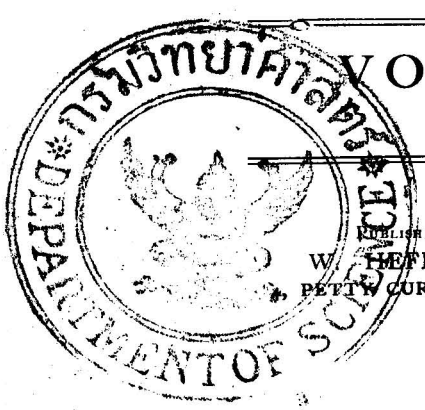
THE ANALYST

THE ORGAN OF THE

Society of Public Analysts and other Analytical Chemists

A MONTHLY JOURNAL DEVOTED TO THE ADVANCEMENT OF ANALYTICAL CHEMISTRY



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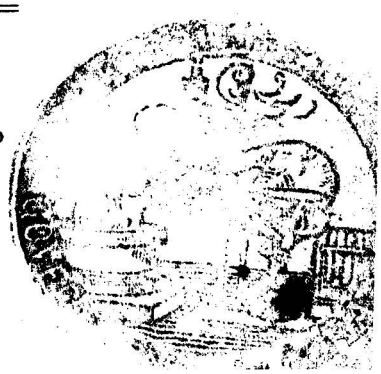


JANUARY, 1928. Vol. LIII., No. 622.

THE ANALYST

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

AN Ordinary Meeting of the Society was held at the Chemical Society's Rooms, Burlington House, on Wednesday, December 7th, the President, Mr. E. Richards Bolton, F.I.C., being in the chair.

Certificates were read for the first time in favour of George Reginald Barnes, B.Sc., Cecil Abell Bassett, B.Sc., A.I.C., Ethel Irene Beeching, M.Sc., A.I.C., Harold Pease Buttrick, A.I.C., Cecil Owen Harvey, B.Sc., A.R.C.S., A.I.C., Harold Vivian Horton, B.Sc., A.I.C., Thomas Howard, M.Sc., A.I.C., Harold McKee Langton, M.A., B.Sc., A.I.C., William Alfred Nottage Markwell, Walter George Messenger, B.Sc., A.I.C., Edward John Newby, B.Sc., Horace Samuel Rooke, M.Sc., A.I.C., Claude Trevine Symons, B.A., F.I.C., David Rees Thomas, M.B., Ch.B., S. Sera, and Wilfred A. Whitley.

Certificates were read for the second time in favour of Alfred Harry Bateman, B.Sc., A.I.C., Arthur Owen Blackhurst, M.D., William Clayton, D.Sc., F.I.C., Charles William Cornwell, B.Sc., A.I.C., and Thomas Riley, A.I.C.

The following were elected Members of the Society:—Leslie V. Cocks, A.I.C., Frederick Dixon, B.Sc., A.I.C., David Michael Freeland, A.I.C., Desmond Geoghegan, Claudius George Hyde, A.R.C.S., F.I.C., Vernon James Tilley, F.I.C., Leonard Wild, B.Sc., and Hugh A. Williams, A.C.G.F.C., A.I.C.

Demonstrations were given by G. W. Monier-Williams, O.B.E., Ph.D., F.I.C., of an Apparatus for determining Benzoic Acid in Foods, and by T. McLachlan, F.I.C., and A. W. Middleton of a Sodium Flame for Polarimetric Work.

The following papers were read and discussed:—"Oil Bromide Films and their use in determining the Halogen Absorption of Oils," by Harold Toms, M.Sc., A.I.C. (work done under the Analytical Investigation Scheme); "Tests for Impurities in Ether," by G. Middleton, B.Sc., A.I.C., and F. C. Hymas, B.Sc., A.I.C.; and "Arsenic in Coated Papers and Boards," by Harold J. Stern, B.Sc., Ph.D., F.I.C.

The Biological Test for Blood.

By SIR WILLIAM WILLCOX, K.C.I.E., C.B., C.M.G., M.D., F.R.C.P., F.I.C., D.P.H. (Medical Adviser to the Home Office).

(*Read at the Meeting, November* 2, 1927.)

Until 1901 the examination of blood stains depended upon macroscopic chemical, spectroscopic, and microscopic tests.

By means of the microscopic examination of blood, stains which had been carefully prepared by fixation and staining with a special stain, such as haematoxylin and eosin, the species of animal from which the blood had been derived could not be stated with any certainty.

Mammalian blood could be differentiated from that of birds, fishes, reptiles and amphibia, since the red corpuscles of the latter four groups are nucleated, oval and larger in size, whereas mammalian red corpuscles are non-nucleated. The red corpuscles of mammalian animals differ somewhat in size, but the differences are so slight that one cannot, with certainty, differentiate the blood of man from that of other mammals by measurement of the diameter of the red corpuscles. It may be mentioned that the red blood corpuscles of the camel tribe are oval in shape and non-nucleated.

About 1900, and onwards, great attention was paid by research workers to the possibility of the use of biological tests for human blood. These tests depend on the production of anti-bodies in a living animal when foreign substances of protein nature of biological origin are injected into the living animal. It is interesting to consider the type of research which led up to the discovery of the Biological Tests for Blood.

In 1890 Behring discovered that antitoxic substances were developed in animals, following the injections of doses of diphtheria toxin, and, as a result of this work, diphtheria antitoxin, which has proved of so great therapeutic value, was discovered. Ehrlich, in 1891, produced antitoxic substances in animals, which were capable of neutralising the poisons ricin and abrin which are present in castor oil seeds and jequirity seeds, respectively. Experiments with snake venom, whereby antitoxic substances were produced, were made by Sewall in 1887, Calmette in 1894, and Fraser in 1895. Then Ehrlich propounded his beautiful theoretical explanation of the phenomenon occurring in the production of anti-bodies, which is still at the present time of great value and wide application.

About 1896 it was discovered by Durham and Widal that the injection or infection with specific bacteria caused the development in the body of anti-bodies which caused agglutination of these bacteria. On this work was founded the Agglutination Test for Typhoid Fever, which bears the name of Widal, and which has for many years been of universal use as a diagnostic test. It has applications in many other diseases, such as the para-typhoid group, Malta fever, etc.

In 1898 Bordet found that haemolytic bodies were produced in the sera of animals when foreign red blood corpuscles were injected into their circulation.

It was found that these bodies "Haemolysins" were specific in nature. In 1900 Deutch proposed a haemolytic test for human blood. This depended on the removal of some of the red blood corpuscles from a suspected stain by means of carbolised normal saline.

The suspension of the corpuscles of the suspected stain was treated with an antiserum which had been prepared by the injection of human red blood corpuscles into a rabbit, and which contained haemolysins destructive to human red corpuscles. If the corpuscles in the suspension of the suspected stain were haemolysed, it was thought that this was proof that the blood was human.

This test has never come into use for forensic purposes, owing to the many fallacies to which it is liable; for example, the age of the stain and the atmospheric conditions to which it has been exposed may cause disintegration of the red corpuscles and solution of the haemoglobin, quite apart from any specific action of the antiserum.

THE PRECIPITIN TEST.—It was found that the injection of special proteins into animals (rabbits usually being chosen for this purpose) caused the development of anti-bodies in their blood serum. The serum of the animal, after a suitable series of injections, became very sensitive to the protein used for its immunisation, and would cause the formation of a precipitate when mixed with even high dilutions of the protein in question. Thus Kraus, in 1897, produced precipitins in the serum of animals submitted to injections of milk. Bordet, in 1897, confirmed this work. Myers, in 1900, produced specific precipitins from the injection of egg albumin and of serum albumin. Uhlenhuth, about 1900, did a great deal of work on the precipitin test, and was the first to apply it for medico-legal purposes in 1901.

Nuttall and his collaborators, Graham Smith and Sanger, were working at Cambridge at this time on the biological tests for various species of blood. In 1904 Nuttall published his masterly treatise on Blood Immunity and Blood Relationship.

As a result of the above work on the precipitin tests and of the improvement of technique, a test of great value and reliability has been devised, whereby it may be stated with certainty the species of animal from which a blood stain has been derived. Dr. Roche Lynch deals fully with the technique of this test (*infra*) and can speak with the knowledge derived from great practical experience.

The only contribution I would like to make to this part of the subject is to emphasise the great importance of working with perfectly clear solutions of the suspected stain. A turbid solution of a few drops of the stain which has been teased and soaked in normal saline can be readily clarified by placing it in a small glass pipette, the commencement of the narrowed part of which has been packed with a few shreds of torn filter paper. If a rubber teat is now placed on the open wide end of the pipette, compression of it readily drives through the turbid liquid and clarifies it so that it is ready for the application of the test.

Dr. John Glaister, jnr. read before the Medico-Legal Society last year an excellent paper dealing with the technical details of the precipitin test in its application for medico-legal purposes.

The precipitin test has been used by the Scientific Analysts for the Home Office since 1908, and I could quote numerous cases in which it has been successfully applied. In 1905 experiments were made by the late Sir Thomas Stevenson on the medico-legal application of the test. It was not until 1908 that the test was used as a routine test in the examination of blood stains for forensic purposes by the Home Office. By this time much had been learnt as regards the preparation of very sensitive antisera, and it was found that the reliability of the test was beyond dispute.

The Staffordshire Case.—A blue serge jacket was sent to me from Staffordshire in 1912. It had on the sleeves some stains which gave the chemical test for blood, the microscopic test for mammalian blood, and the precipitin test for horse blood. Inside the jacket pocket was a handkerchief with stains upon it of mammalian blood, and the application of the precipitin test showed these stains to be human blood. On examination of the coat with a lens several short bay hairs were discovered, and also two or three long black hairs. These hairs had the characteristics of horses' hair. The coat belonged to a man who was accused of horse maiming, his victim being a bay mare with black points. At the time of arrest the accused had a recently cut finger. The scientific findings entirely confirmed the charge against the accused.

The test is of great value for forensic purposes, and has a universal application. A modification of the precipitin test promises to be of great value in food analysis, *e.g.*, in the determination of the kind of meat entering into the composition of potted meats, sausages, etc. Anti-sera containing precipitins specific for the muscle juices of different animals can be prepared and made use of for testing the specific nature of meat stuffs. The late Dr. W. A. Schmidt, in 1908, published a valuable paper in the *Cairo Scientific Journal* on this subject.

Before leaving the subject of the Biological Tests for Blood I should like to express the opinion that these should only be carried out by experts familiar with all the technique of the processes, and who have themselves prepared the anti-sera which they use in their tests. Anti-sera for the precipitin test are on the market, but they are difficult to obtain. Their sensibility is often very variable, and, on this account, it is best for an expert to work, as far as possible, with anti-sera which he has himself prepared, and of which he knows the exact limit of sensibility.

COMPLEMENT FIXATION TEST.—Bordet introduced this test. It depends on the prevention of haemolysis of the red blood corpuscles of an animal which has been sensitised to the inactivated serum of a particular animal, the complement being derived from the solution of the suspected stain and being fixed by an anti-human blood serum.

This test is stated to be a hundred times more delicate than the precipitin test. It has not come into general use for forensic purposes, because the

haemolysis of red blood corpuscles is susceptible of so many additional factors, and it has been felt that the test is not sufficiently safe for forensic deductions to be made from it. The test may be used as a confirmatory test to the precipitin test.

BLOOD GROUPING TESTS.—The blood of human beings varies in certain properties, and individuals belong to one of four groups as regards the agglutinating properties of their serum and corpuscles. The particular group to which an individual belongs is permanent for his lifetime. Dr. Martley speaks with experience of the application of this test. The Blood Grouping Test may be applied in cases of alleged paternity.

It is thus seen that the Biological Tests for Blood enable the forensic expert to say from what species of animal a given blood stain is derived, and, if the blood is human, he may state to what human blood group the stain belongs.

The Technique of the Precipitin Test and Its Forensic Value.

BY G. ROCHE LYNCH, O.B.E., M.B., B.S., D.P.H. (Senior Official Analyst to the Home Office.)

(*Read at the Meeting, November* 2, 1927.)

THE precipitin reaction for the identification of the source of a sample of blood is the application of a test which, from a scientific aspect, has a much wider significance.

When a solution containing protein is placed within an animal by any other means than *via* the alimentary canal, the blood of the animal becomes immunised against that protein, provided that the protein is foreign to that animal. The protein is called the *antigen*, and the substance developed in the blood and producing the immunisation is called the *anti-body*. This applies to nearly every known protein, and in the majority of cases the anti-body takes the form of a precipitin, although in some cases it may have additional properties. The antigen must be a native protein uncoagulated and in solution, which, of course, is colloidal in character. When the blood of the animal, that is to say, the serum moiety which contains the anti-body, is mixed with a solution of some of the same protein used for injection into the animal a precipitate forms. This action is probably a means of throwing out of solution the foreign protein in the living animal, thus rendering it harmless and in a state suitable for the action of enzymes whereby it can be destroyed. The anti-body is specific to the antigen, that is to say, precipitation will only occur when the anti-body is mixed with the same kind of protein which in the first place evoked the formation of the anti-body in the

blood of the animal concerned. This general statement is, in the main, true, with the exception of certain minor group reactions, which will be dealt with later. The anti-body appears in the blood a few days after injection, and disappears when a second injection is made, only to reappear again. If the animal receives no more injections the anti-body soon disappears; how is not known, but it is not excreted, as such, in the urine.

The source of the antigen may be bacterial, animal, or vegetable, and the anti-body production is, no doubt, a response to the injection of a poison, for, although varying in degree, all foreign proteins are toxic to the animal organism.

Precipitin formation requires the presence of electrolytes, and will take place within a fairly wide range of hydrogen ion concentration, which is stated to be from P_H 4·5 to 9·5, and within this range the bulk of the precipitate formed is the same, other conditions being equal. Excess of antigen may inhibit precipitin formation, but excess of the anti-body has no effect in this direction.

When the production of anti-bodies from an antigen consists of mixed proteins such as we have in blood serum, a number of anti-bodies will be produced equal in number to the number of proteins in the material injected. Further, if the various proteins of, say, blood serum were separated and injected into a number of animals, we should obtain one anti-body in each case which was capable of reacting only with the particular antigen from which it was produced.

With these few preliminary remarks concerning the general properties, I will proceed to a detailed description of the complete technique of the test as used for the detection of blood stains, and will conclude with a short discussion of the other applications of the test.

For convenience I will divide the test into four parts:—(1) Preparation of the antiserum; (2) preparation of the extract of the unknown stain; (3) the test; and (4) deductions, fallacies and other applications.

