for more than

two or three months, if they habitually give figures for the percentage blood sugar reduction (as defined below) of less than 15 or more than 45, and if at any time they have received doses varying greatly from 0·5 unit per kilo. Pregnant females or animals which have had litters should not be used. Further details on the selection of rabbits are given by K. Culhane (*Quart. J. Pharm.*, 1928, **1**, 517).

The test is performed in two parts, the first on one day, and the second three or four days later.

*First Part of Test.*—An even number of rabbits, in any case not less than ten, is selected, and all food withdrawn from their cages 22 hours before the test. On the morning of the test the animals are weighed and divided into two groups, in such a manner that for each animal of a given weight in one group there is one of approximately corresponding weight in the other group, so that both the total weights and the distribution of individual weights in the two groups are as far as possible the same. A sample of blood is withdrawn from the ear vein of each rabbit, 2–5 mgrms. of potassium oxalate being added to each c.c. to prevent clotting, and the percentage of blood sugar determined. We have found it convenient to use a modification of the Folin–Wu method, in which the sugar in the protein-free filtrate is estimated colorimetrically. The method is simple and rapid, and allows one observer to carry out as many as 120 estimations in one day. It is known that the readings of the colorimeter are not quite proportional to the ratio of the sugar content of the test solution to that of the standard solution. To correct for this error we have constructed a curve relating the reading of the colorimeter to the amount of sugar present when the standard is set at 20 mm. The use of this curve considerably increases the accuracy of the method, and is quicker than other means of correction that have been proposed. An accuracy within ± 2–3 mgrms. glucose per 100 c.c. of blood is attained.

Each rabbit in the first group receives a subcutaneous injection of 0·5 unit per kilo. body weight of the standard preparation, and each animal of the second group a dose of the sample under test expected to be equivalent. Both sample and standard are diluted so that each animal is given a volume of 0·25 c.c. per kilo., and all injections are made at regular intervals of three minutes. Samples of blood are withdrawn from the ears of each of the rabbits at the end of the first hour after the injection, and at each of the subsequent four hours. It is convenient to pool the five samples from one rabbit; 0·2 c.c. of blood is, therefore, delivered into a test tube containing 8 c.c. of $N/12$ sulphuric acid, and the mixture is shaken thoroughly at each sampling. After addition of the last sample, 1 c.c. of a 10 per cent. solution of sodium tungstate is added, the solution well shaken, and the estimation of the sugar carried out on the filtrate after removal of the precipitate. No glycolysis occurs over the five hours of the experiment.

The difference between the initial blood sugar and the average blood sugar for the five hours, expressed as a percentage of the initial figure, is referred to as the "percentage blood sugar reduction."

*Second Part of the Test.*—The same animals are again prepared for test three or four days later by withholding food for the previous 22 hours. The same doses per kilo. are used, but on this occasion the animals which were given the standard

preparation on the first day now receive the dose of the sample, whilst those which were given the sample on the first day now receive the dose of the standard. The percentage blood sugar reductions are determined as before.

The sum of the percentage blood sugar reductions of the animals when given the dose of standard, and the corresponding sum when given the dose of the sample are now determined, and the second figure expressed as a percentage of the first. This figure represents the percentage activity of the unknown solution in terms of the solution of the standard preparation.

While the total percentage reduction is roughly proportional to the dose within certain limits, as in all physiological assays it is possible to obtain an accurate determination only when the dose of the sample produces the same effect as the dose of the standard. In this case the assay may be considered accurate to within $\pm 10$ per cent.; frequently a greater accuracy is attained. When the result of the test indicates that the assumed value of the sample differs from the observed value by more than 10 per cent., the test must be repeated, using a dose of the sample per kilo. body weight calculated on the basis of the first test to contain 0·5 unit.

Success in the method depends chiefly on a proper selection of rabbits and of the dose given to them, and upon a rigid adherence to the exact times for taking blood samples.

(2) MOUSE TEST.—An inbred stock should be employed and should be maintained at a constant environmental temperature and on a constant diet, such as brown bread soaked in water, and mixed with canary, millet, hemp, or linseed in rotation, cod-liver oil and chopped cabbage being added once, and dried cheese twice a week. All mice are starved from 5 p.m. on the day preceding test.

At least sixty mice, weighing between 16 and 22 grms. each, are divided into two groups as uniformly as possible. Each mouse of the first group is then given a subcutaneous injection of 0·02 unit of standard, and each of the second group a dose of the sample expected to be equivalent. Both the standard and sample under examination are diluted with water acidified to $p_H$ 4·0, so that the required doses are contained in 0·1 c.c. of solution. Immediately after injection the animals are placed in small ventilated boxes immersed in a specially constructed thermostat at a constant temperature of 37·5° C. Five mice are put into each box, and it is our practice to inject in groups of five, standard and sample in alternate order. The number of convulsions occurring during the ensuing $1\frac{1}{2}$ hours is noted, and the percentage on standard and sample is determined. Animals developing convulsions are immediately given a subcutaneous injection of 0·2 c.c. of 20 per cent. glucose solution. They suffer no ill effects, and can be used again in subsequent tests.

The result of the assay is obtained from a curve relating dose given to percentage developing convulsions. Such a curve is constructed by injecting the standard preparation in varying doses into a large number of mice, and may be kept as a standard of reference. The curve that we use was obtained by injecting about 240 mice for every point.

In calculating the result of a particular test, the dose of standard corresponding with the observed convulsions on the standard is read off on the curve. The ratio

between this and the actual unit value of the dose injected gives a factor for the day variation. The dose of standard that would correspond with the number of convulsions given by the sample is next read from the curve, and the result is divided by the day variation factor.

Thus if $x$ and $y$ are the percentage numbers of convulsions observed on the sample and standard respectively, and from the curve, B is the "characteristic" dose of standard to give $y$ per cent. of convulsions, then the ratio $\frac{B}{0{\cdot}02}$ gives the Day Variation Factor, 0·02 unit being the actual dose of standard given.

If A is the dose from the curve corresponding with the $x$ per cent. of convulsions obtained on the sample, the potency is then given by $A \times \frac{0{\cdot}02}{B}$, for the number of units contained in the 0·1 c.c. injected.

The potency of the original solution is calculated from this result by reference to the dilution made.

With the use of such a "characteristic" curve and of thirty mice on the standard preparation and on the sample, the error of the result may be considered to be $\pm 10$ per cent., provided that the number of convulsions on the standard is approximately equal to that on the sample. If this is not the case, the test should be repeated, of doses of standard and sample being used which are estimated to produce the same number of convulsions in both groups of mice. The result shows the greatest accuracy when the number of convulsions falls on the straight part of the curve (30–50 per cent.). Tests in which a very high or very low value is obtained should be repeated after adjustment of the dose.

## PITUITARY POSTERIOR LOBE EXTRACT

Since it is possible to separate the different activities of this extract, it is necessary to carry out separate tests for each activity. The same International Standard is used for each one; from 0·5 mgrm. of this preparation, 1 unit of specific activity can be obtained. The standard should be kept in a weighing bottle *in vacuo* over phosphorus pentoxide. For the test a small quantity is accurately weighed out into a test-tube, and 0·25 per cent. acetic acid is added, a volume of 1 c.c. being used for each mgrm. of powder taken. The test-tube is plugged with cotton-wool, heated on the boiling water-bath for 3 to 10 minutes, and the extract, which contains 2 units of activity per c.c., is filtered through dry paper.

Pituitary posterior lobe extract is used clinically for its activity in stimulating contraction of the uterus, raising the blood pressure, and inhibiting the secretion of the kidneys. Tests should be carried out for each of these properties. Although we have found that the oxytocic or uterus-stimulating power and the pressor or blood pressure-raising power usually vary together, it is generally considered that they are properties of two different substances which can be separated; the principle which inhibits kidney secretion is the same as, or closely related to, that acting on the blood pressure.

Test for Oxytocic Potency.—The excised uterus of the virgin guinea-pig is used as test object; the technique of the test is described by J. H. Burn and H. H. Dale (*Reports on Biological Standards*, I, *Pituitary Extracts*, Medical Research

Council: Special Report No. 69, London, 1922). Attention must be directed particularly to the following points:—Guinea-pigs weighing 200–250 grms. are the most suitable. They should have been kept away from contact with males from the time of weaning. The vagina should be closed, indicating that the animal is not in oestrus. It is killed by a blow on the head, and one horn of the uterus, together with the ovary at its upper pole, is carefully dissected out without stretching, the lower end being cut away from the vagina. It is then set up in a bath of warm oxygenated Ringer–Locke solution, the lower end being attached to a fixed hook, and the upper hook passing through the ovary and being attached to a frontal writing lever. The bath, which should contain about 100 c.c. of Ringer–Locke solution, is surrounded by a water-bath maintained at a constant temperature of 37° C. The preparation is left undisturbed for about 20 minutes, during which time it relaxes to a constant level. A suitable uterus should show small irregular contractions. If these are large, it should be discarded and a fresh one obtained. A suitable formula for the Ringer–Locke solution is as follows:—Sodium chloride, 9; potassium chloride, 0·42; calcium chloride, 0·24; magnesium chloride, 0·005; glucose, 1·0; sodium bicarbonate, 0·5 grms.; water (distilled), 1 litre.

It is absolutely essential that the salts and water used should be free from traces of the heavy metals.

The apparatus is so designed that the Ringer–Locke solution can be repeatedly run off and replaced by fresh solution at the same temperature. The lever should be provided with a Bowden cable, such as is used for the shutter release of a camera, to hold it steady during the time the solution is being changed. The lever should be weighted with 0·5 to 1 grm.; the magnification need not be more than two diameters.

In carrying out the test the standard solution should be diluted 100 times. The first dose may be 0·5 c.c. of this dilution, that is, 0·01 unit, but less or more may be required according to the uterus. A dose must be selected which gives a contraction slightly less than the maximum. Alternate doses of the standard and the solution under test are then added to the bath at regular intervals, which may vary in different experiments from 10 to 20 minutes. As soon as the contraction has reached its maximum after each addition, the Ringer–Locke solution is changed twice, to wash away the active principle still remaining in the bath. The object of the test is the determination of a dose of the sample which gives a contraction equal to that produced by the dose of standard. Since the contractions given by the same dose may not always be exactly the same, confirmation of equality is obtained by finding the doses of sample which produce contractions slightly less and slightly greater than that given by the dose of standard. In each case it is essential, for an accurate assay, that two pairs of doses be used for the comparison, since the size of a contraction is frequently influenced by that of the preceding response, especially when the doses given are not exactly equal. It is sometimes advisable to add the doses in the order standard, sample; sample, standard, rather than alternately. When the comparison has been carried out on six pairs of doses in this manner, the result may be considered accurate to within 15 per cent. Frequently a greater accuracy is attainable.

The uterus of the guinea-pig also contracts to histamine, which may be present as an impurity in extracts of the posterior lobe. It can be detected by treating them with *N* sodium hydroxide solution for one hour at room temperature, neutralising to litmus, and re-testing. The oxytocic activity of the pituitary extract is destroyed by this treatment, any remaining stimulant power being due to the presence of histamine. Not more than 5 per cent. of the oxytocic power of the extract tested should be due to the latter, or not more than 0·1 mgrm. of histamine should be present per 10 units of oxytocic activity.

The United States Pharmacopoeia requires the use of this method for the assay of solution of pituitary; the standard, which is prepared from the posterior lobes of the pituitary bodies of cattle by the method of M. I. Smith and W. T. McClosky (*U.S. Public Health Service Hygienic Laboratory, Bulletin No.* 138, Washington, 1924), has since been accepted as the International Standard.

TEST FOR PRESSOR POTENCY.—This may be carried out on the decapitated cat or the anaesthetised atropinised dog. Both methods are described by the Health Organisation of the League of Nations. We have found it convenient to use the decapitated cat. The method of preparation is described by J. H. Burn (*Methods of Biological Assay*, London, 1928, p. 46).

In brief, the animal is anaesthetised with ether, the carotid arteries are tied and the spinal cord is exposed by an incision in the midline of the back of the neck, followed by removal of the arches of the upper cervical vertebrae. The spinal cord is then cut across in the upper cervical region and the brain destroyed by a probe. Preparations are made for injecting the extract into a femoral vein, and for recording the blood pressure in a carotid artery on the kymograph. It is, of course, necessary to supply artificial ventilation of the lungs after the brain has been destroyed.

The object of the assay is the determination of the dose of the preparation under test which produces a rise in blood pressure exactly similar to that given by the selected dose of standard. Doses can be given only at infrequent intervals. L. T. Hogben, W. Schlapp, and A. D. McDonald (*Quart. J. Exp. Physiol.*, 1924, **14**, 301) recommend hourly injections, but we have found it possible to give them as frequently as every half-hour. When one dose follows another too quickly the preparation has not recovered from the effects of the earlier dose, so that successive injections of the same dose produce successively smaller rises in blood pressure. Injections may be commenced about an hour after the destruction of the brain, when the blood pressure has reached a constant low level. A suitable dose for injection into a 3–4 kilo. cat is 0·25 unit. The dose of standard should be one which gives about half the possible maximum rise of blood pressure. Alternate doses of the sample under examination and the standard are given with the object of determining the dose of sample which gives the same rise of blood pressure as the dose of standard. The error of the assay may be taken as 20 per cent., but in many cases is not more than 10 per cent.

TEST FOR ANTIDIURETIC POTENCY.—Assays have been carried out on human beings, dogs, mice, and rats. The Health Organisation of the League of Nations recommends the use of the unanaesthetised dog, the normal man, or the patient

with diabetes insipidus (E. Knaffl-Lenz, *Publications of the League of Nations*, III, *Health*, 1928, III, 10, Ch. 734, p. 55). The method involving the use of rats described by J. H. Burn (*Quart. J. Pharm.*, 1931, **4**, 517) appears the most suitable. It is based on a method originally described by O. S. Gibbs for use with mice (*J. Pharmacol. Exp. Therap.*, 1930, **40**, 129).

Sixteen rats, weighing 120–140 grms. each, are employed for each test, divided into four groups of four. Males give better results than females. The test is divided into two parts. Food is withdrawn from all the animals from 5 p.m. on the day preceding the test, but water is left in their cages. Each group is placed in a metabolism cage resting on a large funnel, and given warm tap-water by mouth, with the dose of pituitary extract subcutaneously immediately afterwards. Five c.c. of water per 100 grms. body-weight are administered by means of a gum elastic catheter passed through the central hole in a small wooden gag held in the animal's mouth. Two groups are injected with the standard extract, and two with the sample under examination, a suitable dose being 0·006 unit per 100 grms. body-weight. The amount of urine excreted by each group is noted every 15 minutes, and the time taken to reach maximum excretion is thus determined. Two days later the test is repeated after a preliminary fasting period as before, but the two groups which were given the standard are now injected with the sample, and the two which were given the sample are injected with the standard.

The average times to maximum excretion for all four groups on the standard and on the sample are then determined. The relationship between the doses giving these effects is obtained by reference to a curve relating dose to effect. This is obtained in the same way as the mouse insulin curve by experimentation on a large number of animals with doses of standard ranging from 0·002 to 0·016 unit per 100 grms. body-weight, and is kept for reference (see under Physiological Assay of Insulin by the Mouse Convulsion Method for the use of such a curve). The error of the test is not more than 20 per cent.

## ADRENALINE

Adrenaline can be determined chemically by a method described by Folin, Cannon, and Denis, depending upon a colour reaction, which is sensitive to 1 in 3 million (*J. Biol. Chem.*, 1913, **13**, 479). It is not, however, absolutely specific. Briefly, the method is as follows:—Two grms. of the sample of suprarenal glands are digested with 15 c.c. of 0·1 *N* hydrochloric acid and 45 c.c. of water for one hour. The mixture is gradually heated to boiling, 5 c.c. of 10 per cent. sodium acetate solution are added, and the mixture is boiled for two minutes. It is then transferred to a 100-c.c. flask and diluted to the mark with water. As much of the solution as is required is filtered or centrifuged. Five c.c. of the clear solution are transferred to a 100-c.c. flask, and 2 c.c. of sodium tungstate reagent are added, followed by 20 c.c. of saturated sodium carbonate solution. The sodium tungstate reagent is prepared by boiling gently for two hours 100 grms. of sodium tungstate, 80 c.c. of 85 per cent. phosphoric acid, and 750 c.c. of water; the solution is made up to one litre when cold. After addition of the sodium carbonate solution, the mixture is shaken, and, after two to three minutes, is made up to volume with water. One c.c. of a freshly-prepared 0·1 per cent.

uric acid solution is put into a second 100-c.c. flask, and 2 c.c. of the reagent and 20 c.c. of saturated sodium carbonate solution are added. After two to three minutes the standard solution is also made up to volume, and the two solutions are compared in the colorimeter; the result is calculated from the known fact that uric acid gives a colour which is identical in kind with, but is only one-third as intense as that given by adrenaline. The results by this method agree well with, on the whole, those obtained by the physiological test.

The optical rotation of a sample of adrenaline should be a good indication of its purity. We have found, however, that many samples showing rotations from $-49°$ to $-53°$ with the sodium line, when assayed physiologically, show only 64–88 per cent. of the standard. It is therefore necessary to carry out a physiological test, especially when the adrenaline has been prepared synthetically. The standard used is a sample of pure crystalline laevo-rotatory adrenaline issued under the auspices of the Health Organisation of the League of Nations. For the test either the decapitated cat or the anaesthetised atropinised dog may be used. We have employed the former preparation, which is made as described for the test of the pressor hormone of pituitary posterior lobe extract.

The standard preparation should be dissolved in water, brought to an acidity of $p_H$ 2 by means of hydrochloric acid, at a concentration of 0·1 per cent. The further dilution of this and other preparations of similar concentration is usually 1 in 100, and should be made with 0·9 per cent. sodium chloride solution, acidified to $p_H$ 2 with hydrochloric acid. Such a dose of the standard should be selected as will just produce a submaximal rise of blood pressure; 0·0005 mgrm. is commonly the most suitable dose, but this may vary between 0·002 and 0·01 mgrm.

The test differs from that of the pressor hormone of pituitary posterior lobe extract in the fact that doses can be given at intervals of a few minutes. Once the standard dose has been chosen, it should be given throughout alternately with a dose of the sample under examination. It is essential to repeat each dose at least once, and an assay can be considered satisfactory only when doses of the unknown have been found which give rises of blood pressure just less than, equal to, and just greater than, those produced by injections of the dose of standard.

The United States Pharmacopoeia recommends the use of anaesthetised atropinised dogs. The general principles of the assay are exactly the same as when the decapitated cat is used. The sample under examination is compared with a solution of standard epinephrine.

J. C. Munch and W. A. Deckert (*J. Amer. Pharm. Ass.*, 1930, **19**, 354) found that procaine increased the sensitiveness of the dog to a subsequent injection of adrenaline. In carrying out the test, therefore, a series of doses of the solution of standard adrenaline is first given to determine the sensitivity of the animal, and this is followed by a single injection of the solution under test. The pressor response given by the latter is then equated to that given by one of the doses of standard.

## THYROXINE AND DRIED THYROID

As we consider the chemical test a more suitable method of assay, we shall not do more than mention that three different methods of physiological assay

are available, namely, the acetonitrile test, the tadpole test, and the mouse metabolism test. In the first the potency of the preparation is determined by its power of increasing the resistance of mice to acetonitrile, in the second by its effect in accelerating metamorphosis of tadpoles with corresponding decrease in size, and in the third by its influence in stimulating the output of carbon dioxide and consumption of oxygen. The errors of these methods are about 30 per cent. The Health Committee of the League of Nations have recommended the acetonitrile test, and have proposed the use of a preparation of thyroid gland containing 0·2 per cent. iodine in specific combination as the standard. (E. Knaffl-Lenz, *Publications of the League of Nations*, III, *Health*, 1928, III, 10, Ch. 734, p. 35.)

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# Notes on the Use of the Hortvet Apparatus in Determining the Freezing-Point of Milk

BY H. C. LOCKWOOD, B.Sc., A.I.C.

(*Read at the Meeting, April* 6, 1932)

THE Hortvet apparatus,* as supplied, lacks various refinements necessary for rapid and accurate determinations. The following modifications are therefore suggested.

ELIMINATION OF PARALLAX.—Accurate readings to a thousandth of a degree are difficult with a solid-stem thermometer, owing to marked parallax. This is aggravated by the attached lens, especially as the lens can be swivelled in all directions. The error was avoided in the following manner:

A horizontal pointer, AB, made of sheet metal, was fixed radially on the frame of the lens at C, the point nearest the thermometer stem. The pointer extended across the face to the centre of the lens, and the other end was of sufficient length just to touch the thermometer when turned to its nearest position by swivelling the bracket, D. The lens must always be used in a vertical plane, parallel with the thermometer, and all adjustment is obtained by swivelling and sliding on the main supporting pillar. Any adjustment which throws the plane of the lens out of the vertical must be avoided. Some of the fitments supplied appear to be superfluous.

* A general description of the Hortvet apparatus is given by Elsdon and Stubbs (*J. Soc. Chem. Ind.*, 1931, **50**, 135–141T, and they append a comprehensive list of references.

A convenient way of arranging the thermometer parallel with the main lens support, E, is as follows:—The pillar, E, is sighted with each of the two parallel edges of the back-board of the instrument and should be found parallel by construction. If not parallel, the pillar should be adjusted.