(1) PREPARATION OF THE ANTISERUM.—Of all the available animals for experiment, only two are of use to us for this test, namely, the chicken and the rabbit. Although precipitins are developed in other animals, the response is comparatively feeble and the serums of little value for our purpose. Of the chicken, I have had no experience, but it has been used to some extent in America and to a larger extent in India by Sutherland and his successors. The rabbit is a convenient animal, and, as a rule, gives good results. It is possible to purchase antisera, both in this country and from Germany, but some samples I have had have been unsatisfactory. All antisera should be kept on ice, handled as little as possible, and warmed up as slowly as possible; consequently the home-made variety, whose history is known, is obviously more suitable. Incautious handling may result in loss of titre (that is, the greatest dilution of the antigen which will produce precipitin formation with the anti-body or antisera), and may also result in loss of specificity.

The principle of the preparation of the antisera is to inject, either intravenously or intra-peritoneally, a series of doses of the protein against which you wish to

produce anti-bodies—in our case human blood. After a variable time has elapsed the blood of the rabbit is tested, and, if found potent, is bled, and the serum collected and preserved. The dosage and time intervals recommended are legion, and I can only refer to a few; generally speaking, the intra-peritoneal methods yield antiserum with the highest titre, but the intravenous route produces a workable antiserum in the shortest time. Willcox, I believe, used intra-peritoneal methods, injecting about 5 c.c. every 5 to 6 days, giving 5 to 6 injections, and testing the animal a few days after the last dose. I have used the intravenous route, giving 1, 2, 3, 4, and 5 c.c. at three-day intervals, and testing 3 days after the last dose; also giving 2 c.c., waiting 6 days, then 2 c.c., waiting 6 days (both intravenously), then giving 5 c.c. intra-peritoneally, and commencing to test about 6 days later. I have also tried Hektoen's method, giving 5–10–15 c.c., intra-peritoneally on three successive days, and testing about 10 days later. Lloyd, in India, who probably employs this test to a greater extent than anyone, and who tells me that he examines over 100 articles a week, always gives on the first day 4 c.c. intravenously, 8 c.c. on the fourth day, and kills on the twelfth day. If the serum is not satisfactory it is discarded and fresh animals immunised. I have obtained good and poor results with all methods, but, of them all, I am inclined to favour the methods which employ both intravenous and intra-peritoneal routes. Generally speaking, if an animal does not develop good anti-bodies within the limits laid down, it is useless to give further injections, and it is better to start afresh with another animal. During the process of injection there is a troublesome feature which is liable to occur, especially after the third dose, namely, the liability of the animal to collapse and die within a minute or two after receiving the injection. The condition is known as anaphylactic shock. Whether it is due to too large doses being given I am not prepared to say, but regarding the schemes of dosage I have given, it would be wise to modify the dose a little according to the size of the animal, assuming that the doses given are for an animal weighing 2 kilos. The condition of anaphylactic shock may often be avoided by giving 2 drops of the serum intravenously, waiting 30 to 60 minutes, and then giving the remainder, and I always do this before every third and fourth dose. This process is called de-sensitisation. The protein solution for injection must be fresh, sterile, and according to Lloyd, inactivated by heating to 55° C. for 30 minutes. Convenient amounts of blood may easily be obtained by drawing it off by means of a syringe from the vein in the forearm and separating the serum. Many workers recommend the use of whole blood for injection purposes, so that, in addition to the serum precipitants, one is able to obtain the haemoglobin precipitants as well. There appears to be little advantage in this in practice. If whole blood is used it must be either defibrinated or citrated and intra-peritoneal routes only used.

In the rabbit the vein at the edge of the ear is a convenient one for injection, and the injection is made with a syringe and needle. For the peritoneal route a syringe and needle is used, the needle being inserted through the abdominal wall and the material injected. When a satisfactory titre has been obtained the animal is anaesthetised and bled into sterile tubes from the carotid artery, or by

and heart puncture with needle and syringe. Clotting takes place, the serum is removed and stored in sealed sterile tubes on ice after centrifuging. It is best not to add preservative, but, if there is a doubt about the sterility, a little carbolic acid may be added, equivalent to about 0·5 per cent. Chloroform should not be used, as has been recommended. Before bleeding, the animal must be starved for 12 to 18 hours, since otherwise the serum will be opalescent, owing to the presence of minute fat globules from the food; such a serum is useless. The serum may also be dried in shallow dishes over sulphuric acid *in vacuo*, and the dried serum reconstituted as required. Such a serum often gives good results, but the process generally results in loss of titre. If successful, a dried serum will keep longer than that kept in liquid form. On the whole, I do not recommend this. A serum to be of use for the test must be capable of precipitin formation with, at least, a 1–5000 dilution of human serum in normal saline (0·9 per cent.) in 20 minutes at room temperature, and a really satisfactory serum should give a positive result in a dilution of 1 in 20,000. Lloyd, in India, tells me that he never uses a serum unless the titre is 1 in 40,000. The serum must also be tested for specificity, and should fail to give a positive reaction with the sera of other animals (except closely related species) in dilutions of 1–100 or less. Unless it satisfies this condition the antiserum must be discarded. Nearly every antiserum will show a slight reaction with any sera, but in dilutions of not more than 1 in 50.

(2) PREPARATION OF THE UNKNOWN EXTRACT.—A small fragment of the article containing the stained area is cut into small pieces, placed in a small tube with a few drops of saline, and incubated at 37° C., to enable the proteins of the stain to dissolve. It is, of course, assumed that the stain has already been proved to be blood. After sufficient time has elapsed for the proteins to be dissolved (which will vary with the age of the stain and other factors) the fluid is withdrawn and filtered. A convenient device for the filtration of small amounts of liquid without appreciable loss is shown in Fig. 1. This filtered extract generally contains haemoglobin or one of its derivatives, and may also be used, prior to the precipitin test, for examination in the microspectroscope. The next stage is to test the extract in order to obtain some idea of the concentration of the proteins present. This may be done in three ways:—(A) Foam test; (B) nitric acid test; and (C) salicyl-sulphonic acid test.

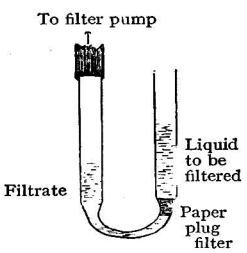


Fig. 1.

(A) *Foam Test.*—This test, which is probably used by a majority of workers, is based on the fact that if air is bubbled through a dilution of normal serum of 1 in 1000 in saline, bubbles will persist for at least 10 minutes, but in greater dilutions the foam disappears almost at once. If the bubbles do not persist, the unknown extract is assumed to be of a concentration of less than 1 in 1000. If the bubbles persist, the extract is further diluted until the bubbles disappear

within 10 minutes. One source of error from this test is that often the clothing to be examined is very dirty, and other substances may be extracted which will greatly modify the reaction. Further small variations in the reaction of the distilled water and the purity of the salt used for making the saline solution have their influence, and, lastly, different bloods show variation. I have often found a blood failing to give the test in a dilution of 1 in 500.

(B) *Nitric Acid Test.*—This test consists in layering strong nitric acid beneath the extract of the stain, and at the junction of the liquids, if the dilution is about 1·1000, a faint opalescent ring will appear, whilst dilution greater than this will scarcely show any opalescence. The test is rather troublesome in practice, and only enables a very general idea of the concentration of the protein to be obtained.

(C) *Salicyl-sulphonic Acid Test.*—This test takes advantage of the fact that salicyl-sulphonic acid precipitates protein, giving a delicate opalescence in dilutions of 1 in 12,000, and upwards, of blood. If a series of dilutions of the unknown be compared with known dilutions of blood, the degrees of opalescence may be matched, and a very fairly accurate estimate of the concentration of the protein obtained.

A portion of the unknown extract is diluted a known number of times until its colour, *i.e.* of haemoglobin, is just visible. This solution is then diluted 1:2, 4, 6, 8, 10, 12, 16, and a unit volume is placed in these tubes. Dilutions of blood (1:2000, 4000, 6000, 8000, 10,000, 12,000, and 16,000) are placed in similar tubes in unit volumes, and about 1/20th of the volume of 50 per cent. salicyl-sulphonic acid is added to each and the contents mixed. The opalescence is then matched, and, by a simple calculation, the concentration of the original extract is obtained.

(3) THE TEST.—There are four ways of making the test, viz., (A) Layering; (B) dropping the antiserum, drop by drop, on to the unknown extract, so that it mostly sinks to the bottom; (C) running the antiserum down the side of the tube, which has been previously moistened with the unknown extract; and (D) capillary tube method suggested by Colles (especially convenient when large numbers of samples of stains have to be examined and economy of antisera is necessary).

Of these four methods, I recommend the first, and if the unknown has been carefully layered above the antiserum so that a sharp ring is formed at the junction of the liquids, and if the tube is examined in an oblique shaded light against a black background, the faintest trace of precipitin formation will easily be detected.

The antiserum is used undiluted, but the antigen or unknown is diluted, so that the concentration is not greater than 1 in 1000.

A concentration of 1 in 1000 will completely avoid any negative reactions due to zone reactions, and will avoid false positive results from group reactions, except in the case of very closely related species. Generally one puts up a series of dilutions of the unknown extract from 1:1000 to 1:10,000, so that one can observe the gradual fading of the reaction.

The antisera and extract must be absolutely clear, which is ensured by filtration or centrifuging, or both. The complete test, no matter which technique is used, requires the following tubes for control:—

(1) Antiserum and saline used for dilutions.
(2) ,, portion of article under examination free from blood.
(3) ,, known human blood (1:1000).
(4) ,, animal blood, say, horse (1:100).
(5) ,, ,, ,, sheep (1:100).
(6) ,, ,, ,, dog (1:100).
(or any animal likely to be put forward by defence.)
(7) Normal rabbit's serum and extract of stain under test.
(8) Antiserum and borax or cyanide solution if such was used for rendering the stain soluble.
(9) ,, unknown extract estimated dilution (1:1000).
(10) ,, ,, ,, ,, (1:5000).

The tubes are allowed to stand at the laboratory temperature for 20 minutes, and are then read. With good antisera, tubes 3, 9, and 10 will have given positive results if the stain is human. The other tubes (Nos. 1, 2, 4–8) should be completely negative, but at the end of an hour or more it is possible that tubes 4 to 6 may show some slight traces of precipitin formation.

If borax or potassium cyanide has been used for extracting the stain it is usual to neutralise the extract with tartaric acid, and these chemicals must appear in the list of controls.

(4) FACTORS INFLUENCING THE REACTION.

Favourable.

(*a*) Stains which have been rapidly dried at room temperature and have been kept in a cool, dry place.

(*b*) Recent stains, as against old stains; but stains 50 years old have given positive results.

Unfavourable.

(*a*) Stains which have been heated, whereby the protein is coagulated or decomposed.

(*b*) Stains which have been in contact with some protein coagulant, *e.g.* formalin, picric acid, alcohol, mercuric chloride, tannic acid, etc.

In this connection it must be especially remembered that substances of this type, if present, may go into solution during the process of extraction and produce

false positive results, but this is controlled by tube 2, which would give a positive result. When stains on leather, and possibly on certain woods, are under examination the stain should, if possible, be detached from the article prior to the extraction, so that errors from this source may be avoided.

(*c*) Any chemical treatment, whereby the proteins are denatured.

(*d*) Action of proteolytic enzymes, *e.g.* pepsin.

(*e*) Putrefaction may alter the protein, so that a negative result is obtained, but a stain of this type is always worth testing.

(*f*) Age of stain alluded to above.

USES OF THE TEST.—Positive results will be obtained with all body fluids and tissues, with the exception of tears, crystalline lens, semen, and milk. These substances either do not contain the proteins of the serum, or (in the case of the crystalline lens) there is no blood circulation. In each case special antisera have been made against these proteins, but this is of no practical value. Albuminous urine, pericardial, pleural and peritoneal fluids, cerebro-spinal fluid, blister fluids and menstrual blood all give positive results, as they all contain the same antigens as the blood of the particular species. Bones, if well pounded and extracted with ether before making the saline extract, will give positive results. In medicine there are two uses for the reaction, *viz.* the detection of occult blood in the faeces and the addition of egg albumin to the urine to simulate disease. The former is often difficult to decide by the ordinary chemical test, as unless careful dieting is carried out prior to the test, blood derived from the food may give positive chemical tests for blood.

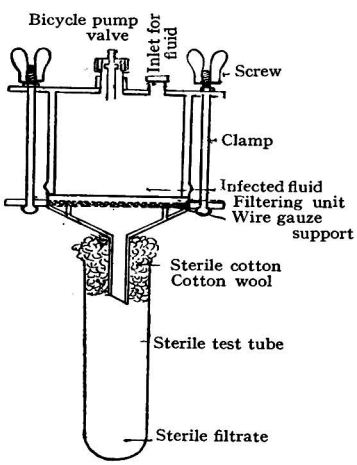
The test, apart from the examination for human blood, is, of course, valuable, but unless some idea is given to the worker, the determination of the source is likely to be very laborious. It has been successfully used in cattle-maiming cases, and has substantiated defences in cases where the butcher stated that the stains came from his trade, and in a motor car accident where the defence was that the blood was from a hare which the defendant had shot and placed in the car. In all these cases the blood was found not to be human, and in some of them the test was carried further and the blood source determined.

In America it has been used for the enforcement of the game laws (in certain States rare species of animal are preserved) and successful convictions have been obtained.

EXAMINATION OF FOOD.—The examination of food preparations for egg protein, presence of horse flesh in food, foreign cereals in flour, and the genuineness of honey are all practical applications of the test, and have been carried out by various workers. Regarding the differentiation of beef, mutton, goat, and pork, there are difficulties, owing to the close family relationship of the ruminants, as mentioned below.

When dealing with meat one works with anti-musculoprecipitant serum and antiserum prepared from blood. These are prepared in the same way by

injection, except that saline extracts of muscle tissue are made. Of course, all muscle tissue contains blood; consequently anti-musculoprecipitant serum will react to some extent with blood, but better results will be obtained with muscle extracts. The technique for the preparation of these extracts is as follows:—Fresh muscle tissue, free from fat, is minced, pounded up with sharp sand, and extracted with saline. After being strained through muslin, the extract is filtered through cotton wool and centrifuged, the supernatant fluid being carefully removed. This extract is full of micro-organisms, and, if injected in this state, would probably kill the animal. It is therefore filtered through some apparatus, such as that shown in the diagram, and a sterile filtrate obtained.



The approximate concentration of protein may be determined by the salicyl-sulphonic acid method, and the dosage regulated to be similar to that given in the case of serum. Any of the above series of doses may be used. With regard to honey, it is obvious that the difficulty of obtaining the proteins in pure condition will be great, and that a relatively large quantity of honey will be required to obtain sufficient protein for injection, but there is no theoretical objection to the process. Cooked meats are, of course, unsuitable for examination, and if pies were required to be examined, arrangements would have to be made for their seizure prior to cooking.

GROUP REACTIONS.—It is a well-known fact that anti-human serum will react to a less extent with the blood of closely related monkeys. Anti-hare serum will react almost as efficiently with normal rabbit serum as it does with normal hare serum, as these animals are still more closely related. The ox, the sheep, the goat and the pig are all fairly closely related animals, and the anti-sera or the anti-musculo sera will give group reactions in (it is stated) fairly high dilution. I can give you no personal experience of this, but workers such as Sutherland, whose experience of the test was second to none, state that the differentiation is quite practicable. The methods of approaching this difficulty are four in number.

(1) *Weichardt. Adsorption.*—To prove, for example, the presence of ox blood: Prepare the anti-ox serum from a rabbit, add to it normal sheep serum. A precipitate forms. After a time filter and again test against sheep serum. A negative result is now obtained. Now test adsorbed serum with known ox serum. Precipitate occurs; finally, test with the unknown extract. If a positive result is obtained, the unknown extract is ox and not sheep.

(2) *Uhlenhuth. Cross immunisation.*—To prove ox blood: Inject a sheep with ox blood, and use the antiserum for testing the unknown. The use of a related animal for the preparation is stated to avoid group reactions. Other workers have been unable to confirm this.

(3) *Sutherland. Dilution of the antiserum.*—Here the antiserum, prepared, say, from a rabbit, is diluted with normal rabbit serum until it no longer gives the group reaction. The unknown is then tested, it being realised that a rather lower titre is to be expected.

(4) *Hamberger* relies on the rate and amount of precipitation from similar dilutions of the antigen and related antigens.

I have said nothing about the complement fixation test for the identification of an individual protein, chiefly because I have no experience of it, but from what I have read of the subject, it would seem that the complexity of the test renders it almost impracticable, and it has also been stated to be almost too sensitive. It has never come into regular use in this country, Germany, or America. It is, of course, possible that experimental work may, in the future, provide a practical test.

In conclusion, I think it is fair to say that the test is now absolutely established, so far as the examination of blood is concerned, chiefly owing to the pioneer work of Sir William Willcox. In other countries a very limited use has been made of the test in other directions and convictions have been obtained, but little or nothing has been done in this country, so far as I am aware. In spite of the group reactions referred to above, I believe that the test is a practical proposition, but further experimental work must be done before it can be safely introduced into legal work. It must be realised, however, that the examination of food on these lines will necessitate the constant preparation of fresh antisera and the keeping of a considerable number of varieties in stock. This alone will render it impracticable for the analyst engaged in routine practice, as such preparation would make serious calls on his time. Further, in cases where no serum of a particular kind is available about three weeks must elapse before a supply can be obtained from an animal.

In these days, when there is a growing demand for manufactured food, it does seem desirable that there should be control over the nature of the ingredients, and in the present state of our knowledge it seems that the only possible means of attacking such a problem is from the biological aspect.

The Use of the Blood-Grouping Reactions in Forensic Investigations.

By F. C. MARTLEY, M.A., M.D., F.R.C.P.I.

(*Read at the Meeting, November* 2, 1927.)

A few generations ago blood-letting was probably the most frequently employed therapeutic treatment, but it is only of late years that its converse transfusion has come into general use. With modern equipment it is a comparatively easy operation, and has proved its value, but at the same time many cases have occurred in which the results have been unfortunate. On making investigations one very marked reason was found which might account for the failure, namely, the interaction between the blood-serum of the recipient and the blood-corpuscles of the donor. When different corpuscles are mixed with different sera one of two results is found to follow; either the corpuscles remain scattered discretely, or else they are agglutinated into clumps, showing that when a strange blood is injected into the veins of a patient it is a chance whether the new corpuscles introduced take their place alongside the indigenous ones and share their work, or whether they are agglutinated into clumps which not only prevent them from doing useful work, but by their size are caught up in the capillaries and damage them by mechanical blocking.

Blood Groups.—The question was gone into, and it was found that all bloods might be divided into four groups, the cells of any one group not clumping with any sera of that same group, but acting in varying ways with the sera of other groups. The interactions of the cells and sera of these four groups are shown in Table A; the crosses indicate clumping, the dashes absence of it.

Table A.

	Corpuscles			
	1	2	3	4
Serum I	—	—	—	—
,, II	+	—	+	—
,, III	+	+	—	—
,, IV	+	+	+	—

A member of Group I is sometimes known as a universal recipient, as his serum is incapable of clumping any kind of corpuscles, and one of Group IV as a universal donor, as his corpuscles are not agglutinable by any serum. The relative frequency of the various groups is something more than 40 per cent. in Group II, and about the same for Group IV; about 10 per cent. for Group III, and 1 or 2 per cent. for Group I; this is for European countries; the ratios differ in

different parts of the world, notably so in the East, where the numbers of Group III are much increased.

DETERMINING THE GROUP.—To test the group of a blood it is usually sufficient to have some known II and III sera. A volume of each is put on a glass slide, and the corpuscles of the unknown blood are added to each and mixed; in most cases it is enough to add a trace of the blood to be investigated, but to insure getting clear-cut results it is advisable to wash the corpuscles in saline to rid them of any traces of their own serum, and also to employ them very dilute—say, one in a hundred. After a couple of minutes the slide should be rocked gently from side to side, and the clumping shows itself; it can be seen by the naked eye, as if some grains of red pepper had appeared in the specimen, but, personally, I prefer to examine the preparation under a low power of the microscope (⅔ inch objective); the appearance of clumping thus seen is very characteristic and is readily to be distinguished from extensive rouleaux formation, which at times almost suggests true clumping.

This is enough to group the unknown blood; if both sera clump the corpuscles the case is a I; if only one does so it is a II or a III, as the case may be; and if neither does so, it is a IV. In the case of a I, II or III I am well satisfied with the result so obtained, but if it is apparently a IV, I prefer to proceed further so as to avoid making a diagnosis on negative evidence only; therefore I now take some of the serum of the case to be investigated and mix it with II or III corpuscles, which will, of course, be clumped by it if it is from a IV, and thus I shall have positive evidence in support of my finding. This is usually sufficient, but in some special cases it is advisable to complete the cross reactions, putting up the patient's corpuscles with the various known sera and the patient's serum with the various known corpuscles.

In this way the blood group of any patient requiring a blood-transfusion and of any prospective blood donor can be tested; certain would-be donors can be rejected at once, their blood-groups being incompatible with the patient's; but even when they are apparently suitable it is not safe to use them till their corpuscles have been tested directly with the patient's serum; this is because occasionally the serum of a person belonging to a certain group is found to clump the corpuscles of a member of the same group, and if one strikes such an agglutinable donor the results are unpleasant. Such cases are comparatively rare, but I have come across them; that they do exist suggests that there may be more than four groups.

THEORY OF THE GROUPS.—Now the theory that is used to explain the four groups is shown in the accompanying Table B.

TABLE B.

Group	I	*A*	*B*		
,,	II	*A*			*b*
,,	III		*B*	*a*	
,,	IV			*a*	*b*

The large letters, *A B*, represent factors known as agglutinogens, the small letters, *a b*, corresponding factors known as agglutinins; every blood has two of these factors, but never two of the same letter. The agglutinogens reside in the corpuscles, the agglutinins in the serum; the agglutinin "*a*" clumps corpuscles containing the agglutinogen A, and similarly with *b* and *B*.

Now this table explains all the ordinary reactions, but it will not explain such a reaction as the serum of one number IV clumping the corpuscles of another number IV; if, however, we postulate the occasional occurrence of a third agglutinogen, *C*, with a corresponding agglutinin *c*, and perhaps a fourth pair, *D d*, it is possible to explain the anomalous cases. The Americans have gone into this question on these lines and have decided that theoretically there should be 64 groups, and have found examples of 20 or 30.

On the other hand, another explanation has been put forward by some who postulate only the *Aa* and *Bb* factors, suggesting that an individual, instead of his blood having the composition of, say, *Ab*, really has not a full modicum of *A*, but that the measure is made up by an addition of some "*a*." There is something attractive in this hypothesis, though it necessitates some explanation of such a person's corpuscles not being agglutinated in his own veins; though I have an open mind on the matter, I think that I should like the second explanation to prove the correct one.

Other points of interest are:—(*a*)The extent of agglutination varies within rather wide limits; the same serum can in one case only be diluted down to 1 in 16 to allow of clumping of appropriate corpuscles being demonstrated, whilst with corpuscles from another member of the same group the dilutions may be carried on to 1 in 250 without the clumping effect being destroyed.

(*b*) The amount of the agglutinating power of any serum is finite; when a certain quantity of corpuscles has been clumped the serum has no further such action on a further supply.

(*c*) The clumping action takes place better in the cold than in the heat; sometimes when the reaction is slight it can be more easily demonstrated by keeping the preparation for some hours in a moist cell in an ice chamber.

(*d*) The blood of a new-born infant does not usually give the reaction at birth; it only appears gradually after some months.

BLOOD GROUPS IN STAINS.—Now, to go to a subject which will appeal more to analysts. Can any method be devised which will enable one to tell with certainty the group of a dried bloodstain? The importance of this in forensic matters will be self-evident.

There appeared to be two methods of approaching the matter:—(*a*) By trying to make an extract of the stain, in the hope that some of the original agglutinins could be recovered and be demonstrated by their clumping suitable corpuscles; and (*b*) by exhausting or diminishing the agglutinising power of a known serum by the agglutinogens recoverable from the stain.

By the first method I have found it difficult to get definite results, and it has only been in a minority of cases that I have succeeded in getting an extract which will give an agglutination, and it is generally only when I have left the preparations for some hours in an ice-chest.

The second method has been more promising: I first take a known test serum and make dilutions of $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ and so on to 1 in 500 or 1 in 1000; I then place equal volumes of these dilutions on a slide and add to each an equal volume of 1 per cent. agglutinable washed corpuscles and note down to what dilution the clumping will be manifest. I next repeat the process with the same serum incubated with a piece of the same material, free from stain, as that on which the stain has been found; the clumping may not now extend so far down the dilutions—perhaps two or three places less than in the previous experiment. Afterwards I proceed to incubate the same serum with the actual blood-stain, and, if the stain came from a group which the test serum would agglutinate, the serum will now either cease to clump the test corpuscles, or else its power will be extinguished several places sooner than in the previous experiment.

If definite positive results are obtained in the two sets of experiments, I should feel justified in identifying the blood-group of the stain; if in one set I got definite positive results, and in the other corroborative negative results, I should still feel confident in my diagnosis, but I should never be satisfied to give an opinion on purely negative evidence.

Possible Applications of the Tests.—I must now refer to another interesting field in which the blood-grouping reactions have great possibilities; it has, in addition, a certain human interest which will appeal to the man as well as to the analyst. I refer to their possible importance in paternity cases. Now the blood-group of the child from the mating of any couple depends directly on those of the parents, and follows strictly Mendelian laws. One may put it briefly, to memorise it, that a parent can hand on his or her agglutinogen, or in its place the corresponding agglutinin; and can also transmit his or her agglutinin, but not the corresponding agglutinogen. If this rule is applied, it is possible to calculate the possible and impossible progeny of any mating, and Table C shows these results tabulated. I have just said "impossible" results, and it is with these that the blood-grouping tests are of importance; when, for instance, two IV's have a II child, this is an impossibility, and only to be explained by the intervention of a third party.

Table C.

Mating.	Possible Children.	Impossible Children.
I + I, II, III or IV II + III	I, II, III or IV	None
II + II or IV	II or IV	I or III
III + III or IV	III or IV	I or II
IV + IV	IV	I, II or III

Discussion on the Three Papers.

Mr. G. Rudd Thompson congratulated the three authors on their papers upon a subject which would probably contain much that was entirely novel to the majority of the members of the Society. He desired to accentuate the statement of Dr. Roche Lynch as to the unreliability of many of the so-called "Anti-Human Sera" in being non-sensitive and misleading, yet could not help alluding to the fact that it was not the lot of every analyst who might be called upon to use them at times, to be able to make what was evidently a clear and proper production under personal control. He wished to ask Sir William Willcox whether, in his opinion, if a serum obtained from reliable sources gave, under proper conditions of control, satisfactory negative reactions to the ordinary domestic animals, but at the same time a definite and positive reaction to human blood of known origin, with an equally definite positive reaction to that from a stain under examination, was the analyst justified in giving a positive opinion as to the identity of the stain under examination, even if he had not actually prepared the serum as described as being the method of the Home Office experts. He referred, as one of the early workers upon the subject, to the magnificent work of Willcox, who was undoubtedly one of the best, if not the best, authority on blood tests, and one whose investigations had done so much to place the precipitin test in the forefront of "fool-proof" tests, and to make it one of the most valuable aids in forensic cases; he admitted, however, that there were many pitfalls if the test was carried out by inexperienced workers.

Sir William Willcox, replying, said that in special cases it was quite permissible for an analyst to use bought sera for the testing of suspected human blood stains. If these were used, the expert should not take it for granted that the serum supplied to him had exactly the qualities professed for it. He should test for himself its sensibility to human serum and the serum of other animals. It had been shown that transit in post might seriously affect the qualities of a serum which had been prepared for the testing of the blood of different animals, and the expert should satisfy himself by experiment that the serum was sensitive to human blood and to that of the respective animals to which it had been sensitised.

Determination of Small Quantities of Benzoic and Cinnamic Acids, with some Notes on the Colorimetric Determination of Salicylic Acid.

By JOHN RALPH NICHOLLS, B.Sc., F.I.C.

Introduction.—The methods in general use for the determination of benzoic acid depend upon either weighing or titrating the extracted acid after appropriate purification. When the quantity of acid is small much material is needed to obtain a satisfactory result. A method capable of determining small quantities of benzoic acid would be of particular advantage in the examination of foodstuffs for preservatives, where the use of large amounts of material increases considerably both the time and difficulty of the extraction of benzoic acid.

Several attempts have been made to use quantitatively the test of Moeller (*Bull. Soc. Chim.*, 1890, **3**, 414), generally as modified by von der Heide (*Z. Nahr. Genussm.*, 1910, **19**, 137), but the results have varied in the hands of different workers. Experience in this laboratory has shown that the test is liable to wide variations, probably owing to the difficulty of ensuring comparable conditions of nitration. Too short or too long a time gives low results, and in certain cases the test may fail altogether. In addition, the colour produced is not directly proportional to the quantity of benzoic acid present, thus necessitating a series of standards for each determination. Several other substances give a colour under the conditions of the test.

In view of the delicacy of the colour reactions given by salicylic acid, the methods for the production of salicylic acid from benzoic acid were reviewed to see if any could be adapted to quantitative work. The processes fall into two main classes:—(1) Fusion with alkalis, and (2) oxidation, usually with hydrogen peroxide. In the former case extreme care is required, with the closest attention to manner and time of fusion. This presents obvious difficulties, so that attention was directed to the second class.