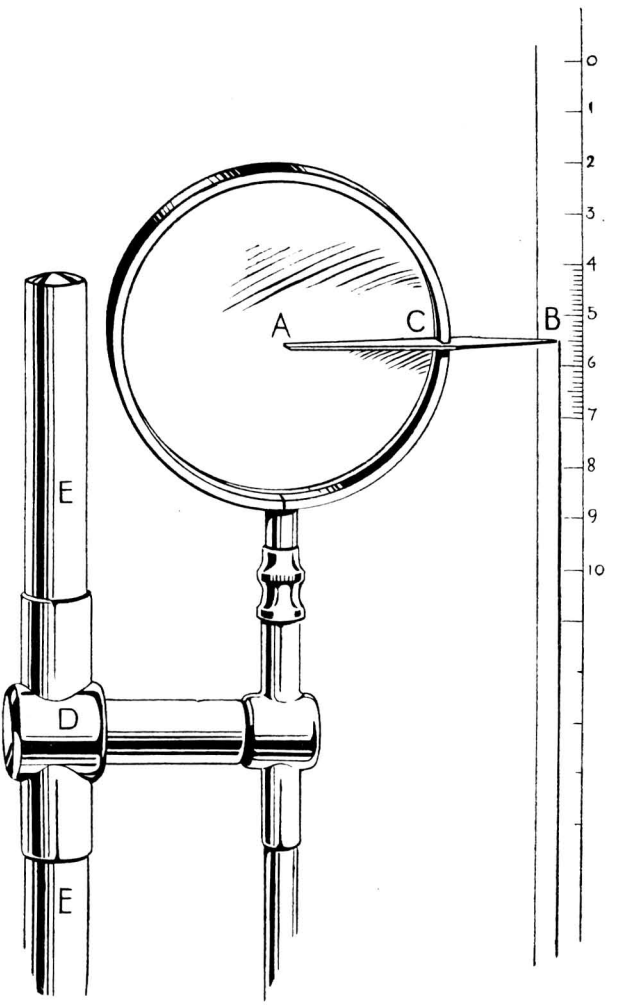


Fig. 1

When the thermometer is inserted, it is necessary to sight it against each edge of the back-board of the instrument, and adjust it until parallel. A certain amount of adjustment is given by the freezing-point tube, but large deviations would have to be overcome by re-fitting the large metal tube in the cork, or by packing the Dewar flask. The correct position, having been once found, is easily re-obtained for subsequent determinations. In Fig. 1 the pointer, AB, being at right angles to the pillar, E, is made, therefore, to move at right angles to the thermometer, and when the lens bracket, D, is swivelled, the pointer, AB, moves

in a plane at right angles to the thermometer. Thus, if B, the end of the pointer, touches the thermometer opposite the top of the column of mercury, and the bracket is then swivelled on pillar, E, as described later, the pointer, AB, is in the plane at right angles to the stem of the thermometer containing the top of the column of mercury. If the reading is then taken through the centre of the lens at A, the eye, A, and the top of the column of mercury are in a straight line at right angles to the stem of the thermometer, and parallax is thereby avoided.

In the determination the column of mercury is watched until stationary after the necessary tapping of the thermometer. The lens is then adjusted by turning and sliding the split sleeve, D, on the main support, E, so that the pointer touches the thermometer stem and is level, as near as can be judged, with the column of mercury. The inaccuracy introduced here is of the same order as that due to parallax, which may amount to *plus* or *minus* five-thousandths of a degree. The main pillar, E, is then firmly clasped by the left hand, the thumb-nail being placed under the sleeve so that it acts as a guide when the lens bracket is turned through approximately 90°. The eye is then placed at such a distance from the lens (say 6 inches),that it can focus the thermometer and yet see the pointer at the centre of the lens. The eye is then moved up or down until the centre point corresponds with the top of the thread, when the reading can be made conveniently with complete avoidance of parallax. By this method the reading is taken at right angles to the stem of the thermometer, and different observers agree to a thousandth of a degree.

The increase in the accuracy of the reading can be calculated, as follows, by consideration of Fig. 2, where the dimensions shown are based on the particular apparatus at present in use:

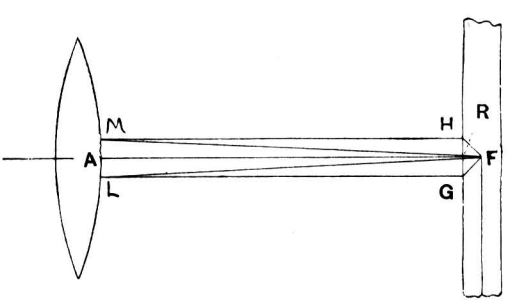


Fig. 2

In Fig. 2 F is the top of the column of mercury, and R is the correct reading to be obtained, the line RF being at right angles to the stem of the thermometer. By actual experiment it was found that the inaccuracy due to parallax may amount to ±0·005°, which, in actual measurement, equals 1 mm. (1° C. = 10 cm.). The pointer, AB, of Fig. 1, can be made to touch the thermometer near the correct reading, R, with this degree of accuracy, and this is shown exaggerated in the diagram as HG, where HG represents 1 mm. or 0·01° C. The lines HM and GL are at right angles to the stem of the thermometer, and represent the movement of the pointer, AB, when the lens-bracket is turned on the main supporting pillar. The points M and L give the maximum variation of A, the centre of the lens,

when taking the reading of F. By joining M and L to F, two lines are given which cut the graduations on the stem of the thermometer a little above and below R, the correct reading to be obtained. The accuracy of the reading depends on the amount of scale intercepted by the angle MFL, since the eye is kept within this angle by the pointer at the centre of the lens.

By actual measurement the thermometer stem is 9 mm., so that RF = 4·5 mm., and HG is 1 mm. When the lens-bracket is turned to take the reading, the distance of the centre of the lens to the thermometer thread, AF, is 100 mm. Hence in the triangle MFL, the intercept of $\angle$MFL on G

$$= \mathrm{ML}\,\frac{\mathrm{RF}}{\mathrm{AF}}$$

$$= 1\,\frac{4{\cdot}5}{100}\ \text{mm., since ML} = \mathrm{HG} = 1\ \text{mm. on assumption,}$$

$$= 0{\cdot}045\ \text{mm., } i.e.\ 0{\cdot}00045°, \text{ since } 1\ \text{mm.} = 0{\cdot}01°\ \text{C.}$$

Hence, on the assumption that the reading can be taken unaided to 0·01° C., the accuracy, by the use of the pointer, is increased to 0·45-thousandths, an increase of at least twenty times. The reading is, therefore, easily obtainable to a thousandth of a degree.

In this proof the refractive index of the thermometer-stem has not been taken into account, as all the rays are practically at right angles to the thermometer stem, and thus suffer very little refraction.

STIRRING.—The rate of stirring should be fairly uniform during supercooling, and a speed of approximately one complete stroke a second was found suitable. Under these conditions the liquid under examination was kept in a fair degree of motion, and it was very exceptional for the freezing to start of its own accord before the liquid had been sufficiently supercooled, as often happens with erratic stirring. With the stirrer supplied this was found very tiring, as the arm had to be held out unsupported about 18 inches above bench level while the stirring was maintained for five or ten minutes during a determination.

A small pulley was attached to the thermometer-stem a short distance above the zero, and a light string was fastened to the stirrer, and passed over the pulley and down through a guide to the bench. A loop was made in the end of the string, so that when the loop touched the bench at a convenient place by pulling, the stirrer was at the top of its stroke, which could be pre-determined by noting the position of the handle with regard to the graduations of the thermometer. By inserting a finger in the loop it was necessary only to raise the finger three inches above the bench and then lower it, to obtain a complete stroke of the stirrer. This operation can be regularly maintained without fatigue. A small coil-spring was fitted on the stirrer between the handle and the guide to prevent the stirrer from bumping on the bottom of the tube. This way of stirring is much less tiring, but still has the disadvantage that during the tapping of the thermometer and the seeding, the stirring has to be discontinued for a short time, as both hands are engaged.

VOLUMES OF LIQUID NECESSARY FOR THE TEST.—To obtain identical conditions it is necessary to use the same volume of liquid for each test, and, to save

measuring the liquid in a cylinder, whereby the cooled liquid would be warmed considerably, a mark has been made on the boiling-tube to indicate the volume necessary so that when the thermometer is inserted, the bulb will be submerged to the lower mark.

It is also difficult to judge the amount of alcohol in the metal tube, but a suitable depth having been found, a graduation can be made on the ether gauge-tube, which is then used as a dipstick.

THE USE OF PRESERVATIVES.—It is frequently most desirable that milk samples should be submitted from the laboratories in the milk-producing areas to the main laboratory for confirmation.

The potassium chromate preserving pellets, often used, were tried with regard to their suitability for the freezing-point test. It was found that one tablet, weighing 0·117 grm., when dissolved in half-a-pint of water or milk, lowered the freezing-point, on an average, by 0·008° C., and also increased the acidity by 0·4 c.c. $N/10$ sodium hydroxide solution for 20 c.c. Even when three chromate tablets were used with half-a-pint of milk the acidity began to increase after the second day of storage at room temperature. The addition of three tablets to the half-pint of milk would lower the freezing-point by 0·024° C., representing 4 or 5 per cent. of water, and the analytical figures would also be influenced, giving an enhanced figure of 0·4 on the density, resulting in an increase of 0·1 per cent. in total solids, though the fat would remain unaltered. Too many corrections are necessary for this preservative to be adopted in determining the freezing-point of milk.

It was considered desirable to obtain a preservative having a freezing-point of −0·55° C., so that its addition to pure milk would probably not affect the freezing-point. After several preliminary experiments, a solution of formaldehyde was found which satisfied various requirements and had the great advantage of being practically neutral to phenolphthalein.

The preparation of the formaldehyde solution was found not to be as required by theory from the simple formula H.CHO, since the 40 per cent. solution contains, besides the volatile H.CHO, such compounds as the hydrate $CH_2(OH)_2$, and polyhydrates such as $(CH_2)_2O(OH)_2$. On dilution of the formalin, decomposition and molecular re-arrangement take place, and is complete in twenty-four hours, as shown, when 2 c.c. of 40 per cent. formaldehyde to 100 c.c. are used.

| Time | $\triangle$ °C. |
|---|---|
| ½ hour | 0·385 |
| 1½ hours | 0·550 |
| 24 hours | 0·630 |
| 4 days | 0·624 |

The 2 per cent. solution was further diluted by the calculated amount to give a freezing-point of − 0·55° C. This solution was found to have good keeping properties as shown below.

| Time | $\triangle$ °C. |
|---|---|
| After diluting first solution | 0·553 |
| 1 month | 0·555 |
| 3 months | 0·555 |

Occasionally a little difficulty is experienced in obtaining good duplicate readings on formaldehyde solutions, and variations may amount to 0·01° C., but, by allowing plenty of time for thawing, or by changing the solution in the boiling-tube, this difficulty is overcome. Such divergence is not of any practical importance, since only 1 c.c. of the solution is added to 100 c.c. of milk.

In preparing the stock solution, 18 c.c. of 40 per cent. formaldehyde are diluted to 1 litre and, after standing for twenty-four hours, the freezing-point is taken and the necessary dilution made. The addition of this solution to pure milk has no effect on the freezing-point, no matter in what proportion it is added. Tests were made with various amounts in order to ascertain the preserving properties.

| Volume of preservative added to 100 c.c. of milk c.c. | Temperature of room during the test °F. | Age of milk when sour Days |
|---|---|---|
| 0·5 | 60–70 | 3 |
| 1 | 60–70 | 4 |
| 2 | 60–70 | 6 |
| 2 | 75 | 3 |

Methyl red is a useful indicator to determine whether a sample of milk is too sour for the freezing-point test. So long as a red colour is not given on adding 0·1 c.c. of a 0·1 per cent. of that indicator to a small amount of milk in a test-tube, the sample can be considered suitable.

Several investigators have attempted, by titrating with $N/10$ sodium hydroxide solution, to apply a correction for the acidity of milk, but their results differed widely, varying from 0·0013° to 0·0021° C. for every 0·1 c.c. of $N/10$ sodium hydroxide solution used for 20 c.c. milk, after subtraction of the amount normally required by the milk (3 or 4 c.c.). Such a variation in the factor makes the correction unreliable, and it is better to preserve the sample.

The effect on the analysis of adding formaldehyde solution was considered, and milk to which 2 c.c. of the solution per 100 c.c. had been added, gave results which, after correction for dilution, agreed with the original sample within experimental error. The specific gravity of the formaldehyde solution was 1·002.

The addition of 1 c.c. of formaldehyde solution to 100 c.c. of milk will make no detectable difference in the determination of fat, and the density can be corrected by adding 1 per cent. of the significant figures; *e.g.* 1032·1 gives 1032·1+0·3=1032·4. This addition of formaldehyde will make practically no difference to the freezing-point of watered milk, since with 20 per cent. of added water it will lower it by only 0·001° C., and for 40 per cent. of added water by 0·002° C.—figures well within the accuracy required. It is considered very unlikely that such gross adulteration as the latter will occur.

In conclusion, I am indebted to Messrs. Cadbury Bros., Ltd., for permission to publish this portion of the research on the freezing-point of milk, which was carried out in their laboratories at Bournville.

# The Separation of Aluminium as Phosphate in the Presence of Calcium Phosphate, with Special Reference to the Action of Milk on Aluminium

By A. G. C. GWYER, Ph.D., and N. D. PULLEN, A.I.C.

There seems to be a great difference of opinion as to the best method of determining aluminium when it occurs as a phosphate in the presence of calcium phosphate. In such case the method of Carnot, or some modification of it, seems to be in general use. As is well known, this method consists in the precipitation of aluminium as the neutral phosphate from a solution rendered slightly acid with acetic acid. In the absence of calcium no particular difficulty arises, but it does not seem to be generally recognised that, in the presence of calcium, the degree of acidity at which the aluminium is precipitated is very important and even crucial.

Aluminium phosphate itself begins to precipitate at a $p_H$ value of about 3·5, whereas calcium phosphate precipitates at $p_H$ 5·7–6·0. It would appear, therefore, that, so long as the solution is rendered slightly more acid than 5·7, aluminium phosphate should precipitate free from calcium. We have found, however, that this is not the case, and it will be shown that, unless the acidity is increased to $p_H$ 4·5 or 4·0, calcium contamination will occur, and the results obtained for aluminium will be rendered worthless.

In the work which we had in hand it was desired to determine small amounts of aluminium in the presence of relatively large amounts of calcium, and the experiments here described were designed with that end in view.

The determination may be carried out in one of two ways. (*a*) Removal of the calcium first and determination of the aluminium left in the filtrate; (*b*) direct precipitation of the aluminium under conditions which ensure that the whole of the calcium remains in solution.

The first method is not entirely satisfactory with only small quantities of aluminium in the presence of relatively large quantities of calcium, as it is rather difficult to examine the calcium precipitate for traces of aluminium carried down with it. But with the second method it is a perfectly simple matter to examine the aluminium precipitate for traces of calcium. For this reason we have confined our attention to the second type of separation.

Experimental.—For the first part of the experiments on methods of separation, synthetic solutions were prepared containing—(A) 0·00139 per cent. of aluminium (as sulphate), and 0·375 per cent. calcium (as phosphate); (B) 0·00139 per cent. of aluminium (as sulphate) only.

Method I: Precipitation of Aluminium by Hydroxyquinoline.—To 100 c.c. of each of the above solutions 10 c.c. of 40 per cent. sodium acetate solution were added, and the liquid was neutralised with ammonia, and then acidified with acetic acid. Three c.c. of 2 per cent. hydroxyquinoline acetate solution in 5 per cent. acetic acid were then added, drop by drop, and the mixture was

maintained at 70° C. for 2 hours, and then allowed to stand for a further 2 hours. The precipitates were filtered off on Jena glass filters and dried at 110° C. before weighing.

| *Taken* | *Taken* |
|---|---|
| A. 0·00139 per cent. of aluminium | B. 0·00139 per cent. of aluminium |
| 0·375 ,, ,, ,, calcium | |
| *Found* | *Found* |
| A. 0·0004 per cent. of aluminium | B. 0·00137 per cent. of aluminium |
| 0·0004 ,, ,, ,, ,, | 0·00126 ,, ,, ,, ,, |

In the solutions A, both calcium and aluminium phosphates are precipitated on neutralisation and remain, in colloidal state, on addition of acetic acid. As a result, practically no hydroxyquinoline compound is formed.

This determination was then repeated with weaker solutions—

| *Taken* | *Taken* |
|---|---|
| A. 0·0005 per cent. of aluminium | B. 0·0005 per cent. of aluminium |
| 0·1 ,, ,, ,, calcium | |
| *Found* | *Found* |
| A. 0·0003 per cent. of aluminium | B. 0·0006 per cent. of aluminium |
| 0·0002 ,, ,, ,, ,, | 0·0005 ,, ,, ,, ,, |

The above results show that, whilst the hydroxyquinoline method is quite satisfactory for solutions of aluminium only, it is useless where calcium is present in relatively large amounts.

METHOD II: CARNOT'S METHOD.—One hundred c.c. of the test solution (*infra*) were neutralised with ammonia to methyl orange, and sulphur dioxide was passed in until the precipitate redissolved (Fe··· also reduced to Fe··). Sodium phosphate (0·2 grm.) and sodium acetate (5 grms.) were then added, and the solution was boiled until free from sulphur dioxide. The precipitate was then filtered off, washed, re-dissolved in hydrochloric acid, and re-precipitated as above, but without the addition of the sodium phosphate. After being washed, dried and ignited, the precipitate was weighed as aluminium phosphate.

*Taken*

0·00139 per cent. of aluminium (as sulphate)
0·375 ,, ,, ,, calcium (as phosphate)

*Found*

| | |
|---|---|
| 1. 0·00312 per cent. (as aluminium) | 3. 0·00184 per cent. (as aluminium) |
| 2. 0·0016 ,, ,, ,, ,, | 4. 0·00124 ,, ,, ,, ,, |

Further examination of these precipitates showed calcium contamination in each case.

METHOD III.—A series of tests was then made with modifications of Method II, the $p_H$ of the solution being carefully adjusted to 4·5 by means of acetic acid and sodium acetate.

As the results show, the amount of acetic acid to be added is critical, depending upon the amount of calcium present, and, unless sufficient acid is present, erroneous results will be obtained.

For preliminary tests the following solution was prepared:—0·00137 per cent. of aluminium (as sulphate); 0·05 per cent. of calcium as phosphate (approx.).

The aluminium was precipitated by adding 1 c.c. of acetic acid and 1 c.c. of saturated sodium phosphate solution, and dropping 40 per cent. sodium acetate solution into the hot mixture until the precipitate formed began to flocculate. The solution was then heated for a while, and the precipitate was separated, dried, ignited, and weighed as aluminium phosphate.

With this solution it was found that, provided the amount of acetic acid added was not less than 1 c.c., practically theoretical results were obtained.

A second solution was then prepared containing the same amount of aluminium, but twice the amount of calcium. The results from this were interesting, in that they showed that, unless the amount of acetic acid added was increased to 5 c.c., a large white precipitate of calcium phosphate came down, although the $p_H$ of the filtrate still remained below 5·0.

As this appeared to be the solution of the problem, the experiments were repeated under more carefully controlled conditions.

*Taken.*—0·00137 per cent. of aluminium as sulphate; 0·1 per cent. of calcium as phosphate.

*Found.*—(*a*) With 1 c.c. of acetic acid, 1 c.c. of sodium phosphate solution, 10 c.c. of sodium acetate solution.

Weight of ppt. (1) 0·3414 grm. containing 0·0764 grm. calcium, 22·4 per cent.
(2) 0·3123 ,, ,, 0·0704 ,, ,, 22·5 ,, ,,
Filtrate (1) 0·0168 grm. of calcium ($p_H$ 4·9).
(2) 0·0226 ,, ,, ,, ($p_H$ 4·85).

(*b*) The experiments were then repeated, this time with the use of 5 c.c. of acetic acid, instead of 1 c.c., with the following results:

Weight of ppt. as aluminium phosphate—
(1) 0·0067 grm. = 0·00132 per cent. of aluminium.
(2) 0·0069 ,, = 0·00134 ,, ,, ,, ,,

Calcium in filtrate—
(1) 0·0964 per cent. of calcium ($p_H$ 4·5).
(2) 0·0956 ,, ,, ,, ,, ($p_H$ 4·5).

Actual calcium content of original solution 0·0964 per cent.

These results show definitely that complete separation of aluminium and calcium phosphates can be effected, provided that the correct amount of acetic acid is added to give a $p_H$ between 4·0 and 4·5; with higher values for $p_H$ calcium phosphate begins to come down, giving unreliable results.

We wish to draw particular attention to these results for the following reasons: The solution, as made up, contains practically the same amount of calcium as is found in fresh milk, *viz.* 0·1 per cent. The determination, made as under (*a*), gave a white precipitate weighing some 0·34 grm., and, considering that the precipitation was made in a solution of a $p_H$ value* below 5·0, there would appear to be a certain amount of justification for assuming such a precipitate to consist of aluminium phosphate where the actual amount of aluminium present

* The $p_H$ was determined colorimetrically, bromphenol blue being used as indicator. The standard was prepared from a similar solution checked against a hydrogen electrode.

was unknown. Actually the precipitate is shown to consist of calcium phosphate, but, on the assumption that it is aluminium, then, by calculation, an aluminium content of 120 grains per gallon in the original solution is obtained.