Oxidation with Hydrogen Peroxide.—The first worker to record the production of salicylic acid by the action of hydrogen peroxide on benzoic acid was Hanriot (*Compt. rend.*, 1886, **102**, 1250), and the method was applied by Peter and Le Bach (*U.S. Dept. of Agric., Bur. Chem., Bull.*, **65**, 160) to the detection of the acid in foodstuffs. Many others have used such an oxidation process, employing very varying conditions. In some cases ammonium benzoate has been oxidised (Dakin and Herter, *J. Biol. Chem.*, 1907, **3**, 419), and in others the acid has been used in the presence of acetic acid (Chauvert, *Ann. Falsif.*, 1925, **18**, 31), iron salts (Jonescu, *J. Pharm. Chem.*, 1909, **29**, 523; Neuberg, *Biochem. Zeitsch.*, 1910, **27**, 271; Biernath, *Chem. Zentralbl.*, 1912, **1**, 1929; Fleury, *J. Pharm. Chim.*,

1913, **8**, 460; Chelle, *Ann. Falsif.*, 1925, **18**, 134, etc.), or copper salts (Dubaquie, *Ann. Falsif.*, 1925, **18**, 149). In no case is salicylic acid the sole product, varying amounts of meta- and para-hydroxybenzoic acids and of dihydroxybenzoic acids being also produced.

It was thought that conditions might be found under which a constant proportion of salicylic acid would be produced, so that its colorimetric determination would serve to give the benzoic acid originally present.

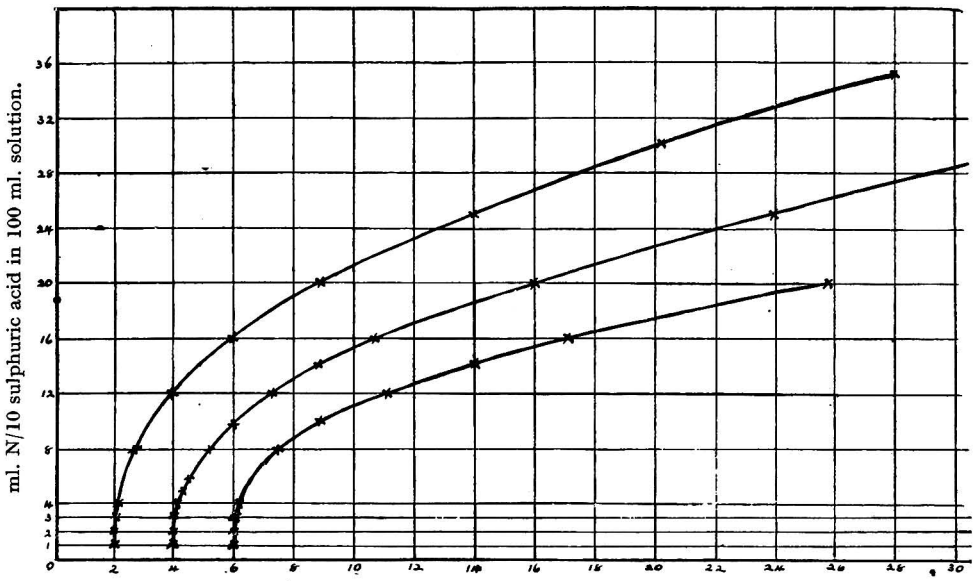
PRELIMINARY INVESTIGATION OF THE TESTS FOR THE DETECTION OF SALICYLIC ACID.—Two of the most delicate tests for salicylic acid which have been applied for quantitative work are: (1) the violet colour given with ferric salts, and (2) the red colour given in Jorissen's test (*Bull. de l'Acad. Royal des Lettres et des Beaux-arts de Belgique*, 3rd series, **3**, 259). In the ordinary examination of foodstuffs for salicylic acid the former test is not reliable, as colours indistinguishable from that of ferric salicylate are given by maltol (Brandt, *Ber.*, 1894, **27**, 806) and isomaltol (Backe, *Compt. rend.*, 1910, **150**, 540, and **151**, 78). Maltol and isomaltol are likely to be found in foodstuffs which have been baked, or they may be produced in the course of analysis if saccharine material is submitted to steam distillation from acid solution. As they are of acid character and are soluble in the same solvents as salicylic acid, they would be extracted with it (see Sherman, *J. Ind. Eng. Chem.*, 1910, **2**, 24, and Chapman, ANALYST, 1927, **52**, 215).

Maltol and isomaltol, like salicylic acid, can readily be broken down by oxidation, so that the process for removing salicylic acid before testing for benzoic acid will also remove them. It is permissible, therefore, to use the ferric salicylate test for detecting salicylic acid produced from such a purified benzoic acid. Neither maltol nor isomaltol gives a colour under the conditions of Jorissen's test. This test appears to depend upon the production of a quinone-oxime colour, and therefore the only substances likely to interfere with its use for detecting salicylic acid are phenolic bodies with a free ortho-position. It is improbable that any substance of this nature would be present in the extracted and purified benzoic acid from foodstuffs. Phenolphthalein gives a strong reaction in Jorissen's test and should not therefore be used as an indicator at any stage of this test.

Both of the above tests have been examined in connection with the present investigation, and under the conditions specified later either can be relied upon for the determination of salicylic acid, provided that interfering substances are absent.

(*a*) *The Ferric Salicylate Test.*—Contrary to the usual statements in the literature, this test should not be applied to a neutral solution of a salicylate, as the colour so produced is not of satisfactory shade. To obtain a good tint the solution should be slightly but appreciably acid, the intensity of colour from a given quantity of salicylic acid decreasing with increasing acidity. The minimum acidity necessary for producing a good violet colour is about $N/1000$ of mineral acid. The following curves illustrate the effect of acidity and show the quantities of salicylic acid required to produce a standard intensity of colour in solutions of

varying acidity. The three standard colours used were those produced with 2, 4 and 6 ml. of 0·01 per cent. salicylic acid solution, respectively, in 100 ml. of water containing 1 ml. of $N/10$ sulphuric acid.



From these curves it is apparent that a given quantity of salicylic acid gives the deepest colour with an acidity of 1 to 3 ml. of $N/10$ acid per 100 ml. Greater acidity gives a weaker colour, and less acidity gives a bad tint. In subsequent tests the acidity was adjusted to about $N/500$. For the purposes of colorimetric work the quantity of salicylic acid should not exceed about 0·8 mgrm. per 100 ml., or the colour will be too strong for accurate comparison.

(*b*) *Jorissen's Test.*—The details usually given for this test do not specify accurately the quantities of reagents to be employed. It appeared desirable, therefore, to standardise the conditions to be used for quantitative work. Each reagent was varied in turn, and the colours produced were compared. It was found that the proportions of ingredients could be varied within very wide limits without appreciable differences in the colours produced with a given quantity of salicylic acid. As, however, it was desired to detect the smallest possible quantity of salicylic acid the following conditions were finally adopted:—

Add to 10 to 40 ml. of a neutral solution, containing not more than 1 mgrm. of salicylic acid, 1 ml. of a 2 per cent. solution of sodium (or potassium) nitrite and 1 ml. of a solution containing 0·3 per cent. of crystallised copper sulphate and 10 per cent. of acetic acid. Place in a boiling water bath for 15 minutes, cool, and dilute to 50 ml.

By this process the colour produced was found to be directly proportional to the quantity of salicylic acid present, and could be matched by adding a standard

colour solution from a burette to a blank consisting of water to which had been added 1 ml. of the acid copper solution used in the test. The standard colour employed throughout this work was prepared by carrying out the above test on 5 ml. of 0·1 per cent. salicylic acid solution diluted to 40 ml., 5 ml. of each of the reagents being used, and the final solution being diluted to 100 ml. (1 ml. of this standard colour = 0·05 mgrm. salicylic acid).

PRELIMINARY TESTS ON THE OXIDATION OF BENZOIC ACID WITH HYDROGEN PEROXIDE.—Since all the monohydroxy benzoic acids are easily oxidised further, it appeared desirable to limit the amount of hydrogen peroxide present, to avoid production of dihydroxy acids, either from the benzoic acid present or from the salicylic acid produced from it. Theoretically, 1 part of hydrogen peroxide oxidises 3·6 parts of benzoic acid to monohydroxy acid, and therefore for the preliminary tests approximately 1 mgrm. of peroxide and 3 mgrms. of benzoic acid were used. The oxidation was tried in alkaline, neutral and acid solutions, with and without metallic salts, at various temperatures, and under the influence of the light from a mercury lamp. Among the metallic salts employed were those of iron, copper, nickel, cobalt, manganese, cerium, molybdenum, titanium and the alkalis. In many cases it was necessary to remove the metal present before testing for salicylic acid, on account of the colour of the solution. From these preliminary tests it appeared that little, if any, salicylic acid was produced unless the oxidation was carried out in slightly acid solution containing appreciable amounts of iron or copper salts, the former giving more salicylic acid than the latter. The results obtained by the oxidation of 3 mgrms. of benzoic acid in the presence of iron salts gave promise of yielding a satisfactory method.

OXIDATION IN THE PRESENCE OF IRON SALTS.—(*a*) *With the use of the Ferric Salicylate Test.*—The same proportions of benzoic acid and hydrogen peroxide were again employed. It was found that increase of temperature had a marked accelerating influence on the rate of oxidation, and that by heating the solution just to boiling oxidation was complete and consistent results could be obtained. In many instances a preliminary indication of violet colour was later masked by a deepening in colour of the iron solution, but, on removal of the iron, salicylic acid was detected. Good results were obtained when the acidity of the solution was such that, on boiling, most of the iron came out of solution, so that, on filtering, a clear violet filtrate was obtained. Such a filtrate had an acidity greater than $N/500$, as the iron was precipitated as ferric hydroxide, leaving free acid in solution. It was found most convenient to control this acidity by the addition of alkali immediately before filtering. With sufficient alkali added to give an acidity of $N/500$ practically all the iron came out of solution, carrying with it the *p*-hydroxybenzoic acid produced at the same time as the salicylic acid. This had the advantage of avoiding a modification of the colour of ferric salicylate by the brown colour given by ferric *p*-hydroxybenzoate. The filtrate required the addition of a further drop of ferric chloride solution before the full colour due to the salicylic acid was obtained.

Equally good oxidation was obtained by exposure of the solution for several minutes to the mercury lamp instead of by heating; but, as it was necessary to remove the iron afterwards by some such process as the above, this method of oxidation was abandoned on account of the increased time required.

By varying the quantity of iron salts present it was observed that a certain concentration gave a maximum yield of salicylic acid. Similarly, there was an optimum concentration of free acid and of hydrogen peroxide. Near these concentrations the salicylic acid produced was approximately constant, but with much less, or more, reagent there was a marked decrease in yield. With 3 mgrms. of benzoic acid in a total volume of 45 ml. the maximum quantity of salicylic acid was obtained by using 1·4 to 1·6 ml. of $N/1$ ferric chloride, 3·5 to 4·5 ml. of $N/10$ sulphuric acid, and 2·5 to 3·5 ml. of 0·1 per cent. of hydrogen peroxide. With a different total volume the optimum quantities of the reagents varied proportionately, showing that concentrations, and not actual amounts, were effective.

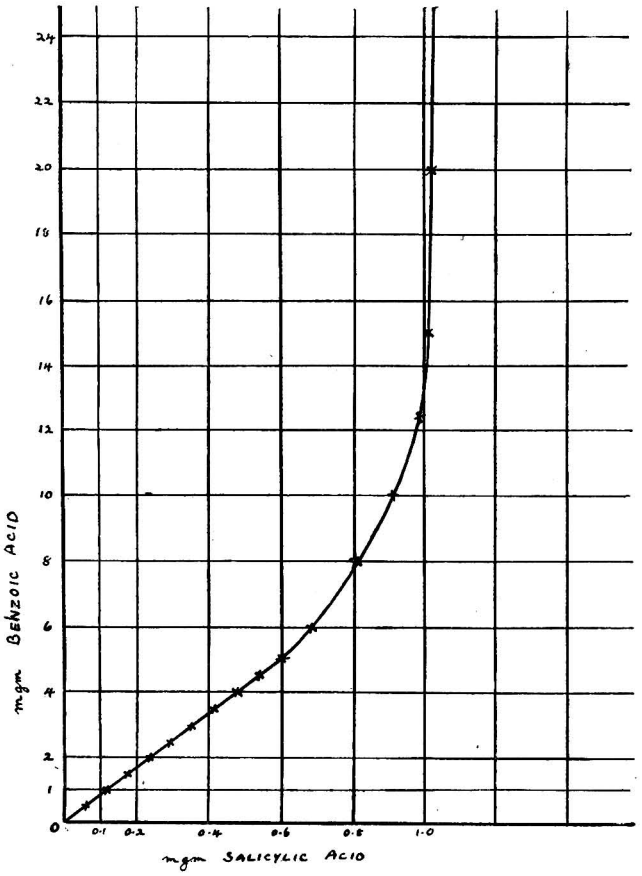
It was found convenient to make up an iron solution containing 50 ml. of $N/1$ ferric chloride and 13 ml. of $N/10$ sulphuric acid in 100 ml., and to use 1 ml. of this solution for every 15 ml. of solution tested. A practically neutral filtrate was obtained by adding 1 ml. of this solution to 15 ml. of water, boiling, and adding 0·6 ml. of $N/1$ alkali and filtering. By reducing the amount of alkali added the acidity of the filtrate could be made $N/500$.

The conditions which gave the best results with 3 mgrms. of benzoic acid were now used with varying amounts, and it was found that up to a certain maximum the colour produced was directly proportional to the benzoic acid present. Above this maximum the proportion of salicylic acid produced was smaller, owing to insufficient hydrogen peroxide. The colour obtained was identical with that produced by adding ferric chloride to a slightly acid solution of salicylic acid.

The following table shows the results obtained with varying quantities of benzoic acid. The test was carried out in a total volume of 15 ml., 1 ml. of the iron solution described above, and 1 ml. of 0·1 per cent. solution of hydrogen peroxide being used. After being heated just to boiling point and the addition of 0·5 ml. of $N/1$ sodium hydroxide solution, the liquid was filtered and the filter washed with hot water to make up 50 ml. The colour obtained after the addition of one drop of iron solution to the cooled filtrate was compared with that produced by adding 0·01 per cent. of salicylic acid solution to a blank carried out under identical conditions. With the larger quantities of benzoic acid the solution was further diluted with $N/500$ acid before matching the colour.

Benzoic Acid. Mgrms.	Salicylic Acid. Mgrm.	Benzoic Acid. Mgrms.	Salicylic Acid. Mgrm.
0·5	0·06	5·0	0·60
1·0	0·12	6·0	0·68
1·5	0·18	8·0	0·80
2·0	0·24	10·0	0·88
2·5	0·30	12·5	0·98
3·0	0·36	15·0	1·02
3·5	0·415	20·0	1·04
4·0	0·48	25·0	1·06
4·5	0·54		

The following curve shows the results plotted:—



It will be observed that up to 5 mgrms. of benzoic acid the salicylic acid formed is constantly 12 per cent. of the benzoic acid. This indicates that about 10·5 per cent. of the benzoic acid is oxidised to salicylic acid, the remainder being probably oxidised to a mixture of *m*- and *p*-hydroxybenzoic acids. When using this method, therefore, the test solution must not contain more than 5 mgrms. of benzoic acid in 15 ml., or about 30 mgrms. per 100 ml. If the result of the test indicates a greater proportion of benzoic acid than this, the test should be repeated after diluting the test solution.

The conditions and method of oxidation are summarised as follows:—Dilute an aliquot part of a neutral solution containing not more than 4 mgrms. of benzoic acid to 15 ml. with water. Add 1 ml. of iron solution (50 ml. of *N*/1 ferric chloride

+ 13 ml. of $N/1$ sulphuric acid to 100 ml.) and 1 ml. of 0·1 per cent. hydrogen peroxide (1 ml. of 20 vol. hydrogen peroxide diluted to 60 ml. = 0·1 per cent.). Heat just to boiling, add 0·5 ml. of $N/1$ sodium hydroxide solutions and filter while hot into a 50 ml. Nessler tube, washing the precipitate with hot water. Cool the filtrate, dilute to the mark, and add 1 drop of iron solution. Match the colour produced by adding a 0·01 per cent. solution of salicylic acid to a blank carried out in an identical manner.

It is advisable to standardise the salicylic acid solution for the particular conditions of the test by carrying out the process on a known quantity of benzoic acid—say, 2 or 3 mgrms. Normally 1 mgrm. of benzoic acid is equivalent to 1·2 ml. of 0·01 per cent. salicylic acid solution.

If desired, the total volume of solution may be varied, provided that the quantities of iron solution and peroxide are varied in the same proportion. Also, the volume of the filtrate may be varied, provided that the acidity is adjusted to about $N/500$ by the addition of the necessary alkali before filtration.

(*b*) *With the use of Jorissen's Test.*—The above method of oxidation is equally suitable for applying Jorissen's test. It is advisable, however, to obtain a neutral filtrate after oxidation by the addition of 0·6 ml. of $N/1$ sodium hydroxide solution before filtering. The filtrate and washings are made up to 30 to 40 ml., and the test applied in the manner already specified. If desired, the blank may be carried out on an equal quantity of the test solution, treated in an identical manner, but omitting the addition of sodium nitrite.

The following quantities of salicylic acid were obtained by oxidising benzoic acid and applying Jorissen's test:—

Benzoic acid, mgrms.	1·0	2·0	3·0	4·0	5·0
Salicylic acid, mgrm.	0·12	0·24	0·355	0·48	0·60

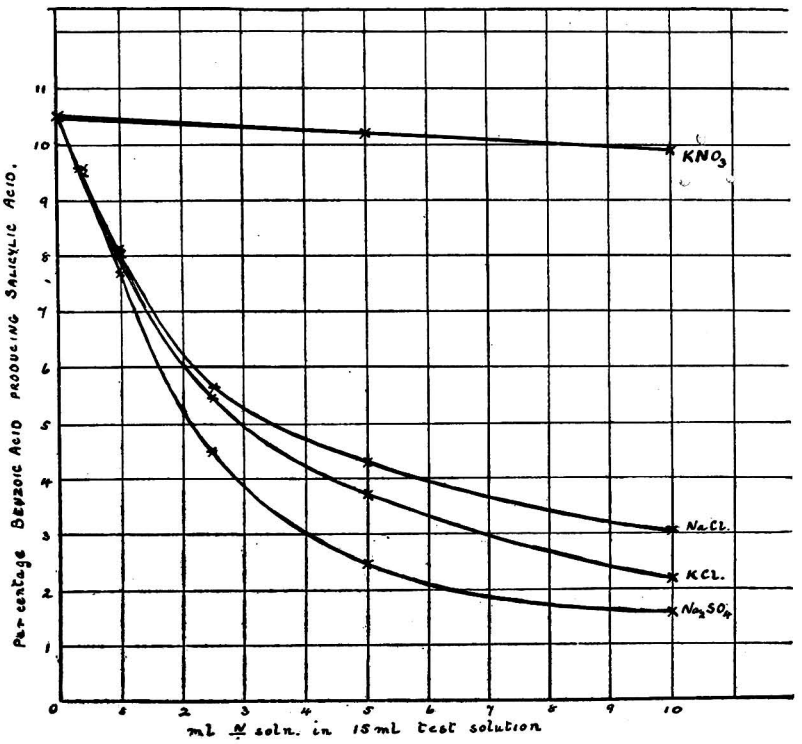
These results are identical with those obtained by using the ferric salicylate test.

In practice, the ferric salicylate test was found to be much simpler and more convenient to apply than Jorissen's test. It has therefore been adopted as the normal test, confirmation being obtained when considered necessary by Jorissen's test.

General Applications.—Other substances may interfere with the test in the following ways:—(1) By altering the proportion of salicylic acid formed from the benzoic acid; (2) by using up the peroxide before it can oxidise the benzoic acid; (3) by giving a colour which interferes with the determination.

(1) Certain salts materially reduce the proportion of salicylic acid formed. Chlorides and sulphates, in particular, have a marked effect, whilst nitrates, unless present in large amounts, have no appreciable influence.

The following curves illustrate the effect of some salts:—



When the test is applied in the presence of these interfering salts, iron compounds separate before the solution reaches boiling point, and it is possible that the lower results are due to this removal of soluble iron before the oxidation takes place. Since it is usually necessary to extract benzoic acid before attempting to determine the amount, the removal of these interfering salts is easy. Where an alkaline extract of benzoic acid requires to be neutralised before applying the test it is advisable to use nitric acid.

In view of this effect of chlorides and sulphates, it appeared probable that the ferric chloride and sulphuric acid might have some inhibiting effect. Attempts to replace them by other salts and acids, such as ferric nitrate and nitric acid, were unsuccessful, the amounts of salicylic acid produced being materially reduced. As, however, any effect of these reagents would be constant, it does not affect the utility of the method.

(2) Many oxidisable substances would be removed during the extraction of the benzoic acid. Others might be extracted with it. Most of these can be

removed by treatment with alkaline permanganate, which has no action on benzoic acid. Such a process, however, cannot be employed if cinnamic acid is present, as this would be oxidised to benzoic acid. Extracts containing cinnamic acid must be purified by methods appropriate to the particular case. The purified mixture of benzoic and cinnamic acids can be treated as described later for the determination of each acid. Phenolphthalein is oxidised by hydrogen peroxide, and therefore must not be used as indicator of neutrality; litmus paper can be used. Traces of immiscible solvents must also be removed before applying the test.

(3) Salicylic acid must, of course, be removed before applying the test, and the same process will remove maltol and isomaltol. Saccharin interferes with the test, as it is oxidised, and, in addition to using up the hydrogen peroxide, the product gives a faint red colour with ferric chloride. Benzoic acid may be separated, however, by carbon tetrachloride, in which saccharin is almost insoluble.

APPLICATION TO FOOD PRODUCTS.—Benzoic acid can be extracted from many materials by means of an immiscible solvent. It may be necessary to treat the material first with an appropriate clearing agent, and any large excess of acid should be partly neutralised. Either chloroform, methylated ether, or a mixture of equal parts of methylated ether and petroleum spirit is suitable. The solution of benzoic acid should be washed with water to remove soluble acids, and the washings should be re-extracted to avoid loss of benzoic acid. Three extractions should then be made with dilute alkali (10, 10 and 5 ml. of *N*/20 alkali are usually sufficient), and the combined alkaline extracts should be boiled to remove dissolved solvent. In certain cases it may be necessary to treat the alkaline solution with potassium permanganate. For this purpose the solution is warmed to about 50° C., and a solution of permanganate added until a persistent pink colour is visible. After acidification, a clear solution is obtained by the addition of a little oxalic acid. Sulphites should not be used, as sulphur dioxide would be extracted with the benzoic acid, and would use up the hydrogen peroxide in the test. The benzoic acid is extracted with an immiscible solvent, and the solution, after washing with water, is extracted with alkali as above described. After removal of the dissolved solvent the alkaline solution is cooled, neutralised to litmus paper with dilute nitric acid and made up to a convenient volume. An aliquot part is then used for the test.

Where a direct extraction is not possible the material should be submitted to steam distillation from an acid-salt solution (see Monier-Williams, *Reports on Public Health and Medical Subjects*, No. 39, Ministry of Health, 1927; ANALYST, 1927, **52**) or acid and calcium chloride solution, the distillate being collected in sodium hydroxide solution. The solution, or a known fraction of it, should be treated with permanganate and subsequently extracted, as above described, to obtain a neutral solution.

As a qualitative test the method lends itself to rapid application. By giving only one extraction on each occasion the whole process of shaking with immiscible solvent, washing, extraction with alkali, purification with permanganate, re-extraction with solvent and alkali, and applying the test may be completed in

less than half-an-hour. In this connection it may be mentioned that the distribution of benzoic acid between equal quantities of methylated ether and water has been found to be approximately 97·5 per cent. and 2·5 per cent., respectively; so that, if the first extraction with this solvent is efficient, over 90 per cent. of the benzoic acid in the quantity of material taken is obtained for the test in this way. This often enables an approximate idea of the quantity present to be obtained.

DETERMINATION OF CINNAMIC ACID.—Cinnamic acid does not give salicylic acid under the conditions of the above test, although the odour of benzaldehyde is apparent during the heating of the solution. It produces, however, a pale yellow colour on the addition of the iron solution to the filtrate, which slightly modifies the violet colour produced by salicylic acid. The colour is not proportional to the amount of cinnamic acid present, probably because most of the colour-producing substance is carried down by the iron precipitate. When benzoic acid is being determined in the presence of cinnamic acid, either Jorissen's test should be applied after oxidation, or the blank should be carried out on a small quantity of cinnamic acid—say, 2 or 3 mgrms., in order to be able to match exactly the colour produced. If large proportions of cinnamic acid are present, a precipitate may be obtained on the addition of iron solution to the filtrate, which prevents a quantitative determination. Not more than about 5 mgrms. in 15 ml. of test solution should be present.

A series carried out with varying quantities of benzoic acid, each mixed with 5 mgrms. of cinnamic acid, gave the following results:—

Benzoic acid, mgrms.	0·5	1·0	2·0	3·0	4·0
Salicylic acid produced, mgrms.	0·06	0·12	0·24	0·36	0·48

These results are identical with those obtained in the absence of cinnamic acid.

Cinnamic acid can be oxidised to benzoic acid in neutral or alkaline solution with potassium permanganate. The following results show the time required for oxidation. In each case a solution containing 5 mgrms. of cinnamic acid was mixed with a known quantity of $N/10$ permanganate and allowed to stand in a closed vessel, at room temperature, for the specified time. After acidification, a known quantity of oxalic acid was added, and the excess of the latter titrated with permanganate.

Time of standing.	Oxidation in weakly alkaline solution. $N/10$ permanganate used.	Oxidation in neutral solution. $N/10$ permanganate used.
Minutes.	Ml.	Ml.
15	2·70	3·45
30	2·95	3·45
45	3·05	3·45
60	3·20	—
90	3·45	—

(3·45 ml. $N/10$ permanganate = 5·1 mgrms. of cinnamic acid.)

A convenient method for the oxidation is as follows:—Add to a neutral solution of the cinnamate at least double the theoretical quantity of potassium permanganate and allow the mixture to stand in a closed vessel at room temperature for 15 to 20 minutes. Just acidify, add a slight excess of oxalic acid, and extract and determine the benzoic acid produced, in the usual manner. Five mgrms. of cinnamic acid treated in this way produced salicylic acid equivalent to 5·0 ml. of 0·01 per cent. solution, = 4·16 mgrms. of benzoic acid = 5·05 mgrms. of cinnamic acid.

In the case of mixtures of benzoic and cinnamic acids the test is carried out before and after oxidation, which enables both to be determined.

SUMMARY.—1. Details are given of a colorimetric determination of benzoic acid by partial oxidation to salicylic acid by means of hydrogen peroxide in the presence of ferric chloride.

2. The method depends upon standardising the concentrations of mineral acid, hydrogen peroxide and ferric chloride, and a rapid oxidation is effected by heating the solution.

3. Under the conditions specified, a constant proportion of slightly over 10 per cent. of the benzoic acid is oxidised to salicylic acid.

4. The oxidation must be carried out on a solution containing no appreciable quantity of salts, other than nitrates.

5. The salicylic acid produced is determined colorimetrically under specified conditions, either by the ferric salicylate test or by Jorissen's test.

6. Cinnamic acid can be determined by first oxidising it quantitatively to benzoic acid; a method for this is described.

The author desires to thank the Government Chemist for permission to publish this method.

GOVERNMENT LABORATORY,
LONDON.

Colorimetric Determination of Small Amounts of Iron in Zinc.

By W. J. AGNEW, B.A.

The following method, based on the purple coloration produced by sodium salicylate in acetic acid solution, was found to give accurate results in determining small amount of iron in zinc:—Two grms. are dissolved in 20 c.c. of hydrochloric acid, a few c.c. of nitric acid added to oxidise any iron present, and the solution treated with 0·01 grm. of uranium in the form of uranyl nitrate solution, to help to agglomerate the iron. Ammonia is added in slight excess, and the liquid heated to boiling and allowed to stand for 20 minutes.

The precipitate of iron and uranium hydroxide is filtered off and dissolved in 10 c.c. of dilute (1 : 1) hydrochloric acid. The solution is transferred to a Nessler glass and ammonia added until the liquid is almost alkaline, and then 1 c.c. of a 10 per cent. aqueous solution of sodium salicylate, followed by ammonia, is now added in slight excess, as shown by the yellow colour due to uranium. Finally, the solution is made acid with acetic acid until the purple colour, due to iron, is developed, at which stage the uranium yellow is destroyed.

Ten c.c. of acetic acid (1 : 1) are now added in excess, and the colour compared by adding a standard iron solution from a burette to a Nessler glass containing a similar quantity of uranium solution of the same acidity. The following results were obtained:—

Zinc taken. Grms.	Iron. Grm.	Total iron found. Grm.	Net iron found. Grm.	Fe added. Per cent.
2·0	Nil	0·00005	—	—
2·0	0·0001	0·00015	0·0001	0·005
2·0	0·0002	0·00025	0·0002	0·010

The standard iron solution is made from ferric sulphate and contains 0·0001 grm. of iron per c.c.