ACTION OF MILK ON ALUMINIUM.—Tests on the action of milk on aluminium have been carried out from time to time by various investigators. In most cases no action is reported, but, from time to time, there have appeared rather alarmingly high results, which appear to show that milk has a marked solvent action on the metal. It would now appear, however, that these results could be readily explained by the effect of the calcium, as shown above. It is true that in some text-books it is stated that, provided the $p_H$ of the solution is kept below 6·0, precipitation of calcium does not take place. But we have shown above that this is incorrect, which makes it appear extremely likely that, had these precipitates been further examined, they would have been found to consist very largely of calcium phosphate and not of aluminium phosphate alone.

Experiments were then carried out by the modified methods of separation given above, to find how much aluminium, if any, is taken up by milk during the normal process of boiling in an aluminium pan.

The tests were made with fresh milk, new pans taken from stock, and an old pan which had been in continual use for more than 12 months.

The experiments were as follows:—A measured quantity of fresh milk (250 c.c.) was heated in the pans to boiling-point, allowed to boil for about two minutes, and then poured into an evaporating dish, evaporated to dryness, and very carefully ashed. The ash from each sample was dissolved in hydrochloric acid, and the solution was neutralised with ammonia and treated with acetic acid, acetate, etc., and the precipitate was weighed as aluminium phosphate.

The following results were obtained when 5 c.c. of acetic acid were used:

| | Weight of final ppt. Grm. |
|---|---|
| Blank on fresh unboiled milk .. | 0·0002 |
| Milk boiled in new pans .. | 0·0002 |
| ,, ,, ,, old ,, .. | 0·0002 |

These figures show that milk, when boiled in aluminium vessels, exerts no action whatever on the metal.

SUMMARY.—Experiments on the gravimetric estimation of aluminium as phosphate in the presence of calcium phosphate show that entirely erroneous results are obtained unless the acidity of the solution in which the precipitation takes place is very carefully adjusted. The $p_H$ should not be less than $p_H$ 4·5, obtained by the use of acetic acid and sodium acetate. It should be noted, however, that with increasing amounts of calcium in the presence of relatively small amounts of aluminium large amounts of acetic acid are required.

Other experiments, on the action of milk boiled in aluminium vessels, have shown that no action whatever takes place. (The modified method given in the text was used for the determination of any aluminium present.)

We wish to thank the British Aluminium Co., Ltd., for permission to publish these results and also Miss M. Bulling for help in carrying out a large number of determinations.

# Notes

*The Editor wishes to point out that the pages of the Journal are open for the inclusion of short notes dealing with analytical practice and kindred matters. Such notes are submitted to the Publication Committee in the usual manner.*

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## THE DETECTION OF ADULTERATION OF INDIAN COFFEE, WITH SPECIAL REFERENCE TO THE EXTRACT METHOD

In the course of investigations carried out on standards for coffee for official adoption under the Burma Food and Drugs Act, the average aqueous extract obtained with Indian coffee was found to be 28·0 per cent., which is somewhat higher than the generally accepted figure of 24·0 per cent. for genuine samples. This led to the assumption that the degree of roasting might appreciably influence the figure.

Accordingly, a graduated series of six samples was prepared from the authentic raw beans, the period of roasting being varied from 15 minutes to an hour-and-a-half, respectively. The samples were ground, and the extract was determined on 5 grms. of the dried sample in the usual way.

The percentage extract thus obtained showed a gradual decrease with increase in the time of roasting, the maximum being 28·8 per cent., and the minimum 27·9 per cent. It cannot be said, therefore, that the degree of roasting influences the result to any marked extent, if carried out within practical limits. It certainly does not account for the discrepancy noted above.

A modification of the usual formula for the estimation of chicory in coffee has, therefore, been found necessary in this laboratory. The average percentage extract of 70 for roasted chicory was substantially confirmed. Under these conditions the percentage of chicory present in a given sample is expressed by the equation: $x = \frac{E - 28}{0{\cdot}42}$, where $x$ represents the percentage of chicory, and E the percentage of extract found. (*Cf.* E. W. T. Jones in Johnson's *"Analyst's Laboratory Companion,"* 1920, p. 157, who, however, used a less complete extract.)

Although chicory is often employed to adulterate coffee in Burma, ground roasted gram (*i.e. Cicer arietinum* L., the common gram or chick pea) is more widely used. Sometimes both these substances are present in varying proportions. Chicory is darker than coffee, whereas gram possesses a rather paler tint. The reason for these mixtures, therefore, will be obvious.

Roasted gram is most readily detected by a microscopical examination of the sample. The palisade cells are of somewhat variable length, and, still remaining more or less attached to each other, are characteristic. The parenchyma and starch grains are similar to those of the common pea, though some of the starch grains are distorted, and present the usual features to be observed after subjection to excessive heating.

The extract method is of no use for the quantitative estimation of gram when used as an adulterant, as the extract figure is too near that obtained for coffee. After being detected by the microscope the adulterant may be estimated by determining the starch present, of which it contains 50 per cent.

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## THE ANTIMONY TRICHLORIDE TEST FOR VITAMIN *A*

WHEN oils are tested for vitamin *A* with Carr and Price's antimony trichloride reagent, the possibility of the presence of dissolved colouring matter which may react with this reagent should not be overlooked.

Certain oil-soluble aniline dyes give intense carmine to violet colorations with antimony chloride. Thus, when "medicinal" paraffin, coloured with an oil-soluble yellow dye to give a tint about equal to that of an average cod-liver oil, was tested with the antimony trichloride reagent in the usual way, a carmine coloration was obtained. "Medicinal paraffin" itself is quite inactive towards this reagent.

It is true that the colours obtained were not the blue which is given by cod-liver oil, but, as only four aniline dyes were tried, it is possible that other oil-soluble yellow, orange or brown dyes may give blue colorations with the reagent. The coloration nearest to the cod-liver oil blue was obtained with turmeric, which gave a bluish-violet colour. The colorations obtained with aniline dyes are stable towards heat and light, but that given by turmeric is unstable.

The dyes tested behaved in the same way towards brominated phenols, which react with oils and fats giving colours with the antimony trichloride reagent, as I have already described (ANALYST, **1931**, **56**, 104).

A. F. MCCARLEY

BIRLING, HARTON LANE,
SOUTH SHIELDS

# Bibliography on Heavy Metals in Food and Biological Material*

*(From the beginning of the year 1921 to date)*

## I. COPPER

ABDERHALDEN and MÖLLER. Iron, Copper and Manganese Content of Blood-Serum. *Z. physiol. Chem.*, 1928, **176**, 95; *B.C.A.*, 1928, 913A.

ANDRIANOFF and ANSBACHER. Copper in the Liver. *Deut. med. Wochenschr.*, 1930, **56**, 357; *C.A.*, 1930, 4544.

ANON. Deposits of Arsenic and Copper on Eating Apples. *J. Ministry Agric.*, 1925, **32**, 549; *C.A.*, 1925, 3559.

ANSBACHER, REMINGTON, and CULP. Copper Determination in Organic Matter. *Ind. Eng. Chem., Anal. Ed.*, 1931, **3**, 314; ANALYST, 1931, **56**, 684. (Colorimetric with potassium ethyl xanthate and other reagents.)

BERG. Occurrence of Unusual Elements in Foods, and in Human Excreta. *Biochem. Z.*, 1925, **165**, 461; *B.C.A.*, 1926, 195A. (Copper, zinc, lead and other metals found in excreta.)

BERRY. Report on (Methods of Determining Copper and Zinc in) Gelatin. *J.A.O.A.C.*, 1924, **8**, 166; *C.A.*, 1925, 547.

BERRY. Report on (Determination of Zinc and Copper in) Gelatin. *J.A.O.A.C.*, 1926, **9**, 458; *C.A.*, 1927, 286.

BERRY. Report on (Determination of Copper and Zinc in) Gelatin. *J.A.O.A.C.*, 1929, **12**, 416; *C.A.*, 1930, 441.

BIAZZO. Rapid Determination of Copper in Preserved Vegetable Foods. *Annali Chim. Appl.*, 1926, **16**, 96; *B.C.A.*, 1926, 382B.

BISHOP. Occurrence of Metallic Elements in Biological Material. *J. Cancer Res. Comm. Sydney*, 1930, **1**, 242; *B.C.A.*, 1930, 632A. (Copper found.)

BODANSKY. The Zinc and Copper Content of the Human Brain. *J. Biol. Chem.*, 1921, **48**, 361; *J.C.S., Abs.*, 1922, i, 194.

CHAPMAN and LINDEN. Presence of Lead and other Metallic Impurities in Marine Crustaceans and Shell Fish. ANALYST, 1926, **51**, 563. (Comparatively large amounts of copper found in some cases.)

* The Publication Committee wish to draw special attention to this Bibliography, which has been compiled by Mr. T. H. Pope at their request. Section II, on Lead, will appear in the December issue.—EDITOR.

CHERBULIEZ and ANSBACHER. Determination of Copper in Organic Matter. *Helv. Chim. Acta*, 1930, **13**, 187; ANALYST, 1930, **55**, 345. (Titration with 1:8-dihydroxy-2-nitroso-3:6-naphthalenedisulphonic acid.)

CLEVELAND and FELLERS. Mineral Composition of Dates. *Ind. Eng. Chem., Anal. Ed.*, 1932, **4**, 267; *B.C.A.*, 1932, 976A. (Small proportions of copper found in edible portion.)

CUNNINGHAM. Biochemical and Physiological Aspects of Copper in Animal Nutrition. *Biochem. J.*, 1931, **25**, 1267; *B.C.A.*, 1931, 1186A.

CURRIE. Determination of Small Quantities of Copper in Tissues. *Biochem. J.*, 1924, **18**, 1224; *J.C.S.Abs.*, 1925, i, 183. (Colorimetric, based on the formation of Scheele's green.)

DAVIES. Observations on the Copper and Iron Contents of Milk and other Dairy Products. *J. Dairy Res.*, 1931, **3**, 86; *C.A.*, 1932, 4385.

DEBORDES. Determination of Copper in Grape Must and Wines. *Ann. Falsif.*, 1929, **22**, 410; *B.C.A.*, 1929, 908B. (Copper separated as sulphide, and determined electrolytically.)

DRABKIN and WAGGONER. Maintenance and Production of Haemoglobin on Synthetic Diets: Determination of Copper. *J. Biol. Chem.*, 1930, **89**, 51; *B.C.A.*, 1931, 247A. [Precautions in using Biazzo's method (*Annali Chim. Appl.*, 1926, **16**, 96) and the potassium ethyl xanthate method.]

DUTOIT and ZBINDEN. Spectrographic Analysis of the Ash of Blood and of Organs. *Compt. rend.*, 1929, **188**, 1628; *B.C.A.*, 1929, 952A.

DUTOIT and ZBINDEN. Spectrographic Analysis of the Ashes of Organs. *Compt. rend.*, 1930, **190**, 172; *B.C.A.*, 1930, 363A. (Copper found in various organs.)

EFFRONT. Toxicity of Salts of Copper. *Compt. rend.*, 1924, **178**, 2152; *J.S.C.I.*, 1924, 689B. (With a meal, relatively large amounts of copper salts are harmless.)

ELVEHJEM and HART. Copper Content of Feeding Stuffs. *J. Biol. Chem.*, 1929, **82**, 473; ANALYST, 1929, **54**, 421. (Copper found in 47 vegetable feeding stuffs.)

ELVEHJEM and HART. Drabkin-Waggoner Modification of the Biazzo Method for Determining Copper. *J. Biol. Chem.*, 1931, **91**, 37; *C.A.*, 1931, 3697.

ELVEHJEM, KEMMERER, HART, and HALPIN. Effect of the Diet of the Hen on the Iron and Copper Content of the Egg. *J. Biol. Chem.*, 1929, **85**, 89; *B.C.A.*, 1930, 242A.

ELVEHJEM and LINDOW. Determination of Copper in Biological Materials. *J. Biol. Chem.*, 1929, **81**, 435; ANALYST, 1929, **54**, 245. (Colorimetric, with chloroform solution of the precipitate formed with potassium thiocyanate and pyridine.)

ELVEHJEM, STEENBOCK, and HART. Distribution of Copper in Blood. *J. Biol. Chem.*, 1929, **83**, 21; *B.C.A.*, 1929, 1096A.

ELVEHJEM, STEENBOCK, and HART. Effect of Diet on the Copper Content of Milk. *J. Biol. Chem.*, 1929, **83**, 27; *B.C.A.*, 1929, 1099A.

EVVARD, NELSON, and SEWELL. Copper Salts in Nutrition. *Proc. Iowa Acad. Sci.*, 1928, **35**, 211; *B.C.A.*, 1930, 638A. (Favourable results noted.)

FLATOW. Ferricyanometric Micro-methods in Blood Analysis. *Biochem. Z.*, 1928, **194**, 132; *B.C.A.*, 1928, 540A. (Determination of copper based on the disappearance of cysteine from a standard solution.)

FLINN and INOUYE. Some Physiological Aspects of Copper in the Organism. *J. Biol. Chem.*, 1929, **84**, 101; ANALYST, 1929, **54**, 758. (Copper in small amount beneficial to guinea-pigs.)

FLINN and INOUYE. Metals in our Food. *J. Amer. Med. Assoc.*, 1928, **90**, 1010; *C.A.*, 1929, 3988. (Experiments on animals.)

FOX and RAMAGE. Spectrographic Analysis of Animal Tissues. *Nature*, 1930, **126**, 682; *B.C.A.*, 1930, 1609A. (Copper of common occurrence.)

FOX and RAMAGE. Spectrographic Analysis of Animal Tissues. *Proc. Roy. Soc.*, 1931, B, **108**, 157; *B.C.A.*, 1931, 756A.

GEBHARDT and SOMMER. Determination of Copper in Dairy Products. *Ind. Eng. Chem., Anal. Edit.*, 1931, **3**, 24; *B.C.A.*, 1931, 316B. (Colorimetric.)

GODDEN. Occurrence of Traces of Certain Elements in Foodstuffs and their Role in Animal Nutrition. *Agr. Progress*, 1931, **8**, 81; *C.A.*, 1931, 4940. (Copper-contents of various foods given.)

GORTER, GRENDEL, and WEYERS. Part played by Copper in the Anaemia of Children. *Maandschrift Kindergeneeskunde*, 1931, **1**, 70; *C.A.*, 1932, 1335. (Copper-contents of bloods given.)

GRENDEL. Copper Contents of Foodstuffs. *Pharm. Weekblad*, 1930, **67**, 913; ANALYST, 1930, **55**, 700. (Colorimetric, with sodium diethyldithiocarbamate [and Henderson's reagent].)

GRENDEL. Micro-Determination of Copper in Blood. *Pharm. Weekblad*, 1930, **67**, 1345; *B.C.A.*, 1931, 378A.

GUILLEMET. Content of Copper in Blood Serum. *Compt. rend. Soc. biol.*, 1931, **108**, 32; *C.A.*, 1932, 3556.

GUILLEMET. Distribution of Copper among the Different Constituents of Normal Blood. *Compt. rend. Soc. biol.*, 1932, **109**, 652; *C.A.*, 1932, 3559.

HAGUES. Metallic Contamination of Beer. *J. Inst. Brew.*, 1931, **37**, 366; *B.C.A.*, 1931, 822B. (Copper determined colorimetrically with ferrocyanide.)

HANAK. Determination of Copper in Green Vegetables. *Z. Unters. Lebensm.*, 1930, **59**, 511; ANALYST, 1930, **55**, 883. (Colorimetric with ammonia or ferrocyanide.)

HARRY. The Application of Piperidinium Piperidyl-dithioformate to the Colorimetric Determination of Copper. ANALYST, 1931, **55**, 736.

HELLWIG and QUAM. Copper Content of Beef and Hog Tissue. *Food Ind.*, 1930, **2**, 411; *C.A.*, 1931, 4631. (Xanthate method used.)

HERTWIG. Determination of Zinc and Copper in Gelatin and Glue. *J.A.O.A.C.*, 1923, **7**, 41; *J.S.C.I.*, 1923, 1034A. (Copper determined volumetrically.)

HESS, SUPPLEE, and BELLIS. Copper as a Constituent of Milk. *J. Biol. Chem.*, 1923, **57**, 725; ANALYST, 1924, **49**, 41. (Colorimetric, with potassium ethyl xanthate.)

HIRANO and MIKUMO. Copper in the Legumes. *J. Pharm. Soc. Japan*, 1925, No. 525, 992; *C.A.*, 1926, 616. (Colorimetric with ammonia.)

HOCHSTADTER. Determination of Ash, Arsenic, Copper and Zinc in Gelatin. *Ind. Eng. Chem.*, 1923, **15**, 1197; *C.A.*, 1924, 30.

HUGUES. Copper Contents of Wines. *Prog. Agr. Vit.*, 1931, **95**, 472; *C.A.*, 1931, 4344.

INOUYE and FLINN. Determination of Copper in Biological Material. *J. Lab. Clin. Med.*, 1930, **16**, 49; *B.C.A.*, 1931, 990A. (Electrolytic.)

JÄRVINEN. Colorimetric Estimation of Small Quantities of Metals in Foodstuffs and the Preliminary Destruction of the Organic Matter. *Z. Nahr. Genussm.*, 1923, **45**, 183; *J.C.S., Abs.*, 1923, ii, 655. (Method for copper given.)

JANICSEK. Copper-content of Drugs arising from Spraying. *Magyar gyógysz. Táras. Ertes.*, 1929, **5**, 317; *B.C.A.*, 1930, 348B. (Electrolytic.)

KEILHOLZ. Determination of Some Metals and of Arsenic in Plant and Human Organs. *Pharm. Weekblad*, 1921, **58**, 1482; *J.C.S., Abs.*, 1921, ii, 708. (Scheme of analysis for arsenic, copper, manganese, zinc, aluminium, and lithium given.)

KING. The Industrial Contamination of Food with Copper. *Ind. Eng. Chem.*, 1927, **19**, 1004; ANALYST, 1927, **52**, 723. (Nickel interferes with colorimetric xanthate determination.)

KING and ETZEL. Copper as an Industrial Contamination in Foodstuffs. *Ind. Eng. Chem.*, 1927, **19**, 1004; *B.C.A.*, 1927, 973B.

KLEINMANN and KLINKE. Copper Content of Human Organs. *Arch. path. Anat. Physiol.*, 1930, **275**, 422; *B.C.A.*, 1931, 1321A. (Ethyl bromide used instead of chloroform in Schönheimer and Oshima's method.)

KREBS. Copper in Human Blood-Serum. *Klin. Wochenschr.*, 1928, **7**, 584; *B.C.A.*, 1928, 1391A.

LAMPITT, HUGHES, BILHAM, and FULLER. Determination of Copper in Foodstuffs. ANALYST, 1926, **51**, 327. (Colorimetric, and gravimetric with quinosol.)

LASAUSSE. Determination of Copper in Preserved Vegetables. *J. Pharm. Chim.*, 1923, **27**, 290; *J.S.C.I.*, 1923, 469A. (Electrolytic and iodimetric methods.)

LENDRICH and MAYER. Occurrence of Arsenic, Lead, and Copper in Fruit and Fruit Products as a Result of Spraying. *Z. Unters. Lebensm.*, 1927, **54**, 137; *B.C.A.*, 1927, 954B.

LIESEGANG. Detection of Small Amounts of Iron and Copper in Linen, Paper, or Animal Tissues. *Z. wiss. Mikroskop.*, **40**, 14; *C.A.*, 1924, 3572.

LINDOW, ELVEHJEM, and PETERSON. Copper Content of Plant and Animal Foods. *J. Biol. Chem.*, 1929, **82**, 465; ANALYST, 1929, **54**, 420. (Copper found in 160 human foods.)

LIPMAN. Chemical Elements indispensable to Plants. *Sci. Monthly*, 1928, **16**, 289; *C.A.*, 1928, 2965. (Copper in small amounts probably essential.)

LOCHTE and DANZIGER. Detection of Poisons in the Ashes of Burned Corpses. *Deut. Z. ges. ger. Med.*, 1922, I, 727; *C.A.*, 1924, 207. (Detection of copper considered.)

LYNCH, MCDONNELL, HAYWOOD, QUAINTANCE, and WAITE. Poisonous Metals on Sprayed Fruits and Vegetables. *U.S. Dep. Agric.*, 1922, *Bull.* 1027, 1; *C.A.*, 1922, 2006. (Copper [and lead] found in only small amounts, if spraying properly executed.)

MCHARGUE. Copper, Manganese, Zinc, Nickel, and Cobalt as Vital Factors in Soils, Plants, and Animals. *J. Agric. Res.*, 1925, **30**, 193; ANALYST, 1925, **50**, 409.

MCHARGUE. Significance of the Occurrence of Copper, Manganese, and Zinc in Forage Crops and Foods. *J. Amer. Soc. Agron.*, 1925, **17**, 368; *C.A.*, 1925, 3330.

MCHARGUE. Association of Copper with Substances containing the Fat-soluble *A* Vitamin. *Amer. J. Physiol.*, 1925, **72**, 583; *J.C.S., Abs.*, 1925, i, 1515.

MCHARGUE. Report on (Determination of) Less Common Metals in Plants. *J.A.O.A.C.*, 1930, **13**, 225; *C.A.*, 1930, 3964. (Method for copper.)

MAYRHOFER. Rôle of Certain Elements occurring in the Organism. *Pharm. Presse*, 1924, **29**, 69, 78, 106, 114, 122; *C.A.*, 1924, 3629. (Copper determined microchemically with mercuric ammonium thiocyanate.)

MEHURIN. Determination of Ash, Arsenic, Copper, and Zinc in Gelatin. *Ind. Eng. Chem.*, 1923, **15**, 942; *J.C.S., Abs.*, 1923, ii, 891. (Copper: Colorimetric with potassium ferrocyanide.)

MEHURIN. Determination of Ash, Arsenic, Copper and Zinc in Gelatin. *Ind. Eng. Chem.*, 1923, **15**, 1197; *C.A.*, 1924, 30.

MEHURIN. Determination of Copper and Zinc in Gelatin. *J.A.O.A.C.*, 1930, **13**, 479; *C.A.*, 1931, 615.

MEYER. New Reaction for Dyestuffs containing Copper in Hair and its Use in the Identification of Dye in Hair. *Chem. Ztg.*, 1932, **56**, 95; ANALYST, 1932, **57**, 333. (Effervescence with hydrogen peroxide and ammonia.)

MINISTRY OF HEALTH. Report on the Composition of Commoner British Wines and Cordials, No. 24. ANALYST, 1924, **49**, 387. (Copper found in both alcoholic and non-alcoholic wines.)

MISCALL, CAVANAUGH, and CARODEMOS. Copper in Dairy Products and its Solution under Various Conditions. *J. Dairy Sci.*, 1929, **12**, 379; *C.A.*, 1929, 5512.

MORRISON and NASH. Copper Content of Infant Livers. *J. Biol. Chem.*, 1930, **88**, 479; *B.C.A.*, 1930, 1465A.

MORVILLEZ and DEFOSSEZ. Reduction of Molybdic Reagents by Cherry Laurel Water. *J. Pharm. Chim.*, 1932, **124**, 27; ANALYST, 1932, 581. (Copper constantly present.)

MUTTKOWSKI. Copper: Its Occurrence and Rôle in Insects and Other Animals. *Trans. Amer. Micr. Soc.*, 1921, **40**, 144; *C.A.*, 1923, 2154.

NITZESCU. Content of Copper in the Organs of the Human Foetus. *Compt. rend. Soc. biol.*, 1931, **106**, 1176; *C.A.*, 1932, 3016.

PHILIPPI and HERNLER. Micro-electrolytic Determination of Copper in Organic Substances. *Microchem., Emich Festschr.*, 1930, 241; *B.C.A.*, 1930, 1546A.

POIROT. Detection of Copper in Distilled Water. *J. Pharm. Chim.*, 1924, [vii], **30**, 393; *J.C.S., Abs.*, 1925, ii, 242. (Colorimetric with guaiacum resin, pyridine, alcohol and hydrogen peroxide.)

PYRIKI. Colorimetric Determination of Lead and Copper in Water. *Z. anal. Chem.*, 1924, **64**, 325; ANALYST, 1924, **49**, 491. (With slight adjustment, Winkler's ferrocyanide and sulphide method for determining lead and copper simultaneously is satisfactory.)

QUAM. Copper Contents of Livers and Liver Extracts. *Proc. Iowa Acad. Sci.*, 1929, **36**, 267; *C.A.*, 1931, 1269.

QUAM and HELLWIG. Copper Content of Milk. *J. Biol. Chem.*, 1928, **78**, 681; ANALYST, 1928, **53**, 542.

QUARTAROLI. Determination of Minimal Amounts of Manganese, Copper and Iron in Biological Investigations. *Annali Chim. Appl.*, 1927, **17**, 361; *B.C.A.*, 1928, 96A. (For copper, the catalytic effect of the metal on hydrogen peroxide is used.)

QUARTAROLI. Copper as a Normal Component of Plants. *Annali Chim. Appl.*, 1928, **18**, 47; *B.C.A.*, 1928, 561A.

QUARTAROLI. Problem of Copper as a Normal Component of Plants. *Annali Chim. Appl.*, 1928, **18**, 427; *C.A.*, 1928, 2387.

QUARTAROLI. Copper as an Element necessary to Plants. *Annali Chim. Appl.*, 1929, **19**, 467; *B.C.A.*, 1930, 385A.

RAMAGE. Mushrooms—Mineral Content. *Nature*, 1930, **126**, 279; *B.C.A.*, 1930, 1325. (Copper found in all parts.)

REMINGTON and SHIVER. Iron, Copper and Manganese Content of some Common Vegetable Foods. *J.A.O.A.C.*, 1930, **13**, 129; *B.C.A.*, 1930, 392B.

REMY. Normal Occurrence of Copper and Zinc in Foods. *Z. Volksernähr. Diatkost*, 1931, **6**, 21; *C.A.*, 1932, 4105. (Amounts of copper normally present sufficient to maintain ionic equilibrium.)

RIBÉREAU-GAYON. Iron and Copper in White Wines. *Ann. Falsif.*, 1930, **23**, 535; *B.C.A.*, 1931, 563B.

RICE and MISCALL. Copper in Dairy Products and its Solution in Milk under Various Conditions. *Int. Rev. Sci. Pract. Agric.*, 1923, **1**, 1095; ANALYST, 1924, **49**, 145. (Colorimetric, with potassium ethyl xanthate.)

RICHARDSON. Determination of Minute Amounts of Lead and Copper in Food, Beverages, etc. ANALYST, 1930, **55**, 323. (Colorimetric, with hydrogen sulphide.)

ROSENTHALER. Economical Drug Testing. *Pharm. Ztg.*, 1929, **74**, 1279; *C.A.*, 1930, 462. (Microchemical test for copper.)

ROUSSEL and DUFOUR-DEFLANDRE. Copper Reserves in the Foetal Liver of Vertebrates. *Compt. rend. Soc. biol.*, 1931, **106**, 260; *C.A.*, 1931, 3715. (Detection with diphenylcarbazide.)

SALE. Report on Water. *J.A.O.A.C.*, 1922, **5**, 379; *C.A.*, 1922, 2563. (Copper determined colorimetrically with ferrocyanide.)

SALE. Report on Water. *J.A.O.A.C.*, 1923, **6**, 307; *C.A.*, 1923, 1853. (Methods for zinc, lead and copper, the last colorimetric with ferrocyanide.)

SCHINDEL. Copper Content of Blood. *Klin. Wochenschr.*, 1931, **10**, 743; *B.C.A.*, 1932, 183A.

SCHÖNHEIMER and OSHIMA. Copper Content of Normal and Pathological Organs. I. Method; II. Copper Content of Normal and Haemachromatose Liver, Gallstones, and Whole Blood. *Z. physiol. Chem.*, 1929, **180**, 249; *B.C.A.*, 1929, 341A. (Colorimetric, with ammonium thiocyanate and pyridine.)

SCHOORL. Examination of Tap Water for Small Quantities of Copper and Lead. *Chem. Weekblad*, 1932, **29**, 338; *C.A.*, 1932, 4661. (Colorimetric determination of copper as sulphide and as ferrocyanide.)

SCHUETTE and REMY. Degree of Pigmentation and its Probable Relationship to the Mineral Constituents of Honey. *J. Amer. Chem. Soc.*, 1932, **54**, 2909; ANALYST, 1932, 578. (Copper found in all honeys examined.)

SCHÜTZ. Detection of Lead and Copper Salts in Citric and Tartaric Acids. *Pharm. Ztg.*, 1929, **74**, 1127; *B.C.A.*, 1930, 36B. (Critical study of officinal [D.A.B. VI.] German methods.)

SEVERY. Occurrence of Copper and Zinc in Certain Marine Animals. *J. Biol. Chem.*, 1923, **55**, 79; *J.C.S., Abs.*, 1923, i, 415. (Copper found in 16 marine animals, but not in mammals or clams.)

SHELDON and RAMAGE. Spectrographic Analysis of Human Tissues. *Biochem. J.*, 1931, **25**, 1608; *B.C.A.*, 1931, 1442A. (Copper universally present.)

SHIKATA, TACHI, and HOZAKI. Application of the Polarograph to the Analysis of Abnormal Mineral Constituents. *Bull. Agr. Chem. Soc. Japan*, 1927, **3**, 52; *C.A.*, 1928, 930. (Determination of copper.)

SHOJI. Analysis of Liver Ash by means of the Polarograph. *Bull. Inst. Phys. Chem. Research, Tokyo*, 1931, **10**, 162; *C.A.*, 1931, 4293. (Data for copper.)

SIMPSON. Minor Elements in Living Organisms. *Chem. Eng. Mining Rev.*, 1932, **24**, 323; *C.A.*, 1932, 4612. (Copper and other elements referred to.)

SMITH. Report on Gelatin. *J.A.O.A.C.*, 1923, **7**, 135; *C.A.*, 1924, 716. (Method for copper reported on.)

SPENCER. Report on (Determination of) Metals in Foods. *J.A.O.A.C.*, 1931, **14**, 434; *C.A.*, 1932, 774. (Methods for copper discussed.)

SPRINGER. Electrolytic Determination of Copper in Preserved Vegetables. *Z. Unters. Lebensm.*, 1929, **58**, 651; *B.C.A.*, 1930, 529B.

SUPPLEE and BELLIS. The Copper Content of Cow's Milk. *J. Dairy Sci.*, 1922, **5**, 456; *C.A.*, 1923, 156. (The potassium ethyl xanthate method found best.)

TAVERNE. Content of Copper and Zinc in Normal and Carcinomatous Tissues. *Nederl. Tijdschr. Geneeskunde*, 1923, **67**, i, 2810; *J.C.S., Abs.*, 1924, i, 123.

TITUS and HUGHES. Nutritional Value of Copper in Powdered Dried Milk. *J. Dairy Sci.*, 1929, **12**, 90; *C.A.*, 1929, 1939. (The copper from the vacuum pans may help to prevent anaemia, but tends to destroy vitamins.)

TROTMAN and SUTTON. Determination of the Inorganic Impurities in Gelatin. ANALYST, 1924, **49**, 271. (Method for copper given.)

TURNWALD and HAUROWITZ. Heavy Metals of the Human Liver and their Spectrographic Detection. *Z. physiol. Chem.*, 1929, **181**, 176; *B.C.A.*, 1929, 591A.

VAN LEEUWEN. Copper and Manganese Content of some Vegetable Tissues. *Ann. physiol. physico-chim. Biol.*, 1930, **6**, 178; *C.A.*, 1931, 2171.

VON DER HEIDE. Determination of Copper and Zinc in Wine. *Z. anal. Chem.*, 1925, **66**, 24; ANALYST, 1925, **50**, 296. (Both metals precipitated as sulphides and weighed as oxides.)

WALKER. Report on (Determination of) Copper (in Foods). *J.A.O.A.C.*, 1930, **13**, 426; *C.A.*, 1931, 357. (Iodimetric method given.)

WALKER. Report on (Determination of) Copper (in Foods). *J.A.O.A.C.*, 1931, **14**, 450; *C.A.*, 1932, 775. (Method previously given is satisfactory [*J.A.O.A.C.*, 1930, **13**, 426].)

WARBURG. Determination of Copper and Iron, and Copper Content of Blood-Serum. *Biochem. Z.*, 1927, **187**, 255; *B.C.A.*, 1927, 985A.

WARBURG. Copper in Human Blood-Serum. *Klin. Woch.*, 1927, **6**, 1094; *B.C.A.*, 1928, 317A. (Colorimetric with ferrocyanide, or catalytically by oxidation of cysteine.)

WARBURG and KREBS. Loosely-combined Copper and Iron in Blood-Serum. *Biochem. Z.*, 1927, **190**, 143; *B.C.A.*, 1928, 192A.

WEBSTER and JANSMA. Copper-Content of Wheats of Oklahoma and other States. *Science*, 1929, **70**, 174; *B.C.A.*, 1930, 120A.

WHITE. Copper in Tumours and Normal Tissues. *Lancet*, 1921, **201**, 701; *J.C.S., Abs.*, 1922, i, 399. (Copper found in all animal and vegetable tissues examined, especially in degenerating tumours.)

WILLIAMS. Determination of Copper and Iron in Dairy Products. *J. Dairy Res.*, 1931, **3**, 93; *C.A.*, 1932, 4385. (After wet ashing, the copper is determined colorimetrically by the xanthate or diethyldithiocarbamate method.)

WRIGHT and PAPISH. Inorganic Constituents of Milk. *Science*, 1929, **69**, 78; *B.C.A.*, 1929, 953A. (Spectrographic method; copper in unusually large amount found in dried milk.)

ZANDA. Significance of Copper in the Animal Organism. *Biochim. terap. sperim.*, 1924, **11**, 7; *J.C.S., Abs.*, 1925, i, 719. (Haematoxylin test and phenolphthalein blood reaction for copper.)

ZBINDEN. The Infinitely Small Quantities of Certain Elements in Milk and their Detection by the Spectrographic Method. *Lait*, 1931, **11**, 114; *C.A.*, 1931, 4032. (Copper found regularly.)

ZONDEK and BONDMANN. Copper in Human and Cow's Milk. *Klin. Wochenschr.*, 1931, **10**, 1528; *C.A.*, 1932, 184.

T. H. P.

# Notes from the Reports of Public Analysts

*The Editor would be glad to receive the Annual or other Reports of Public Analysts containing matter of special interest to the Society. Notes made from such Reports would be submitted to the Publication Committee.*

## CITY OF LEEDS

### ANNUAL REPORT OF THE CITY ANALYST FOR 1931

OF the 3043 samples examined during the year, 1975 were of food and drugs, and, of these, 8·6 per cent. were adulterated.

BLACK BEER.—Two samples were taken at a shop at the request of the Ministry of Health, as a sample taken at the brewery by the Board of Customs and Excise contained arsenic in excess of the recognised amount. These two samples contained 1/25 and 1/80 grain of arsenic per gallon, respectively, but no action was taken, as this preparation is used as a beverage only after considerable dilution.

STARCH IN POTTED SALMON.—Two samples were reported against, and, in the case of one, which contained 20 per cent. of starchy filling material, proceedings were taken, and costs were obtained against the retailer (*cf.* ANALYST, 1931, **56**, 658, 742; 1932, **57**, 382).

C. H. MANLEY

# Fourth National Congress of Pure and Applied Chemistry

THE Fourth National Congress of Pure and Applied Chemistry, organised by the Italian Chemical Association, was held in Rome from June 6th to 12th. Dr. Maselli, President of the Association, presided, and there were present more than five hundred members and guests, including representatives of the National Government and of the Governor of Rome, the Prefect, and the delegates of several foreign chemical Societies.

The Society of Public Analysts and Other Analytical Chemists, who had been invited to send a delegate, were represented at the meeting by Mr. A. H. Bennett.

After the speeches of welcome and an account, given by Professor Parravano, of the administration of the fund which had been collected for the promotion of chemical research, the President announced the conferment of the Emanuele Paterno medal on Professor Gustav Tammann, of the University of Göttingen, and Professor Parravano gave a brief account of the medallist's work.

The President's address was devoted principally to a review of the recent progress of chemical industry in Italy, and Professor Tammann delivered a lecture on the behaviour of polonium and thorium B and of their relations with other metals.

The remaining days were occupied by lectures, visits to laboratories and chemical works, and social functions.

# Department of Scientific and Industrial Research

## REPORT OF THE FOOD INVESTIGATION BOARD FOR THE YEAR 1931*

AMONGST the many researches in progress it is possible to mention only a few. Some of the work of the year has already been published, and the Report gives all references.

### PART I

SECTION A. MEAT.—Sheep's liver contains an appreciable amount of vitamin *C*, but when held at −19° C. the activity progressively diminishes. The work of Edsall and Muralt on the physical chemistry of muscle affords good evidence that muscle globulin can be isolated in a condition very little different from that in which it occurs in muscle fibre. The physical changes in muscle when frozen seem to be due to changes in equilibrium between the muscle and its aqueous environment, and the constitution of muscle below the freezing point is mapped out. A study of the kinetics of the oxidation of haemoglobin to methaemoglobin has shown the reaction to be monomolecular with respect to the concentration of haemoglobin, and the rate of oxidation to vary with different samples of blood, and with the hydrogen ion and salt concentration. The work on the Action of Light on Fats (Lea) has been published (*Proc. Roy. Soc.*, **1931**, **108**, B, 175); also that on Rancidity changes referred to in the 1930 Report (ANALYST, 1931, **56**, 531). The effect of free fatty acids on the flavour of fats in the absence of rancidity was tested by adding to the fats proportions up to 15 per cent. of the appropriate fatty acids recovered after saponification. When tasted hot, none of the mixtures (even with 15 per cent. of fatty acids) could be distinguished from the controls, and in the cold no harsh or unpleasant flavour was apparent. Attempts to place the Schiff test on a quantitative basis have not proved successful, but the bisulphate method of Tomoda is proving more hopeful.

SECTION B. FRUIT AND VEGETABLES.—In the biochemical study of senescence in apples a comparison of chemical changes in Bramley's seedling and Worcester Pearmain apples stored at 1° C. has involved the compilation and tabulation of many data, including analyses of the soils of various plantations, chemical analyses of the apple tissues, average rate of loss of material in populations during storage, loss of sugar and acid, nitrogen-content, etc. The third series of experiments in connection with the study of sucrose hydrolysis and respiration is primarily a

* Obtainable at H.M. Stationery Office, Adastral House, Kingsway, W.C.2. Pp. 293. Price 5s. 0d. net.

study of the influence of temperature upon the course of chemical change. All apples appear to be free from starch when the sucrose value (glucose and fructose being considered in terms of sucrose concentration) is at 3·0. The state and mode of operation of the sucrose/enzyme/acid system in the living cell and in the *post-mortem* frozen condition are being investigated. The rôle of acetaldehyde in the respiration of plants has been further elucidated. Work on potatoes deals with temperature and metabolic balance and the effect of ethylene on metabolism.

The possibilities of employing atmospheric control for bananas in storage has received attention. Since it has become needful to store fruit under conditions allowing the critical and independent control of humidity and of air movement, a suitable apparatus allowing of these controls has been devised, and is described in detail. Acetaldehyde and ammonia have both been found successful for preventing the rotting of stored fruit, if introduced into the storage atmosphere.

The preservation of fruit and vegetables in the frozen state has received considerable further attention. Partial cooking before freezing at −10° or −20° C., is successful for peas, and the method has been applied to potatoes and runner beans and less successfully to asparagus. In the case of fruit, raspberries stored at −10° or −20° C. without previous heating were perfect in colour and flavour, but plums and cherries, if frozen raw, turn brown and develop an unpleasant flavour on thawing, but previous heating in syrup prevents this. Quick freezing offers no advantages. The work at the Imperial College of Science and Technology has shown that difference in manurial treatment of apples has a greater effect on the chemical composition of the fruit than difference in stocks. Two substances belonging to the hemicellulose class have been isolated, which, together with starch pectin and unhydrolysed residue, make up the insoluble material in the apple.

SECTION C. PORK, BACON, AND HAMS.—The transport of unsmoked, mild-cured bacon from Australia and New Zealand has been found to be impracticable under present commercial conditions, since the fat is rancid after less than 2 months, even if the storage temperature has been as low as −10° C. However, carcases of frozen pork can be successfully transported and used for the manufacture of bacon. The iodine value of pork fat was in every case lower for the inner than for the outer fat, appearing to depend on the temperature at which the pork is laid down. Fast-growing pigs tend to have fat with a low iodine value, and slow-growing pigs to have fat with a higher iodine value; feeding the pig with cod-liver oil appears to make the fat more unsaturated.

SECTION D. BIOLOGICAL ENGINEERING.—Work has been concerned with the rate of evaporation of water from biological materials in cold storage, particularly apples, eggs and cheeses.

The work dealing with loss of water by stored infertile eggs has been extended to deal with variations in individual eggs, and the effect of such factors as thickness and porosity of shell, and the different types of package. The effect of waxing on loss of weight in cheeses was to decrease the overall loss of weight by 30 to 40 per cent., but the saving occurred wholly during the earlier part of storage. The difference in freezing point of egg yolk (−0·57° C.) and of egg white (−0·45° C.) is found to decrease only very gradually on storage, the effect being to set up movement of water from white to yolk, with some distension of the yolk, but the weakening of the yolk membrane is probably largely due to ageing, which is more rapid the higher the temperature.

SECTION E. CANNING.—The work on the corrosion of iron and tin separately has been continued, particularly on the effects of the products of corrosion on the further corrosion of the metals. Ferrous salts greatly accelerate the corrosion of tin in presence of air, and ferric salts accelerate corrosion of iron in presence of air (ANALYST, 1931, **56**, 315), and the combined effect of air and ferric iron is much

greater than that of either alone, especially at acidities round $p_H$ 4, as found in many fruit syrups. The effect of oxygen on the rate of corrosion of tin plate is that, with small amounts, tin is attacked most readily at low acidity, but with increase of oxygen the rate of de-tinning becomes greatest at high acidity.

PART II (TORRY RESEARCH STATION)

FISH.—The work on storage of brine-frozen fish described in the last Report has been repeated, and the previous results have been confirmed. These emphasise the need of lower storage temperatures in this country (−20° to −25° C.). Fish stored at such temperatures produces excellent smoke cures. The denaturation of the globulin fraction of the proteins of fish muscle is being particularly studied. The chemical survey of fish oils from both fresh water and marine fish has been continued, and tests for vitamin *A* have been made on both liver and body oils. The liver oil of the halibut is 15 to 75 times as potent in this respect as an average cod-liver oil, and a unique pigmentation discovered in the liver of the angler fish may throw light on the relation between the carotenoid pigments and vitamin *A*.

PART III (DITTON LABORATORY)

ENGINEERING.—The experimental hold has been made to act as a calorimeter for making measurements of the various thermal quantities required for a balance sheet from which the rate of generation of heat by fruit under given conditions of storage can be estimated.

PART IV

The chemical and biological work on fruit at the Imperial College of Science and Technology has been continued.