The purple colour, due to iron, is destroyed by hydrochloric acid, nitric acid, and sulphuric acid, but remains stable without any noticeable fading of colour, even when kept for 48 hours, in presence of acetic acid. As little as 1/50 mgrm. can be detected in a volume of 50 c.c., and therefore the sensitiveness of the test is 1 part in 2,500,000.

Research Department,
Royal Arsenal, Woolwich.

Determination of Minute Amounts of Cobalt in Steel.

By W. J. AGNEW, B.A.

THE following method, which was found to give correct results for traces of cobalt in steel, depends on the deep red colour given by cobalt salts with potassium ferricyanide in ammoniacal solution.

One grm. of the steel is dissolved in *aqua regia*, the solution evaporated to dryness, and the residue ignited to eliminate nitric acid. It is then dissolved in hydrochloric acid, the solution evaporated to a small bulk, diluted a little with water, the iron (or chromium, if present) separated by an emulsion of zinc oxide, the volume made up to 200 c.c., and 100 c.c. of this (representing 0·5 grm. of steel) filtered off.

Five c.c. of strong hydrochloric acid are added to the filtrate, hydrogen sulphide passed through it, to remove copper if present, the copper sulphide filtered off, and the filtrate boiled for some time to remove the gas, after which 0·2 grm. of α-nitroso-β-naphthol, dissolved in 10 c.c. of strong acetic acid, is added, and the solution is boiled for a few minutes longer and allowed to stand in a warm place for about half-an-hour.

The precipitate of cobalti-α-nitroso-β-naphthol is filtered off, washed with 5 per cent. hydrochloric acid and hot water, and ignited in a porcelain crucible in a muffle at a temperature of about 800° C. The residue is dissolved in about 10 drops of hot strong hydrochloric acid in the crucible, diluted a little, and filtered (if necessary) into a 50 c.c. Nessler glass. Ten c.c. of ammonia (1: 1) and 5 c.c. of a 0·1 per cent. solution of potassium ferricyanide are added, and the red colour is matched by pouring into another 50 c.c. Nessler glass 10 c.c. of ammonia (1:), about 10 drops of hydrochloric acid, and 5 c.c. of the ferricyanide solution, and adding from a burette a cobalt solution, drop by drop. (1 c.c. of the cobalt solution is equal to 0·001 grm. of cobalt.)

For quantities greater than 1 mgrm. (= 0·2 per cent.) of cobalt, it was found necessary to add 10 c.c. of the ferricyanide solution; but the colour becomes rather intense when 2 mgrms. (= 0·4 per cent.) of cobalt is present, and a gravimetric method is then more suitable.

The only interfering metal likely to be present in the steel is copper, and this must be removed by passing hydrogen sulphide into the hydrochloric solution before adding the nitroso-β-naphthol, as already described. With a steel which contained 2·5 per cent. of nickel, 1 per cent. of chromium, and no cobalt the following results were obtained:—

Cobalt added. Grm.	Cobalt found. Grm.	Cobalt added. Per cent.	Cobalt found. Per cent.
Nil	Nil	Nil	Nil
0·0002	0·0001 × 2	0·02	0·02
0·0004	0·0002 × 2	0·04	0·04

When 10 c.c. of the 0·1 per cent. solution of potassium ferricyanide and 4 c.c. of the cobalt solution (0·4 per cent.) were taken, 0·4 per cent. was obtained.

The cobalt in these experiments was added to the steel in the form of a solution of cobalt sulphate, 1 c.c. of which was equal to 1 mgrm. of cobalt. It is possible to detect 0·1 mgrm. of cobalt in 50 c.c. of solution, so that the sensitiveness of the test is 1 part in 500,000.

RESEARCH DEPARTMENT,
ROYAL ARSENAL, WOOLWICH.

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.

THE COMPOSITION OF FRUIT.

THE literature concerning the composition of fresh fruit is particularly meagre, and, as we find it necessary from time to time to refer certain questions to the ratio of the various constituents of fruits, we have carried out in these Laboratories during the years 1925 to 1927 a certain number of analyses.

The following tables are therefore published with the hope that they may be of some use to others wishing to get an idea as to the average composition of a number of market fruits.

SUMMARY OF ANALYSES OF FRESH FRUIT FOR THE YEARS 1925—1927 INCLUSIVE.

1. APPLES.

	Number of Analyses.	Maximum. Per Cent.	Minimum. Per Cent.	Average. Per Cent.
(*a*) *Cooking Apples.*				
Total solids	11	15·61	10·25	13·04
Total sugars (as invert sugar) ..	11	8·72	3·64	7·11
Pectin (A.O.A.C. method) ..	3	1·60	0·84	1·29
Insoluble solids	3	2·47	1·95	2·17
(*b*) *Eating Apples.*				
Total solids	7	17·98	12·29	15·12
Total sugars (as invert sugar) ..	7	12·58	3·16	9·72
Pectin (A.O.A.C. method) ..	3	0·93	0·71	0·82
Insoluble solids	3	1·91	1·51	1·70

2. CHERRIES (WITHOUT STONES).

	Number of Analyses.	Maximum. Per Cent.	Minimum. Per Cent.	Average. Per Cent.
Total solids	13	24·70	14·74	18·64
Total sugars (as invert sugar) ..	13	15·30	8·28	11·47
Pectin (A.O.A.C. method) ..	4	0·54	0·24	0·35
Insoluble solids	4	3·10	1·29	2·05

3. APRICOTS (WITHOUT STONES).

Total solids	12	14·30	10·13	12·97
Total sugars (as invert sugar) ..	12	7·61	1·57	5·19
Pectin (A.O.A.C. method) ..	5	1·32	0·71	1·03
Insoluble solids	4	2·49	1·57	2·00

4. BLACKBERRIES.

Total solids	11	18·67	13·62	16·24
Total sugars (as invert sugar) ..	11	4·36	2·59	3·48
Pectin (A.O.A.C. method) ..	5	1·19	0·68	0·94
Insoluble solids	5	10·00	6·45	8·13

5. BLACK CURRANTS.

Total solids	13	24·43	15·93	19·44
Total sugars (as invert sugar) ..	13	7·44	3·66	5·50
Pectin (A.O.A.C. method) ..	4	1·79	1·37	1·52
Insoluble solids	4	6·18	4·78	5·51

6. GOOSEBERRIES.

Total solids	14	13·90	7·93	11·38
Total sugars (as invert sugar) ..	14	6·54	2·00	3·98
Pectin (A.O.A.C. method) ..	5	1·20	0·95	1·08
Insoluble solids	4	2·76	1·66	2·26

7. GREENGAGES (WITHOUT STONES).

Total solids	11	18·27	11·01	14·10
Total sugars (as invert sugar) ..	10	9·77	4·68	6·45
Pectin (A.O.A.C. method) ..	4	1·32	0·95	1·14
Insoluble solids	5	1·99	1·40	1·56

8. PLUMS (WITHOUT STONES).

Total solids	20	15·18	9·65	12·87
Total sugars (as invert sugar) ..	20	8·76	2·28	6·31
Pectin (A.O.A.C. method) ..	4	1·48	0·75	0·96
Insoluble solids	4	1·75	1·00	1·22

9. RASPBERRIES.

Total solids	16	24·82	12·38	16·78
Total sugars (as invert sugar) ..	16	8·67	2·54	4·80
Pectin (A.O.A.C. method) ..	4	0·86	0·58	0·71
Insoluble solids	4	6·22	4·23	5·50

10. RED CURRANTS.

Total solids	14	20·72	12·70	16·12
Total sugars (as invert sugar) ..	14	7·88	2·95	5·38
Pectin (A.O.A.C. method) ..	4	1·50	0·91	1·16
Insoluble solids	4	5·65	3·99	4·77

11. STRAWBERRIES.

	Number of Analyses.	Maximum. Per Cent.	Minimum. Per Cent.	Average. Per Cent.
Total solids	21	13·04	8·95	10·80
Total sugars (as invert sugar) ..	21	7·07	3·37	5·56
Pectin (A.O.A.C. method) ..	6	0·725	0·600	0·681
Insoluble solids	3	2·13	1·70	1·90

12. LOGANBERRIES.

Total solids	4	17·11	16·69	16·92
Total sugars (as invert sugar) ..	4	5·92	2·66	4·04
Pectin (A.O.A.C. method) ..	2	0·675	0·615	0·645
Insoluble solids	2	7·25	7·13	7·19

L. H. LAMPITT.
E. B. HUGHES.

THE LABORATORIES,
MESSRS. J. LYONS & CO., LTD.,
CADBY HALL, W. 14.

DETERMINATION OF SALT IN BUTTER AND MARGARINE.

SOME years ago I experienced difficulty in the routine determination of salt in margarine containing certain emulsifying agents. The method for determining salt, which was in use and proved unsatisfactory, was one that depended on the emulsion being broken by heating the margarine or butter with an acid solution of sodium nitrate, and then titrating with silver nitrate an aliquot portion of the aqueous extract. When margarine contained the emulsifying agents referred to above it was found impossible to extract all the salt, on account of the stability of the emulsion, and thus the method invariably gave low results.

The following method was then devised, in which alcohol is used to break the emulsion. This new method can be used with all varieties of margarine or butter. It has been in use for a number of years and has given every satisfaction; it is very rapid, which is a great advantage in routine work.

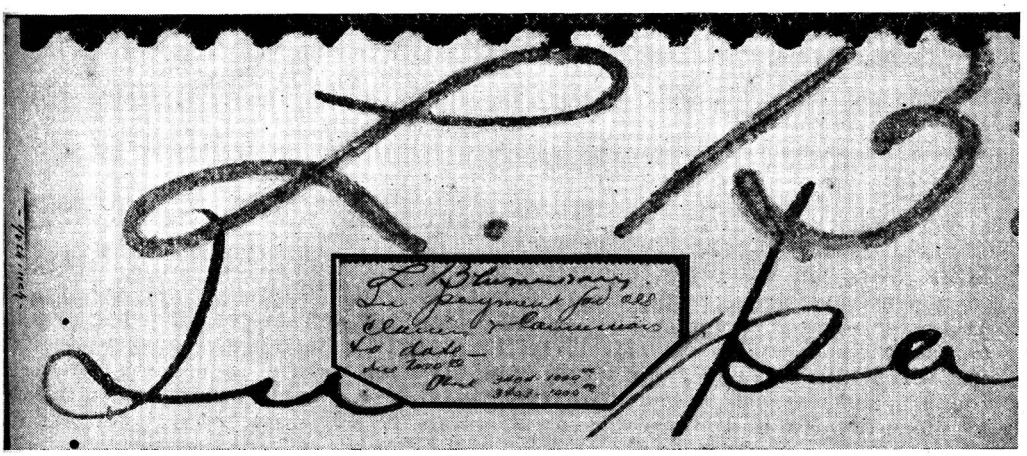
The procedure is as follows:—Weigh out carefully 10 grms. of the margarine or butter into a small wide-necked flask, melt the contents, and add 20 c.c. of industrial methylated spirits, allowing the alcohol to wash down the sides of the flask. Heat the mixture over a small flame, or on a hot plate, to the boiling point, mixing by gentle rotation during the heating. Now add 40 c.c. of distilled water, mix without re-heating, and filter through a folded filter paper. Titrate 30 c.c. of the filtrate with 0·1 *N* silver nitrate solution after the addition of a few drops of chromate solution. The number of c.c. of silver nitrate required, multiplied by 0·119, gives the percentage of salt. The factor is based on a water content of 15 per cent.

G. VAN B. GILMOUR.

SEQUENCE OF STROKES IN BLOTTED WRITING.

ALTHOUGH the paper by Messrs. Mitchell and Ward (ANALYST, 1927, **52**, 580) covers most of the ground, it does not deal with one of the effects which may result when a line is made over another line at a short interval after the writing of that line and is then blotted almost immediately. Under such conditions the blotting may remove not only much of the uppermost writing, but also some of the ink of the first writing, leaving a distinct gap in the line. I called attention to this possibility in my book *Questioned Documents* (p. 381), but at that time I had never met with an actual case. Quite recently, however, a case which was tried in an American Court affords a perfect illustration of this blotting effect.

The facts are interesting. Those who drew the cheque were suspicious of the payee, and wished to make it quite clear what the cheque was for, and they therefore put a note on the back that it was "In payment for all claims." The payee received the cheque, and, as is evident in the photograph, endorsed it above this note—an unlikely place for an indorsement if there was no writing on the back of the cheque.



This endorsement was put upon the cheque soon after it was written, and as clearly appears, was immediately blotted. The writing "In payment of all claims" had not yet become oxidised, and when the blotter was applied it removed not only the ink of the payee's endorsement, but also a large part of the ink of the note, which had been wet by the endorsing ink, with the result that a gap was made in the line. When the first writing had become oxidised it became decidedly black, as appears, whereas the part taken away by the blotting has left the gap mentioned.

The endorser gave evidence that there was no writing on the back of the cheque when he received it, and he claimed that the note had been put upon it after it had come back from the bank. The Court, however, decided, on the basis of the evidence shown by the photograph, that the blotted endorsement had been written on the top of the note.

A. S. OSBORN.

225 BROADWAY, NEW YORK.

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.

COUNTY OF SOMERSET.

Annual Report of the County Analyst and Bacteriologist for the Year 1926.

The total number of samples examined during the year was 7440, of which 1109 were taken under the Sale of Foods and Drugs Acts. Five of these were suspicious, and 29 adulterated.

Arsenic in Apples.—Of the 29 samples of imported apples examined, two contained 1/37th and 1/44th grain of arsenic per lb., respectively. The vendor of the second (a formal) sample was cautioned.

Preservatives in Food.—Twelve of 56 samples of butter contained boric acid (0·05–0·47 per cent.); 6 of 12 margarines, 0·07 to 0·53 per cent. of boric acid; 7 of 16 samples of sausages, 0·08 to 0·5 per cent. of boric acid; and 7 of 10 non-alcoholic wines contained salicylic acid (1·9 to 4·2 grains per pint) or benzoic acid (2·4 to 5·0 grains per pint). Eleven samples of potted meat or fish, 7 of brawn, and 12 of sponge cake were free from preservatives.

Denys R. Wood.

GIBRALTAR.

Report of the City Analyst and Bacteriologist for the Year 1926.

The total number of samples and specimens examined during the year was 4001 (an increase of 363 on the previous year). Of these samples, 137 were food and drugs, taken by Sanitary Inspectors, with the necessary formalities. They included 46 of goat's milk, 3 of cow's milk, 18 of edible fats and oil, 26 spirits and wines, and 13 drugs.

Goat's Boiled Milk.—The statutory limits for goat's milk are 3·5 per cent. of fat and 8·0 per cent. of solids-not-fat. Five of the samples examined were deficient in fat by amounts ranging from 5·7 to 20 per cent. Vendors, when asked for a sample by inspectors, habitually declare the milk to be skimmed. It is the practice of some vendors to remove the scum which rises after boiling, to improve the appearance of the milk; a large proportion of this scum is fat.