D. G. H.

# Government of Madras

## REPORT OF THE CHEMICAL EXAMINER FOR THE YEAR 1931

THE Chemical Examiner (Lt.-Col. Clive Newcomb, F.I.C.) reports an increase in the total number of analyses (6360 as against 5406 in 1931), the increase being in the amount of medico-legal work.

HUMAN POISONING CASES.—Poisons were identified in 150 of the 340 cases investigated. It is interesting to note that opium is now the most commonly found poison (31 cases), whilst arsenic comes second (21 cases), and organic poisons generally are now nearly twice as common as the inorganic, whereas some twenty years ago the reverse was the case. It is probable that the chief cause for this is that, with the increased spread of education, poisoners are learning that, speaking generally, organic poisons give rise to less characteristic symptoms and are less readily detected afterwards.

*Oduvan Poisoning.*—In four cases of poisoning by oduvan leaves there was no difficulty in obtaining the characteristic reaction with the leaves themselves, as described in the Report for 1930 (ANALYST, 1931, **56**, 665), but it was found that, by the time the leaves had disappeared from a stomach by digestion or *post-mortem* decomposition, the characteristic reactions had also disappeared. In one case in which a son had introduced the leaves into a curry, in an attempt to poison his father, the reactions were given by the curry.

*Madar Juice Poisoning.*—The four cases of madar juice poisoning were all suicides. In each examination the stomach-contents were boiled with absolute alcohol under a reflux condenser for an hour and then distilled after the addition of 50 per cent. of sulphuric acid. Each distillate had the peculiar fruity odour which

is not decisive proof of madar, but which is more or less characteristic of it, and there is little doubt that this was the poison used (*cf.* ANALYST, 1931, **56**, 665).

*Formalin Poisoning.*—Cases of formalin poisoning are rare, and there does not appear to be a previous record of one in which formalin was used externally with criminal intent. In one such case during the year a young wife of 15 years of age was severely beaten by her husband and father-in-law, and some corrosive liquid was poured over her head. The liquid caused her great pain, and when she was admitted to hospital some hours later her hair was found to be falling out in locks and the skin of her scalp to be peeling off. The doctor at first suspected that sulphuric or hydrochloric acid might have been used, as the patient's husband belonged to the weaver caste, and this caste also does dyeing and uses these acids in its business. The objects sent for examination were some of the fallen hair, several of the patient's garments stained with the liquid, and a bottle found in the husband's house, and supposed to contain the liquid used. The liquid was formaldehyde of about 31 per cent. strength, and we found formaldehyde on the hair and garments.

*Mercury in Viscera and Bones.*—Mercury was found in the stomach, intestines, liver, and kidneys, and in one humerus of a body which had been exhumed in a stage of advanced decomposition. It is unusual for bones to be sent for examination for poison, and it is interesting that in this case, where one was sent, mercury was found in it. The reason that the doctor gave for sending it was that he suspected, from a bluish discoloration of the stomach and trachea, that copper had been used, but it was clearly a case of mercury poisoning.

FLORENCE'S TEST FOR SEMINAL STAINS.—Florence's test continues to give reliable results with seminal stains. If, however, the stain contains much albumin, as, for instance, when the semen is mixed with blood, the albumin interferes to some extent with the test by reacting in places with so much of the iodine as to leave too little over for the production of Florence's crystals. If but a small amount of albumin is present, some crystals are generally formed, but fewer, other things being equal, than when albumin is absent. When albumin is present in large concentration the thickness of the precipitate that the iodine forms with it tends to prevent the two solutions from mixing and to obscure any Florence's crystals that are formed. The difficulty can be overcome by diluting the aqueous stain extract to give a sufficient bulk, boiling, filtering and evaporating it to dryness on a water-bath. If the residue is now taken up in one or two drops of water, this solution will give Florence's test. The choline—or whatever substance it is which is responsible for the test—is not destroyed by this procedure, but the spermatozoa are disintegrated and must be looked for in a separate portion of the extract.

BULLET HOLE IN COTTON.—In a shooting case a cotton shirt was submitted with a ragged hole through the breast pocket, and of about the size one would expect from a 450-revolver bullet, and round it was a dark grey mark. The defence suggested that the hole in the shirt might have been made by burning with a hot poker. Experimental shots fired from a 450-revolver into a cotton cloth placed on sand-bags produced holes which agreed in size and shape with the disputed hole, and which had grey marks of the same approximate dimensions at the edges. On analysis, the grey stains in each case were found to consist of lead, whilst the ends of the torn cotton fibres round the holes were, for the most part, unstained, in contrast to holes produced by burning. From these results the conclusion was drawn that the hole in the shirt had almost certainly been caused by a soft lead bullet which had struck it obliquely.

ANAESTHETIC ETHER.—The ferrous thiocyanate test described by Middleton and Hymas (ANALYST, 1928, **53**, 201) is admittedly a very rigorous one, and no specimen of ether in the Madras Government Laboratory would pass it. It is questionable whether this test is not an unreasonably high standard for an ether which has had a voyage to India.

## ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

# Food and Drugs Analysis

**Analysis of Mayonnaise and the Variability of its Egg-Constituents. J. L. Perlman.** (*J. Assoc. Off. Agric. Chem.*, 1932, **15**, 466–482.)—The method suggested by L. C. Mitchell (*ibid.*, 1931, **14**, 418) for the determination of lipoids and lipoid-phosphoric acid in eggs may be adapted to the determination of total fat and lipoids in mayonnaise as follows: 5 grms. of the thoroughly mixed material are weighed by difference into a 100-c.c. Kohlrausch sugar flask, which is kept shaken in a machine while 20 c.c. of a mixture of absolute alcohol and chloroform in equal volumes are slowly added. The agitation is continued until the sample is finely divided (5 minutes), 60 to 65 c.c. more of the solvent being then added, and the shaking continued for 50 minutes. The flask is filled to the mark with the solvent and shaken by hand for 5 minutes, the liquid being next filtered rapidly into a 100-c.c. Erlenmeyer flask, stoppered with a plug of cotton-wool, by carefully inverting the flask on to a funnel fitted with a 9 cm. filter paper. Fifty c.c. of the filtrate are evaporated to apparent dryness in a tall 200-c.c. beaker with the aid of an electric fan. The residue of fat is dried for 10 minutes in an oven at 100° C. and dissolved in 10 c.c. of chloroform, the sides of the beaker being rinsed down with chloroform from a wash-bottle and the liquid filtered through a tight plug of extracted cotton-wool into a weighed platinum dish. The remaining fat is transferred from the beaker to the funnel with the help of chloroform and a stirring rod, adhering solid particles being broken up. The funnel and the tip of its stem are washed into the dish, and the filtrate, which should be clear, is evaporated to dryness in a current of air on a steam-bath; the residue is dried at 100° C. until of constant weight (1·5 to 2 hours) and reserved for the determination of lipoid-$P_2O_5$.

The fat thus found includes practically all of the egg-yolk lipoids. Analysis of a mixture of 7 fresh eggs gives the value 37·5:1 for the ratio of lipoid to lipoid-$P_2O_5$, so that multiplication of the $P_2O_5$-content of the fat residue from the mayonnaise by 37·5 gives approximately the lipoid-content, and subtraction of this from the total fat found yields approximately the true vegetable oil-content. This is, however, not correct for samples stored for any appreciable time without refrigeration.

To determine the lipoid-$P_2O_5$: For each grm. of the fatty residue in the platinum dish, 5 c.c. of alcoholic potash (8 grms. of KOH per 100 c.c. of 95 per cent. alcohol) are added, and the whole is evaporated to dryness on a steam-bath, and the residue charred as thoroughly as possible over asbestos. The charred mass is treated with excess of nitric acid (1+2), filtered into a tall 200-c.c. beaker, and washed several times with water. The residue is returned to the dish, incinerated until white at or below 500° C., again treated with the nitric acid, filtered off, and washed. The combined filtrates are evaporated to 50 to 60 c.c., and made distinctly alkaline to one drop of phenolphthalein with strong ammonia; the liquid is then neutralised with the nitric acid, 3 to 4 c.c. of this being added in excess. Thirty c.c. of molybdate solution are added, and the mixture kept at

60 to 65° C. for an hour, with frequent stirring, and filtered through a weighed Gooch crucible; the precipitate is transferred with 1 per cent. nitric acid, washed twice with this acid and finally with water, and dried for 2 hours at 110° C. prior to being weighed. Weight of precipitate $\times 0{\cdot}03784 = P_2O_5$.

It was found that, in some of the older samples of mayonnaise, the lecithin had undergone hydrolysis to some extent, the presence of choline being shown by the formation of its insoluble crystalline poly-iodide, photomicrographs of which are reproduced. By means of this test, definite signs of decomposition were detected in more than one-half of a number of samples of mayonnaise from retail shops, and choline was also detected, in proportions increasing with the time, in mixed eggs kept at either ordinary or incubator temperature. As mayonnaise is prepared by a cold process not lending itself to the maintenance of aseptic conditions, it may contain micro-organisms, but the high acidity tends to retard bacterial and mould growth. Products of bacterial action may, however, be present in the separate ingredients prior to mixing. From decomposing whole eggs markedly deficient in lipoid-$P_2O_5$, bacteria of four different strains capable of hydrolysing lecithin have been isolated. One of these organisms, tentatively named *Bacillus lekitosis*, n. sp., is Gram-positive and spore-forming, and ranges in size from 0·5 × 3 to 0·8 × 6 microns. Besides breaking down lecithin, it ferments dextrose, maltose, and salicin—giving acid but no gas—hydrolyses starch, liquefies gelatin, and effects almost complete proteolysis of milk in about 7 days without giving acid or curd. Under aseptic conditions it destroys the lecithin of mixed eggs almost entirely within about 100 hours at 37° C., this effect being due to an extra-cellular enzyme. T. H. P.

**Detection of Hydrogen Sulphide and the Evaluation of the Degree of Freshness of Flesh Products. F. Budagjan.** (*Z. Unters. Lebensm.*, 1932, **64**, 226–235.)—The sample is disintegrated and 10 to 15 grms. are packed loosely into the wide end of a small tube (similar to an Allihn calcium-chloride tube), the narrow end being partly closed by a notched cork supporting a strip of filter-paper, 2 mm. wide, on which 1 drop of an alkaline solution of lead acetate is placed. Carbon dioxide is then bubbled through a 6 per cent. solution of copper sulphate and passed rapidly through the tube for 30 minutes in the cold, so that any hydrogen sulphide removed from the sample comes in contact with the strip, whilst the flow of gas as a whole is not impeded. The stain varies from yellow-brown to black, according to the amount of hydrogen sulphide removed, and the results obtained after various periods with fresh and salted beef, mutton, horse-flesh, sausage meat and various fish show that the increase in intensity is a measure of the rate of decomposition, no reaction being obtainable with the flesh of cold-blooded animals and mammals when in a sound state. Since the evolution of hydrogen sulphide does not necessarily accompany decomposition, a negative result should be confirmed by other methods, although a positive result is indubitable. J. G.

**Proportion of Tartaric Acid to Free Acid in Natural Moselle, Saar and Ruhr Wine-Musts for the Years 1930 and 1931. F. Seiler.** (*Z. Unters. Lebensm.*, 1932, **64**, 278–282.)—Tables show the alcohol-content, total free acidity

and tartaric acid for 80 of the musts of these wines at various periods after harvesting. The ratio of tartaric acid to total free acid has the mean value of about 50 and 46 per cent., respectively, for musts of the 1930 and 1931 vintages, and is usually highest when the total acidity is lowest. On fermentation the quantity of tartaric acid was usually found to decrease in the 1930 musts, but at a rate which was independent of the amount of alcohol present, whilst in the latter any decreases were usually smaller and some increases are recorded. The data do not enable a reliable distinction to be drawn between the wines and vintage-years, and it is considered that the harvesting-date and storage-temperatures are also important factors. J. G.

**Determination of Lactic Acid in Wines and Fruit Juices. L. Semichon and Flanzy.** (*Ann. Falsificat*, 1932, **25**, 414–416.)—When lactic acid is oxidised in solution by potassium dichromate and sulphuric acid the CH.OH radicle separates as formaldehyde, which undergoes oxidation to carbon dioxide and water, and acetic acid remains. Of the compounds present in fruit juices or their fermented products, only lactic and pyruvic acids, higher fatty acids, and alcohols yield acetic acid under these conditions. The alcohols and the higher fatty acids are readily removable prior to the oxidation, and pyruvic acid has not been found in appreciable proportions in either fruit juices or wines. To determine the lactic acid, 50 c.c. of the liquid are boiled for an hour with excess of milk of lime under a reflux condenser, in order to saponify the esters. The liquid is then concentrated to one-half of its volume and the residue, when cold, is treated with excess of tartaric acid to liberate the acids. After the lapse of two hours, the mass is filtered into a 150- or 200-c.c. Kjeldahl flask, the residue on the filter being washed with a dozen quantities of a few c.c. of water; the total volume of the filtrate and washings should not exceed 50 c.c. Any remaining traces of alcohols and aldehydes, as well as the whole of the volatile fatty acids, are then removed by distillation in a current of steam, the flask being inclined at 45° and immersed in a boiling water-bath. Distillation is continued until the last 50 c.c. of distillate collected gives no indication of acidity with litmus. The residual liquid is then cooled and 1·5 grm. of chromic acid and 2 c.c. of sulphuric acid (sp.gr. 1·71) are added, the flask standing in cold water meanwhile. The contents of the flask are then heated, under a reflux condenser, on a steam-bath for an hour. The acetic acid is then distilled off in a current of steam, and is determined by titrating the distillate with lime water in presence of litmus; 1 molecule of acetic acid corresponds with 1 molecule of lactic acid.

When the wine contains more than 5 grms. of sugar per litre, the amount of oxidising solution used should be increased proportionally. If, during the oxidation, the liquid ceases to show a brown colour, the flask must be cooled and a fresh quantity of the oxidising mixture added. Traces of lactic acid may be carried over with the volatile fatty acids and may be determined in the distillate; it has, however, been found that such traces are negligible in amount. T. H. P.

**Determination of Succinic Acid in Wines and other Fermented Liquids. L. Semichon and Flanzy.** (*Ann. Falsificat.*, 1932, **25**, 416–419.)—Of all the constituents of wine and of fermented liquors in general, only acetic and succinic

acids are unattacked by the mixture of chromic and sulphuric acids used in the determination of lactic acid (preceding Abstract). Moreover, no constituent yields succinic acid on oxidation. The succinic acid may be determined as follows: A long-necked, 150- to 200-c.c. Kjeldahl flask, standing in cold water, is charged with 50 c.c. of the wine, 1·5 grm. of powdered chromic acid and 2 c.c. of sulphuric acid (sp.gr. 1·71), and is then heated for an hour in a boiling water-bath. The liquid is cooled and treated with ammonium hydrosulphide to reduce the excess of chromic acid, and afterwards with ammonia solution to precipitate chromium oxide. After being boiled until evolution of ammonia ceases, the liquid is filtered and the filter washed with water. The filtrate, containing ammonium sulphate and succinate, is concentrated—while kept stirred—to 1 c.c., and treated with 50 c.c. of 95 per cent. alcohol, which precipitates the whole of the sulphate. This is filtered off and washed with alcohol, the filtrate being distilled to recover the alcohol, and the liquid then evaporated to dryness. The residue of ammonium succinate is weighed; if it is not entirely free from sulphate, this is determined as barium sulphate, and the weight of the succinate corrected accordingly.

By combination of this method with that given for determining lactic acid (*loc. cit.*), one sample of wine may be used for the successive determinations of the total volatile fatty acids, lactic acid, and succinic acid. T. H. P.

**Differentiation of Fermentation Vinegar from Artificial Vinegar. C. Bertin.** (*Ann. Falsificat*, 1932, **25**, 412–413.)—Pastureau (ANALYST, 1905, **30**, 274) found that fermentation vinegar always contains methylacetol,

$$CH_3.CO.CH(OH).CH_3,$$

and Denigès (*Précis de Chimie Analytique*, 1931, Vol. II, 307) gives a method, based on the reduction of Fehling's solution, for detecting this compound in vinegar. This method is not very exact, but may be made quantitative by modifying it as follows:—Fifty c.c. of the vinegar are neutralised with anhydrous sodium carbonate, and then distilled, exactly 20 c.c. of distillate being collected. The distillate is poured into a mixture of 10 c.c. of copper sulphate solution (40 grms. of $CuSO_4$ and 1 c.c. of $H_2SO_4$ per litre), 10 c.c. of alkaline Rochelle salt solution (200 grms. of the salt and 150 grms. of NaOH per litre) and 5 c.c. of potassium ferrocyanide solution (50 grms. per litre). The mixture is heated to boiling, and the volume (*e.g.* 11·4 c.c.) of a standard solution of pure dextrose (*e.g.* 3·5 grms. per litre) necessary to reduce the whole of the copper salt present is determined. If the volume of the dextrose solution required for the reduction, under similar conditions, of the mixed copper solution in absence of the vinegar distillate is 13·0 c.c., the amount of methylacetol contained in 1 litre of the vinegar will be 0·112 grm. (expressed as dextrose). [The result given in the original, *viz.* 0·0112 grm. per litre, has been wrongly calculated.—*Abstractor.*] This procedure neglects any methylacetol which may not have distilled over. T. H. P.

**Differentiation of Wine Vinegar from Spirit Vinegar. P. Rudolp and H. Barsch.** (*Z. Unters. Lebensm.*, 1932, **64**, 293–295.)—A method of differentiating wine vinegar and spirit vinegar is based upon the fact that iodine is decolorised more rapidly by the reducing substances in the former than by those

in the latter. (1) To 10 c.c. of the sample 0·5 c.c. of a 0·02 solution of iodine is added, and, after 15 minutes at 50° to 60° C., the mixture is cooled and 10 drops of a 1 per cent. solution of starch are added. In the presence of less than 5 per cent. of wine vinegar a deep blue colour is obtained, but more reduces the iodine so that the final colour is pale blue to bluish-green, or, if more than 20 per cent. is present, the liquid is colourless. If an excess of iodine solution is added and the mixture is back-titrated with 0·02 *N* sodium thiosulphate solution, the method may be made roughly quantitative. (2) The sample (100 c.c.) is decolorised in the cold with 2 grms. of animal charcoal, and 10 drops of a dilute solution of ferric chloride are added to 10 c.c. of the filtrate. Wine vinegar (more than 10 per cent.) appears yellow-green and spirit vinegar red to yellowish-red, but, as the latter colour also turns yellow on exposure to air, the colour should be estimated within 15 minutes. (3) A mixture of 10 c.c. each of the sample and of a reagent prepared from equal volumes of an ammoniacal solution of ammonium molybdate and 30 per cent. nitric acid gives a precipitate changing, through green, to an intense blue ($MoO_2$) in the presence of wine vinegar, whilst spirit vinegar produces a pale yellow-green shade. J. G.

**Determination of Essential Oils in Drugs and Spices. T. T. Cocking and G. Middleton.** (*Pharm. J.*, 1932, **129**, 253–255.)—The apparatus described provides a method of determining the amount of essential oil which may be distilled from a drug, not necessarily the amount contained in the drug. The measuring tube is of such a bore that 1 c.c. corresponds with a length of about 14 cm., and it has a small bulb at the bottom with a capacity of 0·9 c.c., and widens below towards the receiver, in which the oil separates from the aqueous distillate; this may be connected by means of a two-way stop-cock, either with the return-flow tube or with a side-tube through which it may be filled with distilled water. A narrow side-tube with a ground-in stopper between the condenser and measuring-tube serves as a vent. About 300 c.c. of water and a suitable quantity of drug are placed in the flask; the receiver is filled with distilled water from the side-tube, the tap is turned so that the return-tube is filled with water, the stopper is inserted in the vent-tube with a slip of paper under its seating, and the contents of the flask are boiled at such a rate that the receiving-tube does not become warm. Distillation is continued for about 2 hours, and, after the apparatus has drained, distilled water is made to enter the receiver very slowly to raise the layer of oil to the measuring tube. Distillation is continued for another hour, and the distillate is again measured. Oil of turpentine is used as a solvent for oils heavier than water, or for those which crystallise at the ordinary temperature. D. G. H.

**Copper Content of Certain Pharmaceutical Preparations and Chemicals. N. Evers and L. A. Haddock.** (*Pharm. J.*, 1932, **129**, 247–248.)—The proportions of copper (as parts per million) present in various chemical galenicals and miscellaneous substances are tabulated. The determinations were made by the authors' method as described in THE ANALYST (1932, 495), and the figures for various chemicals ranged from 0·4 in hydrochloric acid to 68 parts per million in ferric glycerophosphate, and in certain galenicals, etc., from 4 parts per million for tinct. valerian. ammon. B.P. to 580 for an ext. ergot., B.P. (1914). D. G. H.

**Comparison of Tests for Balsam of Peru. E. M. Smelt.** (*Pharm. J.*, **1932**, **129**, 241.)—The following tests are recommended for the detection of most of the adulterants reported to be used in balsam of Peru. (1) *Odour of the petroleum spirit extract.*—Three grms. of balsam are shaken with 15 ml. of petroleum spirit (boiling below 40° C.), and, after filtration if necessary, the solution is evaporated. No odour of turpentine or benzaldehyde should be apparent, whilst the presence of copaiba or Canada balsam may be suggested.

(2) *Acetic anhydride test.*—Three drops of the residue from (1) are dissolved in 10 drops of acetic anhydride and 2 drops of sulphuric acid are added. If there is no reddish-violet or bluish-violet colour, the absence of Canada balsam, copaiba, colophony, gurjun balsam and storax is indicated.

(3) *Nitric acid test.*—To 4 drops of residue from (1), 2 drops of nitric acid are added, when if a yellow but not green, blue, or purplish-red colour develops round the drops, the absence of balsam of tolu, Canada balsam, copaiba, colophony, gurjun balsam, storax, turpentine, castor or olive oil is indicated.

(4) *Copper acetate test.*—Four ml. of the petroleum spirit solution from (1) are shaken with 10 ml. of a 0·1 per cent. w/v aqueous solution of copper acetate and left to separate. The petroleum spirit layer is not coloured green in the absence of Canada balsam, copaiba, or colophony.

(5) *Alcohol test.*—One volume is soluble in 1 volume of 90 per cent. alcohol, but, on further addition of 2 or more vols. of alcohol, the solution becomes turbid. Artificial balsams, the ingredients of which are completely soluble in 90 per cent. alcohol, are thus detected.

(6) *Chloral hydrate test.*—One grm. of balsam is shaken with a solution of 3 grms. of chloral hydrate in 2 ml. of water, and if, after standing for 15 minutes, there is a clear solution, the absence of castor and olive oils is indicated.

(7) *Carbon disulphide test.*—If three volumes mixed with 1 volume of carbon disulphide give a clear solution, the absence of gurjun balsam (?) olive oil (?) and kerosene is indicated. D. G. H.

**Merz Reactions for Certain New Anaesthetics. H. Szancer.** (*J. Pharm. Chim.*, 1932, **124**, 239–241.)—Merz divides the anaesthetics examined into two groups, the first containing the bases soluble only with difficulty (laracaine, percaine, and pantocaine), and the second group, consisting of novocaine, tutocaine, and psicaine. The diazo reaction, the iodoform reaction after saponification, and the addition to the free base (dissolved in ether) of an ethereal solution of oxalic acid, which may be used as reactions for novocaine and tutocaine, give negative results with psicaine. To identify this substance (which is a pseudo-cocaine) Merck's reaction (*Prüfung der Spezialpräparate*, 1930, 3rd Ed.) may be employed. One grm. of psicaine is heated with 1 c.c. of concentrated sulphuric acid to 100° C., and 2 c.c. of water are added, when the odour of the methyl ester of benzoic acid becomes apparent. Cocaine, which gives a similar reaction, may be distinguished by such tests as those given in Hager's *Handbuch der pharmazeutischen Praxis.* D. G. H.

**Quantitative Methods for the Determination of Theobromine in Diuretine. J. M. A. Hegland.** (*Pharm. Weekblad*, 1932, **69**, 1078–1080.)—(1) A solution of 0·5 grm. of sample in 100 c.c. of water is made neutral to phenolphthalein, and is then shaken with excess of a 0·1 *N* solution of silver nitrate, the nitric acid liberated after 24 hours being titrated with 0·1 *N* sodium hydroxide solution to phenolphthalein; 1 c.c. ≡ 18 mgrms. of theobromine (H. Boie). (2) A mixture of a solution of 0·25 grm. of sample in 2 c.c. of glacial acetic acid with 50 c.c. of 0·1 *N* iodine solution, 6 grms. of sodium chloride and 5 c.c. of dilute hydrochloric acid is diluted to 100 c.c., and, after 24 hours, the theobromine tetraiodide is removed by filtration, and the excess of iodine in an aliquot portion of the filtrate is titrated with 0·1 *N* sodium thiosulphate solution; 1 c.c. of 0·1 *N* iodine solution ≡ 4·5 mgrms. of theobromine (Mahler and Schütz, after Emery and Spiner). A comparison of the results obtained by the two methods in the presence of sodium salicylate shows that (1) gives slightly high results, but is safer and less dependable on variations in conditions than (2), which gives low results.

J. G.

**Colorimetric Assay of Ergot. N. L. Allport and T. T. Cocking.** (*Pharm. J.*, 1932, **129**, 235–236.)—In order to eliminate, in the B.P. 1932 colorimetric test for the standardisation of ergot, the exposure to light of the alkaloidal solution with the *p*-di-methyl-amino-benzaldehyde and sulphuric acid reagent, ferric chloride was used as a catalyst. The reagent consisted of a 0·125 per cent. w/v solution of *p*-dimethyl-amino-benzaldehyde in sulphuric acid (65 per cent. v/v), to which 0·1 per cent. v/v of a 5 per cent. solution of ferric chloride was added, 2 ml. of this reagent being added to 1 ml. of the alkaloidal solution. The full colour is found to develop within 1 minute without exposure to light or further heating, and the reagent may be stored in the dark for about a month without losing its activity. Stress is laid on the importance of using pure anaesthetic ether for the extraction. Ten samples of ergot, liquid extract, percolate and paste were tested with the above reagent, and also by the official reagent, with warming to 45° C. and exposure to light, and the results obtained in the two series of tests were almost identical.

D. G. H.

**Assay of Santonin-bearing Drugs. H. M. Burlage and A. C. Smith.** (*J. Assoc. Off. Agric. Chem.*, 1932, **15**, 491–499.)—Investigation of 17 methods which have been proposed for the determination of santonin has been made, 3 different samples of drugs being used. It was found that Feldhoff's (*Pharm. Z.*, 1925, **70**, 661) and Eder and Schneiter's methods (*Schweiz. Apoth.-Ztg.*, 1925, **63**, 405) gave uniform results and a product of satisfactory melting point, and seemed worth further study. The following modification of Feldhoff's procedure is suggested: chloroform is used in place of ether for extracting the drug and, later, instead of allowing the santonin to crystallise from 15 per cent. alcohol, the alcoholic solution is transferred to a 100-c.c. flask, mixed with 2·5 grms. of potassium hydroxide and, after 15 to 20 minutes, made up to 100 c.c. with 95 per cent. alcohol. A check determination is made with 2 grms. of santonin in the same way, the solution thus obtained being compared colorimetrically with that given by the drug.

A new method, which yields results in good agreement with those of the modified Feldhoff process, makes use of alcohol as the primary solvent, and is carried out as follows:—A weighed quantity of about 10 grms. of the finely powdered drug is boiled for 15 minutes with 50 c.c. of petroleum spirit (saturated with santonin) under a reflux condenser to de-fat the sample. The mixture is filtered by means of suction, and the drug extracted for 3 hours in an extraction thimble in a Soxhlet apparatus with 75 c.c. of alcohol. The extract is evaporated to 10 c.c. and filtered through a plug of cotton-wool, the flask and filter being washed with 25 c.c. of boiling water, and the liquid filtered again through paper into an Erlenmeyer flask. The flask and filter are then washed twice with 10-c.c. portions of hot 15 per cent. (by vol.) alcohol. The filtrate is boiled with 1·5 to 3 c.c. of lead subacetate solution (U.S.P., X, 221), and filtered hot through a plain filter into a tared Erlenmeyer flask, the boiling flask and the filter being washed with two 10 c.c.-portions of hot 15 per cent. (by vol.) alcohol. The filtrate is left for 24 hours in a cool, dark place, and its weight determined. The separated crystals are transferred to a tared filter, and the flask and filter are washed thrice with small quantities of 5 per cent. ammonia solution and thrice with water. The crystals of santonin are dried at 100° C. until of constant weight, this being increased by 0·006 grm. for each 10 grms. of the weighed dilute alcohol solution. T. H. P.

**Assay of Santonin in *Artemisia*. J. Coutts.** (*Pharm. J.*, 1932, **129**, 240–241.)—The following gravimetric method of assay of santonin in *Artemisia* is applicable to the various types of the drug, and gives results comparable with those obtained by the methods of Katz, Fromme and Mouton. Fourteen grms. of the dried, coarsely powdered drug are shaken at frequent intervals for 6 hours with 140 ml. of benzene; 101 ml. of the liquid are filtered off and shaken for 5 minutes with 35 ml. of 8 per cent. sodium carbonate solution. After separation, 80·5 ml. of the benzene solution (8 grms. of the drug) are decanted and evaporated to dryness. The residue is extracted by heating it for 10 minutes with 60 ml. of saturated barium hydroxide solution at 95° C., and the solution is immediately filtered, and the residue is washed with two 10-ml. portions of the hot barium hydroxide solution. The solution is allowed to cool, slightly acidified with 5 ml. of 25 per cent. hydrochloric acid, and left to crystallise for 24 hours. The crystals are collected and washed with cold water, dried and weighed, the weight representing the santonin found in 8 grms. of the crude drug. The benzene used should be completely volatile below 95° C. D. G. H.

**Detection of Solanine. B. Alberti.** (*Z. Unters. Lebensm.*, 1932, **64**, 260–262.)—The usual alkaloid separation is carried out, and a solution of the resulting residue in 1 drop of glacial acetic acid is mixed with 2 drops of concentrated sulphuric acid, and 1 drop of a 1 per cent. solution of formaldehyde (or of 0·5 per cent. hydrogen peroxide) is then added. In the presence of more than 0·0025 to 0·005 mgrm. of solanine or of solanidine a stable cherry or purple-red colour is obtained which deepens on standing (Autenrieth's selenium reagent is sensitive to 0·025 mgrm.). Quillaia or senega saponins, and narcotine, narceine, thebaine, caffeine, strychnine, brucine, digitalin or digitonin do not interfere; morphine

gives the reaction with formaldehyde, but none with hydrogen peroxide. Potato-skins are extracted in the cold with glacial acetic acid. Concentrated sulphuric acid is coloured yellow by solanine. J. G.

**Appearance of Nicotine in Cigarette Smoke. II. C. Pyriki.** (*Z. Unters. Lebensm.*, 1932, **64**, 263–277.)—Modifications of Pfyl and Schmitt's method (ANALYST, 1927, **52**, 728) for the determination of nicotine in tobacco-smoke (*cf. id.*, 1931, **56**, 753; Barta and Toole, *Z. angew. Chem.*, 1931, **44**, 682; Kaperina, *Biochem. Zeit.*, 1930, **219**, 258) are criticised and rejected. The author prefers to extract the acid-absorbing solution with chloroform and to neutralise the aqueous portion before precipitation with picric acid or iodoeosin, the slightly higher results thus obtained being attributed to the inclusion of a higher proportion of decomposition-products. No nicotine is detectable in this way in the smoke from tobaccos containing less than 0·2 per cent. of nicotine; tobaccos containing 0·3 and 0·5 per cent. yield about 5 and 12·5 per cent. of these quantities, respectively, but when they contain 1·2 per cent. or over (up to 2 per cent.) the yield is always about 20 per cent. A number of denicotinising agents (*cf. Z. angew. Chem.*, 1931, **44**, 881; *D.R.P.*, 518,903; and *Pharm. Zentralh.*, 1932, **73**, 257) were examined, and found to be without effect, and, in particular, "Bonicot" (*Chem. Ztg.*, 1932, **56**, 31), which reduces the nicotine content by about 13 per cent., was found to be no better than water, and probably acts by making the tobacco moist. The author's work confirms his previous suggestions (ANALYST, 1931, *loc. cit.*) for the nicotine-contents of tobaccos marked "nicotine-free" and "poor in nicotine," and indicates that the nicotine is carried over in the smoke in loose combination with acid decomposition-products. If 75 per cent. of a cigarette is smoked, the nicotine-content of the residue is increased by 11 to 13 per cent. J. G.

**Polarimetric Method for the Determination of Calcium Gluconate. H. J. Fisher and E. M. Bailey.** (*J. Assoc. Off. Agr. Chem.*, 1932, **15**, 461–466.)—This method is based on the observations that uranium salts considerably enhance the optical rotation of solutions of calcium gluconate, and that, when the solutions are saturated with uranyl acetate, the rotations are a linear function of the concentration of the gluconate, provided this is not high. The presence of sugars in proportions likely to occur in commercial preparations of calcium gluconate does not affect the results appreciably. The calcium gluconate tablets now being sold are chocolate-flavoured, this necessitating slight modification of the procedure. Calcium lactate causes no interference.

Two 0·5 grm.-portions of the calcium gluconate or, with tablets containing 50 per cent. or less of the salt, two 1 grm.-portions are weighed. If chocolate or a fatty base is present, the samples are washed several times on a hardened filter with absolute ether, and the residues are warmed until the ether is expelled. Each portion is warmed with 20 c.c. of water in a 25 c.c.-measuring flask until the calcium salt present is dissolved. To one flask 5 grms. of powdered uranyl acetate are added, the flask being stoppered and shaken in a machine for an hour, during which time the other flask remains untreated. If the sample contains chocolate, a little alumina cream is added to each flask, and both are cooled to 20° C. The contents of each flask are made up to volume, with saturated uranyl

acetate solution for the flask to which this acetate was added, and with water for the other. Each solution is filtered and polarised in a 200 mm. tube, white light being used. If $A$ is the rotation in degrees Ventzke of the solution free from uranyl acetate and $B$ that of the other solution, the percentage of monohydrated calcium gluconate is 4·3 $(B-A)$ if 1 grm. of sample were taken, or double this if 0·5 grm. were used.

The accuracy with which the rotation of solutions can be read in an ordinary saccharimeter is a limiting factor in the accuracy obtainable by this method, and for the pure salt or mixtures containing no calcium compounds other than the gluconate, determination of the calcium by the permanganate method is doubtless preferable. The polarimetric method, although of a lower degree of accuracy, is the only known method applicable either to identification of the gluconate or to its determination in mixtures containing other calcium salts. The presence of tartrates and active malates would interfere with the method, but the low solubilities of calcium tartrate and malate would prevent substitution of these salts for the gluconate. Active calcium lactate is not a commercial product. T. H. P.

**Determination of Titanium in Pomades. E. Kahane.** (*J. Pharm. Chim.*, 1932, **16**, 194–202.)—Titanium dioxide is stated to replace zinc oxide, partly or completely, in certain ointments, cosmetic creams, etc. The following method for the determination of the titanium was found suitable.

*Destruction of Organic Matter.*—Two grms. of the material are introduced into a Kjeldahl flask and 4 grms. of anhydrous sodium sulphate, 10 c.c. of concentrated sulphuric acid, and 5 c.c. of concentrated nitric acid are added. The whole is heated, and, when blackening occurs, a few drops of a mixture of 2 volumes of perchloric acid (sp.gr. 1·6) and 1 volume of concentrated nitric acid are added, from time to time, until no blackening is produced on further heating. The liquid is finally boiled for a few minutes to ensure that the titanium dioxide is dissolved.

*Gravimetric Determination of Titanium.*—Fifty c.c. of water are added to the contents of the flask; the liquid is filtered, neutralised with sodium carbonate solution, then acidified with 1 c.c. of concentrated sulphuric acid, and 6 grms. of sodium thiosulphate are dissolved in it. The solution is added, little by little, to 400 c.c. of boiling water kept constantly stirred during the addition; the boiling is continued for 2 minutes, and the liquid is then cooled rapidly in cold water. The precipitate, consisting of a mixture of titanium hydroxide and sulphur, is filtered off, washed with boiling water, ignited and weighed as $TiO_2$. This method is said to give good results in the presence of the iron and zinc which may occur in the sample, but other metals which precipitate under the conditions used (bismuth, for example) must, if present, be removed by preliminary separation. Details are given for alternative volumetric and colorimetric determinations, which follow established methods. S. G. C.

# Biochemical

**Determination of Methionine in Proteins. H. D. Baernstein.** (*J. Biol. Chem.*, 1932, **97**, 663–668.)—A modification of the technique of Pollak and Spitzer (*Monatsh. Chem.*, 1922, **43**, 113) is described for the determination of the

volatile iodide arising from proteins treated with hydriodic acid. Their observation that the precipitate in the absorber is not all silver iodide, since a portion may be dissolved in nitric acid, is confirmed. When this portion is reprecipitated as silver iodide the combined weight is equivalent to the methionine taken. The tedious treatment of the precipitate is eliminated by a volumetric determination of the silver remaining in the filtrate from the silver iodide precipitate. The modified method is as follows:—About 0·5 grm. of protein is weighed and transferred to a digestion flask, and a small piece of porcelain is added to insure quiet boiling. Ten c.c. of pure hydriodic acid (sp.gr. 1·7) are added, and the flask is connected with a condenser to which one wash-bottle and two absorbers are attached. A figure shows the apparatus. The wash-bottle contains 20 per cent. cadmium sulphate solution acidified with sulphuric acid and with 1 c.c. of red phosphorus suspension added. This removes any hydrogen sulphide, iodine and hydriodic acid which might come over from the flask. The absorbers contain 10 c.c. of a solution of silver nitrate in absolute alcohol. To prepare this solution, 500 c.c. of absolute alcohol and 8 grms. of silver nitrate are boiled beneath a reflux condenser for half-an-hour, and the solution is left to stand in the sunlight for two days, then filtered through very fine paper, and kept in a brown bottle. Washed carbon dioxide is bubbled through the apparatus under a pressure of about 2 pounds. (The gas is washed with silver nitrate and sulphuric acid.) The rate of bubbling is regulated by a screw clamp on the inlet to the flask. The mixture in the flask is brought to boiling with a small flame, and the rate of outflow of gas is then regulated by a second screw clamp between the wash-bottle and first absorber. The bubbling should be just so fast that the bubbles can be counted. Water at about 60° C. is run through the condenser jacket. About 90 per cent. of the methyl iodide comes over in the first hour; the remainder takes several hours more. The author allows the apparatus to run all night (about 15 hours). The absorbers are rinsed carefully into 50-c.c. beakers, which are heated on the water-bath until the volume is reduced to about 10 c.c. The solution is transferred to a 50-c.c. flask, diluted to the mark, and filtered, and 5·0 c.c. of the filtrate are titrated with 0·02 $N$ potassium thiocyanate solution, after the addition of 2 c.c. of nitric acid and 2 c.c. of saturated ferric alum solution, a micro-burette being used. A blank determination is made without the protein, and the difference gives the amount of silver iodide precipitated by the methyl iodide derived from the protein. The method gives nearly theoretical figures (97·6 ± 1·4 per cent.) with pure synthetic methionine. The methionine-content of proteins thus determined ranges from 0·5 to 5·0 per cent. Certain difficulties of interpretation are discussed. Eleven amino acids analysed by the new technique gave negative results. No one has yet isolated an amino acid containing the methoxyl group, and, excepting methionine, none of the amino acids tried by the author (which include representatives of each of the various classes of amino acids), yields a volatile iodide under the conditions described. The author believes that the methyl iodide liberated is a measure of the methionine only. P. H. P.

**Mode of Distribution of Glucose in Human Blood. E. M. MacKay.** (*J. Biol. Chem.*, 1932, **97**, 685–689.)—Glucose, so far as is known, readily permeates

the red blood-cells, and there is no reason to suppose that the conditions which determine the distribution of electrolytes on the two sides of the erythrocyte "membrane" would influence its distribution, so that it is reasonable to believe that the concentration of glucose in the plasma and erythrocytes would be in accordance with the aqueous concentrations of these media. The author has made experiments on this point, since it has not been directly considered in the literature. A table shows the results obtained with 15 specimens of venous blood drawn both from normal and pathological subjects. It is shown that glucose is found in the same concentration in the water contained in the plasma and erythrocytes of freshly-drawn human blood; it is also found equally distributed between the plasma and cell water after the addition of glucose to human blood *in vitro*. The distribution of glucose between the erythrocytes and plasma is apparently, therefore, a process of simple diffusion, and is governed by the aqueous concentrations of these two media. P. H. P.

**Determination of Magnesium in Blood by means of 8-Hydroxyquinoline. D. M. Greenberg and M. A. Mackey.** (*J. Biol. Chem.*, 1932, **96**, 419–429.)—A method is described for the determination of magnesium in oxalated whole blood, oxalated plasma, and serum. The magnesium determination is carried out on a protein-free blood filtrate, prepared either by a modification of the Folin-Wu or the trichloroacetic acid method, and in a dilution of 1:5 so that 5 c.c. of filtrate represent 1 c.c. of blood. Equations show the reactions involved. Magnesium hydroxyquinolate is formed in an ammoniacal solution. The hydroxyquinoline reacts with bromine formed by the reaction of bromate with an excess of bromide in acid solution. More bromate is added than is actually required to brominate the hydroxyquinoline, and the excess is determined iodimetrically by the addition of potassium iodide to the solution to react with the excess of bromate and titration of the liberated iodine. The following reagents are required: *For the preparation of the blood filtrates*, a 10 per cent. sodium tungstate ($Na_2WO_4$) solution and 0·67 *N* sulphuric acid for the modified Folin-Wu, 10 per cent. trichloroacetic acid for the trichloroacetic acid filtrate, and 4·5 per cent. neutral potassium oxalate solution to precipitate calcium in serum. *To precipitate and wash the magnesium hydroxyquinolate*, 1 per cent. 8-hydroxyquinoline solution in 95 per cent. ethyl alcohol (freshly made every 2 weeks), 1 per cent. ammonium chloride solution, concentrated ammonium hydroxide, a 2 per cent. ammonium hydroxide wash solution, and 95 per cent. ethyl alcohol. *For the titration*, concentrated hydrochloric acid, 50 per cent. potassium bromide, exactly standardised 0·01 *M* potassium bromate, 20 per cent. potassium iodide, 1 per cent. soluble starch, and approximately 0·1 *N* sodium thiosulphate solutions. For the potassium bromate solution 1·670 grm. of the pure dry crystalline salt is dissolved in 1 litre of water at room temperature. For the filtration, a battery of Kirk-Schmidt micro-filters fitted into 250-c.c. Pyrex suction filtration flasks is used. Filters with stems 2 inches long were found most suitable.