Unboiled Milk.—It is essential and made compulsory by law that all imported goat's milk shall be boiled before being sold to the public. The reason for this is the occurrence of undulant fever among the goats in Spain. In previous years no sample of goat's milk that had not been boiled was offered for sale in Gibraltar, but in 1926, on two separate occasions, a sample of imported milk was found to contain some unboiled milk (approximately 10 per cent.).

Olive Oil.—Many of the samples examined were of inferior quality, some containing over 19 per cent. of acidity (as oleic acid). There is, at present, in Gibraltar no statutory limitation as to the acidity of olive oil, but the advisability of introducing legislation, similar to that in force in Malta, is under consideration.

CONDENSED MILK.—An Ordinance for Gibraltar, based on the recent English regulations, is in course of preparation. Samples analysed were deficient in fat, total solids, or both.

BACTERIOLOGICAL AND PUBLIC HEALTH WORK.—In all, 3618 samples and specimens were examined. The work included bacteriological tests of blood, blood sugar determinations, renal efficiency tests, cerebro-spinal tests, serological agglutination tests on 205 goats, etc.

A. G. HOLBOROW.

Legal Notes.

Under this heading will be published notes on cases in which points of special legal or chemical interest arise. The Editor would be glad to receive particulars of such cases.

SIGNIFICATION OF THE TERM "DRIPPING."

ON November 10, a tradesman was summoned at Drogheda for selling dripping that was not of the nature, substance and quality demanded. The solicitor for the prosecution stated that, according to the certificate of the Public Analyst (Mr. Fagan), the sample was not genuine dripping, but rendered or refined edible fat. This was based on the fact that dripping is matter which falls from roasting meat, and contains extractive matter of the roasting meat. The predecessor of the present Justice had held that a sample like the present one was not dripping. Justice Goff said that he was aware that his colleague had convicted in a similar case, but that, on appeal, the decision had been reversed by the Circuit Court Judge (*cf.* ANALYST, 1927, **52**, 592); he did not consider himself bound by that decision, as he had not seen a report of the case.

The solicitor for the defendant said that there had been two recent cases in England and Ireland bearing out his contention that the "quality" meant commercial quality. The inspector stated that the sample was labelled "dripping"; he admitted that there was no legal standard for dripping, but when he asked for dripping he did not expect to get "commercial dripping."

Mr. Smyth (for the defence) said that the first time any standard was fixed for dripping was in 1918, when the regulations made under the Defence of the Realm Act set out that "dripping shall be made from raw beef fat or raw mutton fat, or from beef or mutton bones."

The manufacturer of the product said that he had been making dripping for 40 years in Dublin and in England. It was manufactured solely from edible—*i.e.* sweet beef and mutton fat. It was dry cooked, and there was a certain amount of meat in it, though not so much as in dripping from roasted meat. If the public had to depend upon kitchen dripping for their supply of dripping, then all Dublin could not supply the needs of Drogheda—there would be a general shortage. The dripping they supplied was cleaned of all impurities, and had great keeping properties. In fact, the sample taken from the defendant, although nearly eight months old, was well within the specification given by the Food Controller.

The Justice said that he would dismiss the prosecution on three grounds: (1) The defendant acted apparently *bona fide* in selling this product as dripping; (2) on the ground of its commercial quality, although that could be driven too far; and (3) because the word "dripping" seemed to have acquired a much wider

application that it originally had. A confusion had arisen in the ordinary acceptance of the word, and until a change was made in the regulations he must hold that this was dripping. In his view the authorities should make an adequate technical definition. As the definition stood, it was open to any one to use the lowest grade of fat for making this dripping, and that would react to the prejudice of the purchaser. As the merits of the case were with the local authority, who had acted in the interests of the public, he would not allow costs.

EXCESS OF SULPHUR DIOXIDE IN FOOD PRESERVATIVE.

ON November 15 a firm of manufacturers was summoned at Brentford under the Public Health (Preservatives, &c., in Food) Regulations for giving a false label with a supply of food preservative, and also for giving a false warranty.

Mr. R. A. Robinson, for the Middlesex County Council, said that the defendants had supplied a Smithfield firm with a quantity of preservative which was labelled and warranted as containing 7 per cent. of sulphur dioxide, whereas analysis showed that it contained 11·1 per cent. The serious part of the affair was that the Smithfield firm had retailed the powder to several butchers, who had been fined for selling sausages containing an excess of sulphur dioxide.

The defendants stated that the consignment, after being prepared, got wet, and that the constituent parts must have altered during the re-drying process.

A fine of £42 with £5 5s. costs was imposed.

BLOOD TESTS IN PATERNITY CASES.

THE first instance of a blood test being applied in a trial occurred in November, 1927, when a woman was charged in Württemberg with perjury. She had claimed as the father of her illegitimate child a man who denied the paternity, and who asked that blood tests should be applied to his blood, to that of the woman, and to that of the child. The tests were made by representatives of the State Medical Board in Württemberg, and their report was to the effect that the blood of both the man and the woman belonged to Group II, whereas that of the child was Group IV. As the result of this test, evidence was given that the child was not the offspring of the man.

The Court, having heard the evidence, decided that there was no doubt as to the reliability of the blood test, and that it had been proved that the man was not the father of the woman's child. The woman was sentenced to six months' imprisonment.

In December, another paternity case was tried, this time in the highest Prussian Court in Berlin. A woman claimed support for her child from a man who, she alleged, was the father of the child. The man denied paternity and brought medical evidence to show that the "blood group" test supported his statement. The Court would not admit that the 4363 experimental tests upon which the conclusion was based were sufficient to satisfy the requirements of the law. In 1926 there were 2093 tests made, but in one of the cases the blood test proved to be an exception to the rule. Hence the Court considered that this single exception was sufficient to throw doubt upon the certainty of the method, and there was no absolute proof that the man could not possibly be the father of the child. Such evidence, therefore, could not be accepted as the sole proof that a child was not the offspring of a particular man or woman, although it might be admissible as corroborative evidence.

Queensland.

REPORT OF THE GOVERNMENT ANALYST FOR THE YEAR ENDED JUNE 30TH, 1927.

ACCORDING to Dr. J. B. Henderson's Report, the number of samples analysed during the twelve months was 6854, of which 2187 were for the Department of Public Health. Of the 1139 samples taken by inspectors under the Health Acts, 795 were satisfactory, 294 were condemned, and 45 were unsuitable for exact analyst.

LEAD IN SODA WATER.—During the year it was discovered that samples of soda water from soda water fountains contained lead in appreciable quantities. Ordinary soda water in bottles was tested, and all samples were found to be free from lead. Fountain soda water is made in a tin-lined steel vessel called a "carbonator." This vessel has a stirrer, an inlet pipe for water, and one for carbonic acid gas. It was found that, although the use of solder or other alloy containing lead is forbidden by the Health Regulations in any vessel containing moist or liquid food, the interior of nearly all these carbonators was liberally covered with solder where the pipes entered the container, on the stirrers, and on the joints of the tin lining. The solder contained generally about 40 per cent. of lead, and the proportion of the interior surface covered by solder was in one case as high as 15 per cent., and, even in a new carbonator, 7·5 per cent. of the interior was covered with solder. The tin linings themselves were all of good or fairly good tin, the proportion of lead found in the tin being in no case over 0·2 per cent., and generally considerably less.

It was noteworthy that, whilst the comparatively soft Brisbane water readily took up lead from the carbonators, the hard water supply at Toowoomba dissolved no lead from exactly similar carbonators.

A very large amount of work was done by examining tin linings, solders, connecting hose pipes, rubber washers, etc., for lead, in assisting the traders to get over this difficulty. Considerable trouble was experienced in getting the carbonators freed from lead-containing surfaces, and in getting the fountain soda waters freed from lead. Success was finally obtained by using artificially "hardened" water in carbonators containing no lead-solder surfaces.

The extent to which the fountain soda waters were contaminated with lead is shown in the following table:—

LEAD IN SODA WATER FROM CARBONATORS.

Grains of Lead per gallon of Soda Water.	Number of Samples.
Nil	68
1/300th to 1/100th	83
1/100th to 5/100ths	86
5/100ths to 10/100ths	75
10/100ths to 15/100ths	39
15/100ths to 20/100ths	21
20/100ths to 30/100ths	6
30/100ths to 40/100ths	6
60/100ths to 75/100ths	4
75/100ths to 1·1	6
Total	394

It is generally accepted that water containing any proportion of lead greater than 5/100 grain per gallon is dangerous. At least half of the samples tested contained more than this proportion, and, as the table shows, some contained much more.

ACETYLSALICYLIC ACID.—Eleven samples sold under various names (aspirin, etc.) were examined. The weight of acetyl-salicylic acid present in the tablets was very consistent, the greatest variation being only 4 per cent. Only two samples failed to disintegrate in water in two minutes, which has been suggested as the maximum time for the disintegration of acetyl-salicylic acid tablets in water. The British Pharmacopœia gives no quantitative test for free salicylic acid, but the qualitative test given therein allows of about 0·1 per cent. being present. This proportion, however, is not high enough for a tropical climate, and we have found it necessary to adopt a tentative maximum of 0·5 per cent. Only one sample exceeded this limit. One sample contained a sodium salt, probably used under the impression that it would prevent hydrolysis of salicylic acid. It has been suggested that, as the presence of free salicylic acid can be masked by a trace of citric or tartaric acid, a small proportion of one of these acids should be added to the tablets. Two samples contained sufficient of these acids to mask the ordinary sorting-out test for free salicylic acid.

HAIR DYES.—The 31 toilet preparations examined included a number of hair dyes. The vendors of these preparations display no originality in their composition, as the formulas are mostly very old, and are shown in almost every book of chemical recipes. The following list indicates their composition:—(1) Lead acetate and sulphur; (2) henna, wheat starch and pyrogallol; (3) paraphenylenediamine; (4) silver nitrate and ammonium sulphide solution; (5) silver nitrate and pyrogallol solution; (6) pyrogallol and copper salts; (7) silver nitrate and copper nitrate; (8) henna powder and copper salts. The use of paraphenylenediamine as a hair dye has been reported to produce skin eruptions, eczema, nausea or nervous symptoms, sleeplessness, dizziness, weakness and impairment of vision.

BEER.—Of the 26 samples examined 23 represented practically all the brands on the Brisbane market. Most of the Australian beer contained salicylic acid (0·5 to 1·5 grains per gallon); the lager beer from Germany and London was free from preservatives.

TOXICOLOGICAL CASES.—For the Police Department 93 samples were examined in connection with 29 cases of suspected poisoning of human beings, and 7 cases of suspected poisoning of domestic or native animals. In 10 of the human cases no poison was found. Of the other 19 cases, strychnine was found in 10 cases, cresol in 2 cases, prussic acid in 2 cases, arsenic in 2 cases, liniment in 1 case, and adrenalin in 1 case. In one case the blood sample showed that death had resulted from carbon monoxide poisoning. The carbon monoxide had come from the exhaust gases of a petrol engine. A fruit-cake submitted contained 5·4 per cent. of arsenic.

In the 2 cases of poisoning in domestic animals, arsenic was found in 1 case and strychnine in the other, whilst, in the case of native animals, cyanide was found in 3 cases and arsenic in 2.

DANGEROUS DRUGS ACT.—In connection with dangerous drugs administration, 6 samples were submitted, 4 of these being cocaine hydrochloride, 1 cocaine salicylate, and 1 a popular headache powder. Three exhibits of "opium charcoal" proved to be charcoal from other sources and free from opium.

Eighth Conference of the International Union for Pure and Applied Chemistry.*

UNDER the Presidency of Prof. Ernst Cohen, the Conference was opened at Warsaw on September 4th, 1927. Six nations were represented, and reports were presented by the various Commissions. The Nomenclature Commission for Inorganic Compounds considered the Report of the Comité de Travail, as well as the proposition put forward by Grignard and Patterson, and certain proposed amendments.

THE COMITÉ DE TRAVAIL FOR THE REFORM OF INORGANIC NOMENCLATURE.—Chairman, Dr. W. P. Jorissen. (Previous report, ANALYST, 1926, **51**, 192–195.) *Valency.* The indicative number to follow the element in the index. Prefix *di-* is to be kept for such salts as $Cr_2O_2K_2$, where there is more than one acid anhydride for one anhydride base radicle. *Acid salts* are designated, as, for example, acid potassium carbonate, CO_3HK; potassium hydrogen carbonate $KHCO_3$. The use of "thio" for fully substituted sulphur acids is adopted; for example, CS_3H_2 is trithio-carbonic acid. The compound $S_2O_3Na_2$ is to be called sodium thiosulphate; while the acid prefixes *thio, meta, pyro* and *para* are not at present modified; the various discussions on the subject are recommended for consideration. For basic salts the general terms such as basic sulphate, accompanied by the formula, are regarded as sufficient.

COMMISSION FOR THE REFORM OF BIOLOGICAL CHEMICAL NOMENCLATURE.—(1) *Glucosides* are divided into *oses* and *osides*, the former non-hydrolysable reducing agents, and the latter comprising those glucosides which give, on complete hydrolysis, one or more *oses*, accompanied or not by other substances. The *osides* are divided into *holosides*, hydolysing only to *oses*, and *heterosides* giving one or more *oses* accompanied by other non-glucosidic substances.

(2) *Lipoids.* The definition proposed by the Belgium Committee is not adopted.

(3) *Proteins.* These are substances giving, on complete hydrolysis, amino acids, with or without other substances, and are divided into *holo* and *hetero* proteins, the former hydrolysing only to amino acids and ammonia, and being capable of sub-division into protamines and histones, albumins, globulines, glutenines, gliadines, scleroproteins, and keratines. Heteroproteins give amino acids and other substances on hydrolysis, and may be sub-divided into nucleo-proteins, mucoproteins, chromoproteins and phosphoproteins.

(4) *Soluble ferments.* It is proposed to designate these by the termination—*ase.*

COMMISSION ON PURE SUBSTANCES FOR RESEARCH.—The Commission proposes to terminate their work on the codification of a certain number of reagents for the next Conference, and to publish a Codex in 1928. Further work relative to other reagents is then proposed. In view of the desirability of comparing analytical methods from the point of view of results obtained, the Commission propose to be called "The Commission of Analytical Chemistry," and to work in sub-committees:—(1) For pure reagents for analysis; (2) a comparison of analytical methods applied to industrial products; and (3) on the definition of technical products, the first being a permanent sub-committee.

* *Chem. Weekblad*, 1927, **44**, 538–550.