*Procedure for oxalated whole blood or plasma*: Sufficient neutral sodium or potassium oxalate is added to the blood for it to contain about 0·4 per cent. of oxalate; this precipitates the calcium. By the Folin-Wu method a protein-free

filtrate is prepared from the oxalated whole blood or plasma by the addition to one volume of blood of 2 volumes of distilled water, then 1 volume of 10 per cent. sodium tungstate solution, and finally slowly, with shaking, 1 volume of 0·67 *N* sulphuric acid. After addition of the distilled water to whole blood the sample is shaken, and left until the corpuscles have been haemolysed. Addition of a drop of concentrated ammonia to samples that haemolyse with difficulty is helpful. The coagulum is left for 15 minutes or longer, and then filtered or centrifuged off. This gives a completely protein-free filtrate. A trichloroacetic acid filtrate is prepared by the addition of 2 volumes of distilled water to 1 volume of blood; then, after whole blood has been laked, 2 volumes of 10 per cent. trichloroacetic acid are added to coagulate the protein. When a single determination is to be made on a sample, 3 c.c. of blood or plasma are pipetted into a test-tube fitting into a 15-c.c. centrifuge holder, then 6 c.c. of water and 3 c.c. each of sodium tungstate and sulphuric acid, or, alternatively, 6 c.c. of trichloroacetic acid. After standing, the tube is centrifuged, and a 10-c.c. portion of the supernatant liquid is pipetted off for analysis. The 10-c.c. aliquot portion (equivalent to 2 c.c. of the original blood or plasma) of the protein-free filtrate is introduced into a 15-c.c. test-tube, 1 c.c. of 1 per cent. ammonium chloride solution is added, then 6 drops of concentrated ammonium hydroxide, followed by 1 c.c. of the 1 per cent. alcoholic hydroxyquinoline solution. For trichloroacetic acid filtrates the concentrated ammonia added is insufficient for neutralisation, and this can be detected by the green tint of the liquid. More ammonia is added, drop by drop, until the hydroxyquinoline colour in the tube changes to yellow. The contents are mixed, and the tube is placed for 20 to 30 minutes in a water-bath maintained at 70° to 80° C. While the solution is still hot it is filtered through a Kirk and Schmidt micro-filter, gentle suction being used. The precipitate is washed first with 8 c.c. of hot 2 per cent. ammonium hydroxide, then with 8 c.c. of 95 per cent. ethyl alcohol, and finally with another 8-c.c. portion of hot 2 per cent. ammonium hydroxide with the aid of suction, and the filter is then transferred to a clean filter-flask, and treated with 8 c.c. of hot concentrated hydrochloric acid to dissolve the magnesium precipitate; finally, the filter-cup is washed out with water. The titration is carried out in the filter-flask. To the contents 1 c.c. of 50 per cent. potassium bromide is added, then slowly, with a calibrated pipette, 5 c.c. of 0·01 *M* standard potassium bromate solution. The whole is gently shaken for 1 minute to allow of the bromination of the hydroxyquinoline, after which 1 c.c. of 20 per cent. potassium iodide solution is added. The flask is again shaken, and its sides are washed down with water to give a volume of about 50 c.c. To titrate the excess of iodine with thiosulphate, 0·02 *N* sodium thiosulphate is used, 10 c.c. being added from a calibrated pipette, and the rest of the titration being continued with a micro-burette calibrated in 0·02 c.c. The titration is first carried to a pale straw-colour, then 20 drops of 1 per cent. starch solution are added, and the titration is continued to the disappearance of the blue colour. The accuracy of the method depends upon the standardisation of the bromate solution. The strength of the thiosulphate is determined in each series of analyses by a blank titration as follows: Into an Erlenmeyer flask of 200 to 300-c.c. capacity, 8 c.c. of concentrated hydrochloric acid are introduced, followed by 8 c.c. of water

and 1 c.c. of 50 per cent. potassium bromide solution. These are mixed, and the same volume of standard bromate is pipetted in as for the magnesium determinations. The flask is gently shaken for 1 minute, 1 c.c. of the 20 per cent. potassium iodide solution is added, and the sides are washed down, as before, to give a volume of about 50 c.c. The titration is made with pipette and burette, and the differences between that of the blank and those of the magnesium samples give the amounts used up in the bromination. *Procedure for serum*: This depends upon whether or not calcium needs to be determined. If not, 3 c.c. of serum and 5 c.c. of water are placed in a tube, followed by 3 c.c. of 10 per cent. sodium tungstate solution, and 3 c.c. of 0·67 *N* sulphuric acid are added slowly, with shaking. After 5 minutes, 1 c.c. of 4·5 per cent. potassium oxalate solution is added, and the whole is left for 2 hours. The tube is then centrifuged and a 10-c.c. aliquot part of the filtrate (representing a 2-c.c. aliquot part of serum) is taken for analysis, which is carried out as described for plasma. When calcium is to be determined, the filtrate is prepared as for whole blood. Some results of typical experiments showing the accuracy of the analytical method for magnesium and calcium are given.

P. H. P.

**Variations in the Vitamin *A* Content of Butter-Fat. G. S. Fraps and R. Treichler.** (*Ind. Eng. Chem.*, 1932, **24**, 1079–1081.)—Butter fat from cows receiving maize, cotton-seed meal and pasturage contains from 17 to 50 units (U.S.P.) of vitamin *A* per grm., and the content remains high even in dry weather with shortage of grass. After sixteen months' feeding with cotton-seed meal and husks, cows yield butter-fat containing 2 units (U.S.P.) of vitamin *A* per grm.; with the addition of silage to the feed, the vitamin content is 2 to 16 units (U.S.P.) per grm., and rises to about 33 units when the cows have also access to pasturage. Only a small fraction of the vitamin contained in any of the feed is found in the butter-fat.

W. P. S.

**Halibut-liver Oil. Its Vitamin Potency, Physical Constants and Tolerance. A. D. Emmett and O. D. Bird.** (*Ind. Eng. Chem.*, 1932, **24**, 1073–1077.)—Halibut-liver oil has the following characteristics: Sp.gr. at 25° C., 0·927 to 0·928; unsaponifiable matter, 7·44 to 7·90 per cent.; saponification value, 179 to 193; iodine value, 118 to 126. The vitamin *A* potency of the oil varies from 37,500 to 62,500 units, or from 75 to 125 times that of cod-liver oil. The vitamin *D* potency of the oil is also much greater than that of cod-liver oil. The tolerance to large quantities of halibut-liver oil indicates that it can be taken with impunity and accepted as a valuable accessory to human nutrition. W. P. S.

# Bacteriological

**Thermophilic Bacteria in Refined Cane Sugars. W. L. Owen and R. L. Mobley.** (*Ind. Eng. Chem.*, 1932, **24**, 1042–1044.)—Even the highest grades of refined sugars are frequently contaminated by the presence of thermophilic bacteria, and the use of such contaminated sugars is a source of trouble in the canning industry. The spores appear to be confined entirely to the surface of the crystals, and the infection is mainly derived from the dust of the factory,

and sacks. The following summarised data illustrate the results obtained with various grades of sugars from many different sources:

| Grade of sugar | Average flat, sour thermophiles per 10 grms. | Percentage free from flat, sour thermophiles | Percentage having anaerobes | Percentage containing $H_2S$-producers |
|---|---|---|---|---|
| Fine granulated .. | 13·00 | 39·0 | 14·0 | 12 |
| Coarse granulated .. | 8·5 | 10·0 | 33 | 10 |
| Powdered .. .. | 191·0 | 0 | 50 | 50 |
| Raw .. .. | 70·0 | 0 | 28 | 0 |
| Sacks .. .. | 5·5 sq. in. 40 (infected) | | | |

W. P. S.

# Agricultural

**Determination of Catalase in Agricultural Products. A. K. Balls and W. S. Hale.** (*J. Assoc. Off. Agr. Chem.*, 1932, **15**, 483–490.)—The determination of catalase activity in agricultural products is beset by many difficulties, but the method described is satisfactory in so far that, with mixtures of clover and grain extracts, it yields additive results. Under the conditions specified the course of the catalytic reaction is unimolecular, and the constant is proportional to the amount of enzyme preparation used, but, owing to the fact that many factors seem to modify the results, this is not regarded as a proof that the true course of catalase reaction is unimolecular.

To prepare the enzyme material, 2 grms. of the substance are ground with sharp sand and 18 c.c. (20 c.c. if the substance is dry) of a mixture of 95 per cent. glycerol and 0·2 *M* phosphate buffer ($p_H = 7·0$ to 7·2); it is best first to remove the air from the extraction fluid by boiling it for a short time in a vacuum. The grinding is continued for several minutes until the material is very finely divided, the resulting emulsion is centrifuged, and the supernatant liquid is again freed from air. A suitable quantity of the liquid is then allowed to stand for 5 minutes with one-tenth of its volume of boiled liver-juice (*vide infra*) and, if necessary, afterwards diluted with more glycerol and phosphate mixture prior to introducing a measured quantity (1 to 5 c.c.) into the hydrogen peroxide solution for the analysis. The amount of enzyme used should be sufficient to decompose at least one-half of the peroxide in 5 to 7 minutes. It is added from a measuring pipette into a stoppered cylinder containing 1 c.c. of 0·2 *N* hydrogen peroxide solution (*vide infra*), 4 to 6 c.c. of 0·2 *M* phosphate buffer ($p_H = 7·0$), and approximately 1 grm. of freshly-dissolved dextrose in sufficient water to make, with the enzyme solution, a total volume of 50 c.c.; the cylinder and its contents must have been previously cooled in ice. The cylinder is replaced in the ice and, as soon as convenient, a 10 c.c. portion is rapidly pipetted into a flask containing 20 c.c. of 2 *N* sulphuric acid and 5 drops of saturated aqueous molybdic acid solution. Ten c.c. of 10 per cent. potassium iodide solution are added at once, and the liquid is left for 4 minutes, the iodine liberated being then titrated with 0·01 *N* thiosulphate solution.

Another 10-c.c. portion is removed after about 2 minutes, and a third after about 5 minutes, the moment when the delivery pipette is half empty being noted by a stop-watch in each case. The first titration gives the initial concentration

of the peroxide ($a$) and the subsequent ones the amounts of peroxide remaining ($a - x$) at the time of observation ($t$). Substitution in the formula $\frac{1}{t} \log \frac{a}{a-x} = k$ gives the constant, which varies with the amount of enzyme and serves as a direct measure of it. The value thus obtained may also be calculated back to the dry weight of the enzyme preparation (corresponding with the 10-c.c. portion), and thus to the catalase factor, which is the $k$ units per grm. of dried material. Slight variations in the concentration of the hydrogen peroxide solution do not affect the results, and exact standardisation of the thiosulphate and peroxide is unnecessary. The method gives results agreeing to within about 10 per cent.

To prepare liver-juice, fresh liver is ground and mixed with approximately five times its weight of water, the liquid portion being removed and treated with about 1 per cent. of its volume of 0·1 *N* iodine; the mixture should not show free iodine. The liquid is heated rapidly to boiling and filtered, the filtered extract being quickly cooled. This extract retains its activity for several weeks if covered with toluene and kept cool. The hydrogen peroxide used must be free from stabilising preservatives and may be prepared by adding, gradually and with stirring, 7·8 grms. of the purest sodium peroxide to 1 litre of water containing 54·4 grms. of pure potassium dihydrogen phosphate and cooled to 0° to 2°. Practically no oxygen is evolved, and, after the sodium peroxide is dissolved, the solution should have $p_H$ 7·0 to 7·1, and contains 0·2 *N* hydrogen peroxide and 0·2 *M* phosphate buffer. At a low temperature this stock solution keeps well.

T. H. P.

# Organic Analysis

**Determination of Primary Propyl Alcohol in Mixtures of Ethyl Alcohol and Water. O. Noetzel.** (*Z. Unters. Lebensm.*, 1932, **64**, 288–293.)—Values for $n$ (determined by the dipping and Abbé refractometers) and sp.gr. of mixtures containing 60 per cent. of water, and 0 to 40 per cent. of *n*-propyl or ethyl alcohol, or both, are tabulated, and enable the proportion of the former to be determined, although the method does not distinguish it from *iso*-propyl alcohol. The following chemical method was selected after comparisons of the various colour reactions of the oxidation-products of these alcohols:—Five c.c. of the sample, which should be a distillate and should contain about 40 per cent. of total alcohols (*i.e.* sp.gr. 0·95), are placed in a 200-c.c. round-bottomed flask with 6 c.c. of a 25 per cent. solution of chromic acid, 0·5 c.c. of 50 per cent. sulphuric acid and a little pumice, and the mixture is then distilled through a delivery tube, 10 mm. wide, connected with a vertical condenser (*i.e.* similar to a Reichert–Meissl apparatus, but without the bulb). The outlet, which should be pointed, dips into a test-tube marked at 4 c.c. and immersed in ice-water, and the distillation (of 4 c.c.) should be complete in 5 minutes. Exactly 3 drops of a 2 per cent. solution of vanillin in alcohol and 4 c.c. of fuming hydrochloric acid are shaken with 0·6 c.c. of distillate, and, after 6 minutes at 65° C., the mixture is cooled, diluted if necessary with 4 to 8 c.c. of 25 per cent. hydrochloric acid, and the colour is then matched against that from a suitable alcohol mixture treated in the

same way, or against a colloidal solution of copper ferrocyanide. An orange colour indicates 3 per cent. of *n*-propyl alcohol, rose 5, red-brown 10, purple-red to brown 15, and deep purple 20. The accuracy is 3 per cent., and the reaction is unaffected by small amounts of *iso*-propyl alcohol; *n*-butyl alcohol gives a similar colour, but may be distinguished by its different physical constants. J. G.

**Detection of Acetone by means of *o*-Nitrobenzaldehyde. R. Raw.** (*J. Soc. Chem. Ind.*, 1932, **51**, 276T.)—The aqueous solution to be tested is mixed with three volumes of acetone-free methyl or ethyl alcohol and heated to boiling with a few crystals of *o*-nitrobenzaldehyde for about one minute. It is then poured into an equal volume of 10 per cent. sodium hydroxide solution and again heated for a few minutes at 80° to 90° C. In the presence of acetone a precipitate of indigotin appears almost immediately. If the amount is small, the solution should be allowed to stand overnight, the precipitate then settling to the bottom of the tube. The test is specific for acetone. R. F. I.

**Analytical Classification of the Fish-Liver Oils. N. Evers and W. Smith.** (*Pharm. J.*, 1932, **129**, 234–235.)—For the purpose of classification the iodine value of the unsaponifiable matter and the acid phthalic ester value are regarded as the most important to be determined in the case of fish-liver oils, which, commercially, fall into three groups; those of the *Gadidae* family (cod, haddock, etc.), those of the *Elasmobranchae* (shark, dog-fish, skate), and those of a few miscellaneous fish such as hake and halibut. Analytical data are given for a number of authenticated liver oils, and it is pointed out that the difficulty of extracting the whole of the unsaponifiable matter accounts for many of the low published figures. A table is given of mean values and limits for the ordinary tests for a large number of cod-liver oils. Determination of the cholesterol of the unsaponifiable matter groups the oils roughly into their zoological classification. The bulk of the cholesterol is separated in crystalline form by dissolving the unsaponifiable matter in about 10 volumes of absolute methyl alcohol, leaving the solution for 24 hours at 0° C., and filtering off the crystals. The methyl alcohol is evaporated, the residue is dissolved in about 10 volumes of acetone, and any insoluble matter is filtered off after 24 hours. Oils of the *Gadidae* family have definitely higher iodine values than any of the others (except those shark-liver oils containing large amounts of squalene). D. G. H.

**The Yellowing of Oxidised Drying Oils. A. C. Elm and G. W. Standen.** (*Ind. Eng. Chem.*, 1932, **24**, 1044–1045.)—Examination of the transmission spectra of alcoholic solutions of ketohydroxy- and diketostearic acids showed that the yellowing of oxidised drying oils is not due to the presence or formation of ketohydroxy compounds in the oil film. Diketostearic acid is yellow, whilst ketohydroxy stearic acid is colourless. The former has a well-defined absorption band in the blue end of the spectrum, whilst the latter has a less pronounced selective absorption, which, however, is entirely located in the ultra-violet region, and, therefore, cannot produce colour. Even the addition of excess of alkali does not produce a coloured compound. W. P. S.

# Inorganic Analysis

**Solid Carbon Dioxide [and Liquefied Fermentation Gas]. A. J. C. Cosbie.** (*J. Inst. Brewing*, 1932, **38**, 427–439.)—Solid carbon dioxide ("dry ice") is now produced in this country on a commercial scale, being transported in suitably insulated containers, and with only a small loss due to evaporation. An account is given of the author's method of utilising the solid product as a motive force for moving beer from place to place in a bottling department, and as a substitute for "tube" gas for carbonating beer. Solid carbon dioxide has about three times the refrigerating capacity of water ice. The inert constituents (about 0·2 per cent.) in "tube" gas are chiefly hydrogen and nitrogen, whilst in solid gas they are oxygen and nitrogen. The commercial gas from six cylinders did not, in any instance, exceed a purity of 98·5 per cent. of carbon dioxide immediately the cylinder was opened, whilst with the liquefied product from the solidified gas the first sample drawn contained 99·80 per cent. of carbon dioxide. The "tube" gas derived from a brewery differs from that obtained from solid carbon dioxide in containing a large proportion of impurities. Analysis of the drainings from fermentation "tube" gas gave the following results: Sp.gr., 0·895; acidity (as acetic acid), 0·36 per cent.; nitrogen, 0·05 per cent. By fractional distillation the liquid was separated into five fractions ranging from 171·5° F. to 213·1° F., leaving 4 per cent. of an "oily layer," and 3·5 per cent. of sludge. The main fractions consisted largely of ethyl alcohol, with small quantities of aldehydes, ketones and esters, and traces of iso-propyl alcohol. These substances are presumably responsible for the odour of "tube" gas.

**Simple Method for the Determination of Argon. H. Copaux.** (*Bull. Soc. Chim.*, 1932, **51**, 989–992.)—In the following method for the determination of argon in air and industrial gases, heated lithium metal is used for absorbing the nitrogen, etc. The gas is introduced into a Pyrex tube, 40 cm. × 15 cm. in diameter, of wall thickness not less than 2 mm., drawn out at one end and fitted with a sealed-on manometer at the other end, and containing two boats holding, respectively, phosphorus pentoxide and 0·25 grm. of lithium metal. The tube is sealed and the pressure of the confined gas is noted. The tube is heated to bring the lithium to a dull-red heat, until the internal pressure remains constant. The proportion of argon (together with other inert gases, which are usually in insignificant amount) is given by the ratio of the residual and initial pressures. If the gas contains notable proportions of oxygen or hydrogen, a third boat containing reduced copper or copper oxide, respectively, should be placed in the tube. The presence of hydrocarbons in the gas analysed introduces difficulties, unless further refinements, which it was desired to avoid in this simple method, are adopted.

S. G. C.

**Determination of Lead and other Metals in Iron Salts. A. D. Powell and G. F. Hall.** (*Pharm. J.*, 1932, **129**, 247.)—The iron salt, containing from 0·5 to 1 grm. of iron, is dissolved in 20 c.c. of hydrochloric acid (25 to 27 per cent. w/v), and strong nitric acid is added in sufficient quantity to oxidise any ferrous iron (0·5 to 2·5 c.c.). The solution is boiled, cooled, and, if prolonged oxidation

is necessary, the hydrochloric acid concentration is adjusted. The cold solution is treated in an extractor with three 20-c.c. portions of ether, and a fourth extraction is made if the acid solution is still coloured. The acid solution is heated in a narrow-necked flask on a steam-bath until the ether has volatilised, the acidity is almost neutralised with ammonia, potassium cyanide is added, and the lead test is completed by the usual Pharmacopoeial method. Any zinc, copper, nickel, or manganese remain in the acid solution after the extraction with ether. A series of determinations on 19 samples of iron ammonium citrate from various sources showed that the lead varied from 25 to 600 parts per million, but 50 parts is regarded as a reasonable limit. D. G. H.

**Volumetric Determination of Mercuric Chloride by Rupp's Method. H. Brindle.** (*Pharm. J.*, 1932, **129**, 245.)—The chief drawback of the Rupp method for determining mercuric chloride is the difficulty of dissolving the precipitated mercury in the iodine solution, and this was overcome by shaking for one minute with 5 ml. of a mixture of 2 vols. of B.P. ether and 1 vol. of B.P. chloroform before titrating the excess of iodine. D. G. H.

**Determination of Traces of Mercury in the Form of Rings of Mercuric Iodide. A. Delauney.** (*Ann. Falsificat.*, 1932, **25**, 409–412.)—The metal on which mercury has been deposited electrolytically is heated in a horizontal tube in a current of air, the mercury being condensed on a cool part of the tube and afterwards subjected to the action of hot air containing iodine vapour. The mercuric iodide thus formed is driven forward and collects on the walls of the tube as a ring, which is compared with standard rings prepared similarly. The portion of tube with the ring may be sealed off if required. As little as 0·01 mgrm. of mercury is thus detectable. Full details of the procedure are given.

T. H. P.

**Detection of Small Quantities of Bismuth with *o*-Hydroxyquinoline. R. Sazerac and J. Pouzergues.** (*Compt. rend. Soc. Biol.*, 1932, **109**, 79–82; *Ann. Chim. Anal.*, 1932, **14**, 406.)—*o*-Hydroxyquinoline, used in conjunction with potassium iodide, gives a double-iodide reaction with bismuth, which is claimed to be more characteristic than that given by the more commonly used cinchonine or quinine. With a solution containing less than 1 part per 100,000 of bismuth a flocculent, orange-red precipitate is produced; the precipitate appears under the microscope as clusters of orange-red prismatic crystals; with stronger solutions of bismuth the precipitate is partly amorphous, unless formed in presence of about 1 per cent. of nitric acid in the liquid. Iron does not interfere. The reagent is a 2 per cent. aqueous solution of *o*-hydroxyquinoline which is mixed with an equal volume of 4 per cent. potassium iodide solution immediately before use.

S. G. C.

**Determination of Mercury, Zinc, Fluorine and Arsenic in Impregnated Wood. Anon.** (*Chem.-Ztg.*, 1932, **56**, 730–731.)—The following methods have been tested for the analysis of wood impregnated with preservatives as under. The samples of wood used should be cut into pieces about the size of matches. *Mercury compounds* (usually mercuric chloride).—To 100 grms. of the wood are

added 500 c.c. of dilute hydrochloric acid (12·5 per cent.) and 40 c.c. of sodium chlorate solution (20 per cent.). The liquid is boiled under reflux for 1 hour and filtered. This extraction process is repeated twice with weaker acid (2·5 per cent.), and with 20 c.c. of the sodium chlorate solution. The combined extracts, after the addition of 50 c.c. of the sodium chlorate solution, are boiled under reflux until evolution of chlorine ceases, after which the hot solution is transferred to a beaker, and some kieselguhr and 10 c.c. of stannous chloride solution (10 per cent.) are added, with stirring. After 10 minutes the precipitate is filtered off on a Gooch crucible with an asbestos mat. The mercury in the precipitate is dissolved from the crucible by heating it in a beaker with 30 c.c. of concentrated sulphuric acid, and the diluted solution is titrated in the usual manner with 0·1 *N* ammonium thiocyanate solution, with 2 c.c. of a saturated nitric acid solution of iron alum as indicator. *Zinc compounds* (usually zinc chloride).—A sample of the wood is heated at the lowest possible temperature until completely carbonised; ashing is unnecessary. An excess of dilute hydrochloric acid is added to the residue, and the liquid is heated to boiling, then neutralised, made strongly ammoniacal and filtered, the zinc-content being determined in the usual manner. *Fluorine compounds.*—A 10- to 100-grm. sample is saturated with a calcium acetate solution [prepared by dissolving 30 grms. of calcium carbonate in 300 c.c. of dilute acetic acid (25 per cent.), and diluting the solution to 1 litre], and ashed. The ash is wetted with dilute acetic acid (25 per cent.), plus a few drops of 10 per cent. potassium iodide solution (the purpose of the latter being to reduce any manganic oxide), and the whole is evaporated to dryness on a water-bath. The residue is extracted with water, then filtered off and washed, and ultimately ignited. The fluorine in the residue is determined by evolution as silicon tetrafluoride according to Penfield's method, as described in Treadwell's *Analytical Chemistry*, Vol. II.

*Arsenic compounds.*—To 20 to 50 grms. of the wood, contained in a wide-necked 1-litre flask, are added 6 grms. of barium chloride, 10 grms. of ferrous sulphate, and sufficient concentrated hydrochloric acid to cover the wood. The flask is closed by a stopper carrying the following attachments: (*a*) Tap-funnel, (*b*) leading tube, (*c*) splash-bulb attached to a Liebig's condenser. The liquid is distilled in a slow current of air until evolution of hydrochloric acid gas ceases and the liquid boils quietly, the exit end of the condenser dipping below the surface of 300 c.c. of water; a further 100 c.c. of hydrochloric acid are added to the flask and the distillation is continued. The arsenic in the distillate is titrated with iodine, after neutralisation, in the ordinary manner. [The paper is a communication from the Laboratory for Wood Preservation of the Rütgers Co., Berlin.]

S. G. C.

**Electrolytic Determination of Gallium: Prevention of Anodic Platinum Losses. E. Reichel.** (*Z. anal. Chem.*, 1932, **89**, 411–421.)—In the electrolytic determination of gallium (ANALYST, 1932, 334) the anodic solubility of platinum is appreciable. It was found that hydrazine and hydroxylamine act as efficient depolarisers while not interfering with the determination. Correct results were obtained with an electrolyte consisting of water, 100 c.c.; strong ammonia, 25 to 35 c.c.; ammonium sulphate, 20 grms.; and hydrazine sulphate, 6 grms.; and with 1400 R.P.M., 1·7 volt, 5 amp., temperature 70° C., duration

30 minutes. With quantities of gallium exceeding 0·1 grm., the duration and the amount of hydrazine salt added were increased. The addition of hydrazine or hydroxylamine sulphate was likewise beneficial in the case of ammoniacal zinc, nickel, and cobalt solutions submitted to electrolysis. W. R. S.

**Determination of Cobalt by Means of Nitrosonaphthol. C. Mayr and F. Feigl.** (*Z. anal. Chem.*, 1932, **90**, 15–19.)—The precipitation of cobalt by α-nitroso-β-naphthol involves oxidation to the cobaltic state at the expense of the reagent, the reduction products being partly co-precipitated; hence the precipitate is not of stoichiometrically constant composition, and must be ignited to oxide. A pure precipitate, which can be used for gravimetric determination after drying, can be obtained as follows: The cold, weakly-acid chloride, sulphate, or nitrate solution (10 to 20 c.c.) is treated with 5 to 10 drops of hydrogen peroxide (perhydrol) and a slight excess of 2 *N* sodium hydroxide solution, then with 10 to 20 c.c. of glacial acetic acid; the precipitate is dissolved by warming, if necessary. The volume is made up to 200 c.c. with boiling water, and 10 to 20 c.c. of the reagent (a 2 per cent. solution of nitroso-β-naphthol in 50 per cent. acetic acid) are added. The liquid is vigorously stirred and boiled, the precipitate coagulating. After settling, it leaves the supernatant liquid clear and yellow. The precipitate is collected in a tared porous porcelain crucible, and the beaker is cleaned with 3 portions of hot acetic acid (1:3 water). The precipitate is finally washed four times with boiling-hot water, and dried at 130° C. until constant in weight. Factor Co: $[C_{10}H_6O(NO)]_3Co.2H_2O=0{\cdot}0965$. Precipitates containing more than 0·25 grm. of metal are bulky and require several hours' drying. The procedure separates cobalt from nickel, zinc, and aluminium, provided the precipitation is carried out at a higher acidity (up to 50 per cent. acetic acid for high nickel ratios); it provides a better separation from nickel and aluminium than electrolysis.
W. R. S.

**Determination of Rhenium. E. Kronmann.** (*Z. anal. Chem.*, 1932, **90**, 31–34.)—The stepwise concentration of rhenium by fractionation, for which several methods have been proposed, is tedious and rather uncertain. Some rhenium may be lost by volatilisation in the acid attack, while a small amount may remain with the insoluble residue in the subsequent filtration. The author submits the mineral to acid attack combined with distillation in a large all-glass apparatus (illustrated by two diagrams). The powdered mineral (250 to 1000 grms.) is treated with nitric acid, the nitrous fumes passing a cooler and escaping through a specially constructed receiver. The distillation is carried out in a current of air or hydrogen chloride, and the rhenium in the distillate is determined with nitron acetate. The procedure is not given, but reference is made to the papers describing it. W. R. S.

**Selenium as a Catalyst in the Kjeldahl Method for the Determination of Nitrogen in Coal and Coke. H. E. Crossley.** (*J. Soc. Chem. Ind.*, 1932, **51**, 237–238T.)—The advantages of selenium over mercury as a catalyst in the determination of nitrogen by the Kjeldahl process in flour, etc., found by Lauro (ANALYST, 1931, **56**, 813) have now been found to apply also to coal and coke. With the sulphuric acid mixture, as recommended in the Fuel Research Board

method (*Dept. Sci. Ind. Research, Physical and Chemical Survey of National Coal Resources*, Report No. 7), the time taken for a clear liquid to be produced in the digestion of a coal sample was 40 minutes with the selenium catalyst and 87 minutes with the mercury catalyst. The time required for coke was 30 minutes less with selenium than with mercury. S. G. C.

# Microchemical

**Micro-Determination of Carbon and Hydrogen in an Atmosphere of Nitrogen. J. B. Niederl and B. Whitman.** (*Mikrochem.*, 1932, **11**, 274–300.)—The chief source of error in the Pregl method of micro-combustion is the use of lead peroxide, which absorbs the oxides of nitrogen and sometimes some carbon dioxide and water also. When the combustion is carried out in an atmosphere of nitrogen, lead peroxide is dispensed with, the apparatus is simplified, and the time of combustion and sweeping-out of the tubes is reduced from 44 to 22 minutes. The filling of the tube consists of a length of 15 mm. of silver wire or wool, then 70 mm. of fine wire-form copper oxide, a wad of asbestos, 30 mm. of a mixture of equal parts of lead chromate and wire-form copper oxide, another plug of asbestos, and then 60 mm. of wire-form copper oxide, followed by a small plug of silver wire, then 30 mm. of reduced copper, another silver plug, and, finally, 115 mm. of wire-form copper oxide. The apparatus is the same as that used for the Pregl method, except that, as only nitrogen is used, only one gas-holder and pressure-regulator is necessary. The constant-temperature bath may be replaced by a cylinder of copper surrounding the tube, which is heated at one end by the long burner. This maintains the end of the tube at a sufficiently high temperature to prevent the condensation of moisture before reaching the absorption tubes. The combustion, which is carried out at a rate of flow of nitrogen of 5 c.c. per minute, takes 15 minutes, and a further 5 minutes is sufficient to sweep out all the products of combustion. The absorption tubes are then removed from the combustion tube, and 50 c.c. of dry air free from carbon dioxide are drawn through, a Marriotte flask being used, so as to replace the nitrogen by air; this takes only 2 minutes. The tubes are then wiped and weighed. The method of introducing the sample is to be further improved: for volatile or subliming substances about 4 cm. of powdered copper oxide powder are placed in the charged combustion tube, and the substance is introduced into a platinum boat placed close to the copper oxide. Substances of unknown composition and those hard to burn should be mixed with the powder-form copper oxide in the tube. The sample is weighed out into a small platinum scoop, which is dropped into the combustion tube in a vertical position, the scoop is removed with a wire loop, and the sample is mixed with copper oxide with a piece of platinum wire. Liquid samples are weighed out as in the Pregl method. Good results are obtained, and the method is simpler than the Pregl method for those unskilled in micro-combustion.

J. W. B.

**Determination of the Volatile Constituents of Copper-Zinc Alloys. L. I. Weinstein and A. A. Benedetti-Pichler.** (*Mikrochem.*, 1932, **11**, 301–310.)—The sum of the volatile constituents of copper-zinc alloys can be rapidly

determined on samples of a few mgrms. in weight by heating them at 1150° C. for 10 minutes in a gentle current of hydrogen. The hydrogen, which is generated in a Kipp apparatus and washed in dilute sodium hydroxide solution, passes through 1 metre of thin-walled rubber tubing (3 mm. bore) to the quartz combustion tube at the rate of 2 bubbles per second. The tube is heated electrically; it is 35 cm. long and of 8 mm. bore, and is tapered to a capillary of 0·5 to 1 mm. bore and 10 mm. long. The substance is weighed into a porcelain boat and is heated in the heating tube for 15 minutes. Simple alloys of copper and zinc gave good results. Commercial alloys containing a number of constituents gave a loss of weight slightly higher (1 or 2 per cent.) than that calculated for the total weight of volatile constituents. J. W. B.

**New Microchemical Test for Molybdenum, Vanadium and Tungsten. A. Martini.** (*Mikrochem.*, 1932, **12**, 112–113.)—*Molybdenum.*—A drop of a 1 per cent. solution of ammonium molybdate solution is placed on a slide, a little powdered pyrocatechol is added until the solution is orange-red, a drop of benzylamine is added, followed by a drop of aqueous acetic acid (1:5), and the mixture is stirred with a glass rod. Orange streaks appear, which under the microscope are seen as sheaf-shaped crystals. *Vanadium and Tungsten.*—The test is carried out in the same way, 1 per cent. solutions of salts of the metals being used. Vanadium forms pale yellow crystals, tungsten black crystals in the form of rosettes. The reactions take place in even more diluted solutions. Photomicrographs of the crystals are given. J. W. B.

# Reviews

MICROCHEMICAL LABORATORY MANUAL. By FRIEDRICH EMICH. With a section on SPOT ANALYSIS, by DR. FRITZ FEIGL, translated by FRANK SCHNEIDER. Pp. xvi+180 with 83 illustrations. London: Chapman & Hall. 1932. Price

This is a translation of the "Mikrochemisches Praktikum," 2nd edition, already reviewed in this journal (ANALYST, 1931, **56**, 138). It is the only text-book describing in detail both qualitative and quantitative inorganic micro methods, and an English translation was badly needed. The translation has been very carefully and efficiently carried out by Mr. Schneider, and if, at times, the German construction of the sentence has been retained in the translation, the meaning is always clear.

The translator retains the German word "schlieren" in his description of the phenomenon which might have been called "liquid striation" (as in *Recent Advances in Analytical Chemistry*, Vol. II, *Microchemistry*), and this is clearer for readers not familiar with German. Since Professor Emich's companion book, the *Lehrbuch*, has not also been translated, it is a pity that the very useful table of inorganic qualitative tests in that book could not have been included in the English edition of the Laboratory Manual. The Manual is, however, a most valuable and comprehensive book, covering the whole of inorganic microchemistry, including

"spot" tests, on which there is a very brief article by Dr. Feigl, the leading authority on the subject. JANET W. BROWN

WATER ANALYSIS FOR SANITARY AND TECHNICAL PURPOSES. By HERBERT B. STOCKS. Second Edition, revised and enlarged by W. GORDON CAREY, F.I.C. Charles Griffin & Co. 1932. Price 7s. 6d.

This little book was originally (1912) written "For the use of Public Analysts, Medical Officers of Health, and Students who are interested in this branch of Analytical Work." The new edition is very largely a reprint of the first, the main differences being the omission of the description of Frankland and Armstrong's method for the determination of organic carbon and nitrogen, and the addition of paragraphs on the determination of hydrogen-ion concentration, of free chlorine, and of iodides, and a short section on the bacteriological examination of waters. The chemical section of the book runs to 93 pages, the bacteriological section to 10, and eight appendices are contained in 28 pages more. These appendices are respectively: (1) Standards of Purity Recommended by the Rivers Pollution Commission, (2) Standards of Various Authorities for Effluents, (3) Average Composition of Unpolluted Water, (4) Tension of Aqueous Vapour, (5) Reduction of Cubic Centimetres of Nitrogen to Grams, (6) Table for Converting c.cs. of Indigo Solution to Parts of Nitrogen per 100,000, (7) Preparation of Reagents needed for Water Analysis, (8) Preparation of Culture Media for Bacteriological Examination of Water. An index of 5 pages completes the book.

As a text-book for beginners the book will serve its purpose very well; the methods of analysis are described carefully and in sufficient detail, and the inferences to be drawn from the results of analysis are justly and moderately stated. But it is to be regretted that a more thorough revision was not undertaken, and that the reviser has been so tender towards the original content and phraseology, for considerable improvement might have been made, without adding to the bulk of the volume. Burgess' method of comparing the colours of waters is mentioned, but it is not described, nor is a reference to Burgess' paper given; the use of aluminium foil or Devarda's metal instead of the copper-zinc couple in determining nitrates by reduction to ammonia might well have been mentioned, as might also Houston's methods of comparing the lead-solvent and erosive powers of waters. Space for these and for one or two other desirable additions might easily have been found by the omission of the four illustrations, which convey nothing that could not be understood from the descriptions, and in the last of which a bulb described in the letterpress as "pear-shaped" is shown as a very perfect sphere.

A more drastic revision would have allowed the reviser in many places to improve the language in point of accuracy or clearness; the expositions of the method of calculating results are frequently anything but clear—on p. 29, for instance, the word "amount" is used in two successive lines, and means "volume" the first time and "weight" the second, and on the same page the expression "Log of 1 c.c. $N_2$" occurs, as though there could be a logarithm of anything but a numerical quantity.

Our old friends "estimate" and "determine" hobnob with great friendliness throughout the book; on p. 5 we "estimate the amount and determine the

proportion"; on p. 47 "the estimation of hardness comprises the determination of two factors"; and so on. Again, the author is one of those, all too numerous now among the writers of scientific and technical papers and books, who worship "the latter"—on p. 4 we wash with water until "the latter" is neutral; on p. 18 a method for determining organic matter yields figures having no relation to the amount of "the latter"; and on p. 26 we have "of the former," where "indigo" would be not only more forcible and clear, but shorter. The book is full, too, of singular subjects with plural verbs or the converse, and we have on p. 10 a pipette requisitioned which shall be "easily" emptied—what is meant is "quickly," for the "ease" of emptying has nothing to do with the time. These may seem small things, but they irritate, and the writer of a scientific text-book should be scrupulous about them. He is inculcating accuracy (or ought to be), and his language should illustrate it. It is a pity that the opportunity given by revision for improvement in this respect should not have been seized.

Of purely typographical errors there are few—a transposition of letters in "formation" on p. 14, and the omission of N from 1/3200 N on p. 116 are all I have noticed. But a much more important error, possibly typographical, occurs twice on p. 41, in the omission of the brackets that should surround (38—12).

The directions for bacteriological examination are very concise and clear, and perhaps increase one's regret that Mr. Carey has not written a fresh text-book on water analysis instead of being under the restrictions necessarily involved to a greater or less extent in revision—though one may perhaps ask whether the language of the football field has yet crystallised into standard English so far as to permit one to speak of negotiating a reservoir.

Taken with all its minor faults, it is a useful little book, though a better one, of similar size and scope, is much to be desired. J. T. DUNN

THE PREPARATION OF PURE INORGANIC SUBSTANCES. By E. H. ARCHIBALD. Pp. 383. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 1932. Price 23s.

In producing this volume, Professor Archibald, who occupies the chair of Analytical Chemistry at the University of British Columbia, has earned the thanks of all research workers to whom pure chemicals are an absolute necessity, and who themselves have to carry out methods of purification in order to obtain material in as near a state of absolute purity as possible. The book consists of a collection of the methods for preparing pure chemicals employed by the leading scientific research workers during the past thirty years. In the preface the author states that what are thought to be the most dependable and simple of these methods are set forth, and that only those methods of procedure have been included which have been proved to produce what we may consider pure substances.

In Chapter I, purification processes in general, and the most suitable materials for the necessary apparatus, are discussed. Centrifugal draining and washing are recommended as the most efficient way of removing the mother liquor from crystals, and Richards' work on these lines is quoted. In the subsequent chapters, the whole of the elements, with the exception of the following, are dealt with: Ma, Rh, Nd, Il, Eu, Gd, Tb, Yb, Lu, Hf, Re, Os, Ir, Po, Nt, Ac, and Pa. The

chapters are arranged systematically, according to the periodic table, and Chapter II deals with the zero group, helium, neon, krypton, and xenon; Chapter III, with group IA, comprising hydrogen, lithium, sodium, potassium, rubidium and caesium, and so on.

Methods for the preparation of the pure element are described in some cases, of one or more of the pure salts in other cases, or of both element and salts. Copious references to the original papers are given and twenty line drawings of special apparatus are figured; the book is well indexed, there being a subject index and an author's index occupying 13 pages.

This is a very readable book, and will be a useful addition to the shelves of all scientific libraries. The author's style is very lucid, explanations of the various stages of the methods are given in simple language, and, apart from the spelling of sulfur, sulfate, and cesium, the text is free from Americanisms.

T. TUSTING COCKING

ANTIQUES; THEIR RESTORATION AND PRESERVATION. By A. LUCAS, O.B.E., F.I.C. Second Edition. Pp. 240. London: Edward Arnold & Co. 1932. Price 8s. 6d. net.

In the new edition of his practical little handbook Mr. Lucas rightly lays stress upon the importance of chemical knowledge for the successful cleaning or restoration of museum objects. This is now widely recognised, and the directors of the most important museums either have a chemist on their staff or constantly consult one. The curators of small museums, however, can seldom avail themselves of expert assistance, and for all such this book will prove invaluable, not only by giving directions how to treat the various objects, but also by showing what methods should be avoided. As the author points out, some positively injurious methods continue to be recommended, owing to the fact that for the time being they appear to have been successful, and nothing is said about the condition of the objects after the lapse of a year or two.

The new edition follows the general arrangement adopted in its predecessor (*cf.* ANALYST, 1925, **50**, 102), the first two chapters dealing with the general methods of cleaning and preservation, and the third with the application of those methods to objects of specific materials, including metals, plaster, wood and stone. The section on pictures is very complete, and the treatment most suitable for each class (oil paintings, water colours, tempera, etc.) is described in detail. Finally, there is a chapter on simple physical and chemical tests, which will help curators without chemical knowledge to identify the material of which a specimen is composed, and the book concludes with a useful bibliography and a good index.

During the last eight years the author has had almost unrivalled opportunities of studying this subject practically in connection with the preservation of the objects found in the tomb of Tut-ankh-amūn, and of specimens in the Cairo museum, and he embodies the results of his experience in this book, which contains so much new matter that the 135 pages of the first edition have now become 240.

Although, as has been mentioned, the book is primarily intended for those with little, if any, scientific knowledge, it will also be of great use to all chemists who are likely to be consulted on the preservation of structural materials.

EDITOR