COMMISSION ON THERMOCHEMICAL DATA.—The necessity for a uniform method of publication of tables is pointed out, particularly with regard to author's numbers, which should be expressed as calories or kilojoules, so that any necessary corrections may be made, and these should be cited in the tables. In other columns should be tabulated the heat of combustion of 1 grm. and of 1 molecule grm. of substance at constant volume, the same value at constant pressure, and the name of the author determining the correction coefficient. It is desirable that weights *in vacuo* should be recorded.

COMMISSION ON COMBUSTIBLE LIQUIDS.—Immediate circulation of M. Ganes's table is recommended, so that members may indicate which analytical method they may desire to study, and a study of each method of analysis in the different countries would enable comparative tables to be constructed. A study of light products such as essences, medium products such as Diesel oils, and heavy products such as lubricating oils, should first engage attention.

COMMISSION ON COMBUSTIBLE SOLIDS.—Although this Commission has undertaken the co-ordination of methods of analysis of coals, it welcomes the transformation of the Commission of pure reagents for analysis to an Analytical Chemistry Commission.

COMMISSION ON CERAMIC PRODUCTS.—Collaboration in the common work is expected from each member, and a preparation of two samples, either of refractory clay, of washed kaolin, or (in certain countries) of clay and of kaolin is asked for, so that these may be sent to the members for analysis, details of which should be furnished by May 1st, 1928.

D. G. H.

Ministry of Health.

ACTS AUTHORITIES (ENGLAND AND WALES).

CIRCULAR 852.

PUBLIC HEALTH (PRESERVATIVES &c., IN FOOD) REGULATIONS.

THE following circular has been issued to the Clerks of Authorities administering the Sale of Food and Drugs Acts:—

SIR,

1. I am directed by the Minister of Health to remind the Council that the Public Health (Preservatives, &c., in Food) Regulations will come into operation on the 1st proximo so far as they relate to butter, cream and articles of food containing preservative necessarily introduced by the use of preserved bacon, preserved ham, preserved egg yolk or preserved cream in their preparation.

2. It is understood, however, that it may not be practicable for all stocks of preserved butter to be disposed of before the beginning of 1928 and, in the circumstances, the Minister suggests that in the case of butter the Council might follow a course similar to that suggested in paragraph 3 of Circular 751 and paragraph 2 of Circular 806 and refrain, during the first few weeks of 1928, from instituting legal proceedings in any case where they are satisfied that reasonable efforts have been made to clear old stocks and that future consignments will conform with the Regulations.

3. A copy of this Circular is being sent to the Medical Officer of Health and the Public Analyst.

I am, Sir, Your obedient Servant,

R. B. CROSS,

December 30, 1927. (*Assistant Secretary*).

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Volumetric Determination of Lactose in Presence of Sucrose. J. H. Lane and L. Eynon. (*J. Soc. Chem. Ind.*, 1927, **46**, 434–435T.)—In the analysis of sweetened condensed milk the sucrose present affects the determination of lactose by means of Fehling's solution, the volume of lactose solution required to reduce a given volume of the Fehling's solution being reduced. When the authors' method (ANALYST, 1923, **48**, 220) is used, the number of c.c. by which the observed volume of the sugar solution must be increased is given in the following table for lactose solutions containing either three or six times as much sucrose as lactose, and for titrations in which either 10 or 25 c.c. of Fehling's solution is employed.

Observed volume of sugar solution.	10 c.c. of Fehling's solution Ratio of sucrose to lactose		25 c.c. of Fehling's solution Ratio of sucrose to lactose	
c.c.	3:1	6:1	3:1	6:1
15	0·15	0·30	0·30	0·60
20	0·25	0·50	0·30	0·60
25	0·30	0·60	0·35	0·65
30	0·35	0·70	0·35	0·70
35	0·40	0·80	0·40	0·80
40	0·45	0·90	0·45	0·90
45	0·50	0·95	0·55	1·10
50	0·55	1·05	0·60	1·20

At any given part of the table the correction is practically proportional to the value of the sucrose : lactose ratio, and this proportionality holds, up to values of about 10 : 1 for this ratio. T. H. P.

Manganese Content of Plant and Animal Materials. C. W. Lindow and W. H. Peterson. (*J. Biol. Chem.*, 1927, **75**, 169–175.)—The manganese content of 84 samples, covering the principal classes of food materials, is given. In all cases duplicate determinations were made, and in some of them " recovery " determinations were carried out. A table gives detailed data illustrative of the entire procedure. The authors fully appreciate the fact that the figures presented do not necessarily represent the manganese content of these materials generally—merely of the samples. The figures (dry basis) range from none, in fish, to 0·02162 per cent. in northern grown lettuce. The classes of foods in descending order of manganese content are as follows:—Leafy vegetables, cereals, legumes, nuts, roots and tubers, bush and vine fruits, animal tissues, and tree fruits. Leafy vegetables, which are generally high in all mineral elements, contain about 3 times as much manganese as the next two groups—cereals and legumes. Different samples of the same foodstuff vary in manganese content. For example, a sample of lettuce

grown in the north contained more than twice as much manganese as a sample grown in the south. In twenty-two samples of cabbage the manganese ranged from 0·00052 to 0·00159 per cent. Beets and sweet potatoes showed similar variations. Presumably the samples vary with soil conditions. Lettuce, beet greens (tops), blueberries and wheat bran are all unusually high in manganese.

P. H. P.

Detection of Hardened Fat in Beef Fat from the Iodine Value of the Solid Fatty Acids separated by Twitchell's Method. S. C. L. Gerritzen and M. Kauffman. (*Chem. Weekblad*, 1927, **24**, 554–556.)—Twitchell's method (ANALYST, 1921, **46**, 466) has been found suitable for the separation of solid fatty acids from hardened oils. The iodine values of the acids thus obtained from beef fat are usually less than 5, and a value greater than 6 indicates the presence of a foreign substance. For mutton fat the values vary from 1·7 to 12·5, and this probably indicates the incomplete separation of the fatty acids, or the presence of solid, unsaturated fatty acids. The percentage of iso-oleic acid may be calculated from the expression—95.S.(I–5)/9,000, where S is the amount of sample taken, and I the iodine value of the solid, saturated fatty acids. For butter-fat, lard and hardened whale oil the values vary from 4·6 to 5·3, 1·1 to 3·1, and 6·2 to 27·3, respectively. By the addition of known amounts of hardened whale oil to beef fat, the proportion present in a given sample may be determined from a comparison of the iodine values of the separated fatty acids (*cf.* ANALYST, 1924, **49**, 460).

J. G.

Phytosteryl Acetate Test and the Phytosterol of Sunflower Seed Oil. J. Allan and C. W. Moore. (*J. Soc. Chem. Ind.*, 1927, **46**, 433–434T.)—The value of the phytosteryl acetate test, when applied to the detection of animal fats in fats of vegetable origin, has been diminished by the discovery of phytosterols which form acetyl derivatives having melting points ranging from 115° to 250° C., although values below 125° C. are infrequent. It is, however, now found that a phytosterol, $C_{29}H_{49}OH$, occurring in normal amount in sunflower seed oil and constituting the bulk of the phytosterols present, gives an acetyl compound melting at 119–119·5° C. (corr.). Mixtures of this acetyl derivative with those of certain other phytosterols melt at temperatures ranging from 121° to 123·5° C. (corr.), whilst a mixture with an approximately equal weight of cholesteryl acetate shows abnormal behaviour and melts at 121–122° C. (corr.). The difficulty which thus arises in using the phytosteryl acetate test for examining sunflower seed oil or its mixtures for animal fats, is also encountered with hydrogenated sunflower seed oil. The sterols obtained from several other important edible vegetable oils examined yield acetyl derivatives with melting points lying between 122° and 131° C.

T. H. P.

The Lower Fatty Acids of Coconut Oil. E. R. Taylor and H. T. Clarke. (*J. Amer. Chem. Soc.*, 1927, **49**, 2829.)—Systematic fractionation of about 130 kilos. of methyl esters of the fatty acids of coconut oil has been carried out, and the following fatty acids isolated:—Caproic (0·46 per cent.), caprylic (8·7 per cent.),

capric (5·6 per cent.), lauric (45·0 per cent.), and myristic (16·5 to 18 per cent.). The caproic acid (which has hitherto escaped recognition in coconut oil) was identified by converting it, by means of thionyl chloride, into the acid chloride. The product was added, with constant stirring, to concentrated ammonia at 0° C., and the amide produced was washed with dilute ammonia and dried. It was then recrystallised from toluene, and the melting point found to be 98–99° C. An authentic sample of caproic acid was prepared from *n*-butyl bromide by the malonic ester synthesis, and yielded an amide which also melted at 98–99° C. A mixture of the two preparations showed no change in melting point. R. F. I.

Analysis of Jalap. H. E. Dale. (*Pharm. J.*, 1927, **119**, 516–517.)—The resin of jalap, which, according to the British Pharmacopoeia, should be between 9 and 11 per cent., may be satisfactorily determined by completely extracting 10 grms. of powdered jalap with hot alcohol, making up to 100 c.c. with alcohol, filtering, washing, and evaporating 50 c.c. of the filtrate to dryness until completely free from alcohol. The containing beaker is placed in water at 65° C., 15 c.c. of water at 65° C. added, the contents stirred for two minutes and cooled, and the wash water decanted on to an 11 cm. filter paper. The washing of the resin is repeated, with 15, 15 and 15 c.c. of water at 65° C., the resin dissolved in 15 c.c. of alcohol, filtered into a tared beaker and completely transferred with 10, 10 and 5 c.c. of hot alcohol, and the filter paper washed with alcohol. The solution is evaporated, and the residue dried and weighed. D. G. H.

Biochemical, etc.

Effect of Glucose on the Condensation of Formaldehyde. I. Determination of Urinary Sugar by this Principle. F. B. Kingsbury. (*J. Biol. Chem.*, 1927, **75**, 241–245.)—In the development of a new reagent for the colorimetric determination of sugar in normal urine it was discovered that the reagent could be made many times more sensitive to small amounts of glucose by the addition of formaldehyde, due to the production of reducing material (formose) by the condensation of the formaldehyde, and that the amount of this material so produced was proportional to the amount of glucose present. Thus a new analytical method has been devised for the determination of sugar, applicable to urine and blood, based on the catalytic effect of reducing sugar on the condensation of formaldehyde in alkaline solution. The chief advantage of the method is its simplicity, for, on account of the greater specificity of the reagent toward sugars, mere dilution of the urine is the only necessary preliminary step, and thus the method is more rapid. To 1 c.c. of urine, measured by an Östwald pipette into an Erlenmeyer flask, are added 9 c.c. of distilled water by Folin blood pipette, and the contents mixed by twirling. Two c.c. of the diluted urine are transferred to a test-tube (150 mm. × 20 mm.), graduated at 25 c.c., 3 c.c. of the sugar reagent are added, and the tube is gently shaken, without inversion, and then heated in a rapidly boiling water bath for 15 minutes. Simultaneously 2 c.c. each of two glucose

standards (0·005 and 0·01 per cent.) are similarly treated. After heating, the tubes are cooled by immersion in water for 3 minutes, distilled water is added to the 25 c.c. mark, and the contents of each tube thoroughly mixed. The standard solution which more closely matches in colour the contents of the urine tube is placed in the left hand cup of the colorimeter and set at 20 mm.; 15 c.c. of the contents of the urine tube are centrifuged and placed in the right hand cup, and the comparison is made in the usual way. Twenty divided by the colorimetric reading multiplied by the value in per cent. of the standard used, multiplied by 10 (the urine dilution), gives the percentage of sugar present in the original specimen. These directions apply to urines with sugar values from about 0·04 to 0·13 per cent. The new sugar reagent is prepared as follows:—37·5 grms. of sodium carbonate, 25·0 grms. of sodium sulphite, and 5·0 grms. of 2, 4-dinitro-1-naphthol-7-sulphonic acid are dissolved in about 450 c.c. of distilled water, boiled very gently for half an hour, cooled, and the deep, orange-coloured solution diluted to 500 c.c., filtered and bottled. For the final reagent 5 c.c. of freshly prepared 10 per cent. formaldehyde solution in water are added to 100 c.c. of the above stock reagent solution. The formaldehyde is prepared by adding 3 vols. of water to formalin containing 40 per cent. of formaldehyde. The reagent is practically unaffected by homogentisic acid, and thus sugar determinations can be made in alcaptonuric urines. Large quantities of urea affect it, however, so that a modification of the method for urines rich in urea may be necessary. Very sensitive qualitative reagents have been devised on similar lines. One such reagent gave a deep colour with less than 0·001 mgrm. of glucose. The glucose standards may be preserved by means of saturated benzoic acid and also by toluene. P. H. P.

Utilisation of Carbohydrates by Honey Bees. E. F. Phillips. (*J. Agric. Res.*, 1927, **35**, 385.)—The author has made a study of the problem of wintering honey bees by feeding them on a range of pure carbohydrates. Certain of these are assimilated direct; others are only assimilated after being broken down into simpler sugars by enzymes in the bee; others, again, are not assimilated, and cause dysentery. Cages containing about 100 bees were prepared with water and the carbohydrate to be tested. At the end of each 24 hours the dead bees were removed and counted, this being continued each day till all were dead. The following were found satisfactory foods:—Glucose, laevulose, sucrose, trehalose, maltose, melezitose; but galactose, mannose, lactose, raffinose, dextrin, starch, inulin, glycerin, mannite, rhamnose, xylose, and *d*- and *l*-arabinose are unsatisfactory. Bees can live on brown sugar, but practical experience shows that it is an unsuitable food for wintering. The paler honeys form the best material for this purpose. The authors conclude from these results that bees contain invertase, maltase, trehalase, but not lactase, inulase, emulsin or diastase. Dysentery in winter is caused by the indigestibility of the dextrins in the food supply. A full bibliography is given. R. F. I.

Utilisation of Carbohydrates by Honey Bee Larvae. L. M. Bertholf. (*J. Agric. Res.*, 1927, **35**, 429.)—Similar systems of investigation were employed as before (see preceding abstract), *i.e.* the direct feeding method and comparing the length of life with that under normal feeding. The larvae were removed from the comb, adhering food washed off, and the larvae then transferred to a small watch glass containing a solution of the carbohydrate to be tested. The watch glass was placed in a Petri dish containing water to preserve a moist atmosphere, and the whole incubated at 35° C. The larvae were examined every 6 hours, and were considered dead if they no longer responded by muscular contraction when touched with the transferring needle. The carbohydrates are placed in the following order of apparent food values:—Sucrose, laevulose, maltose, melezitose, dextrose, trehalose, dextrin, galactose, lactose (very slight). Starch and glycogen are unsatisfactory. Honeys vary in their food value as follows from greatest to least life-sustaining value:—Basswood clover, white clover, tulip tree, alfalfa, buckwheat, mountain laurel, sage, aster, golden rod, and locust. The reason for the variation is not to be explained on the basis of any known physical or chemical characteristics of the honey. R. F. I.

Synthesis of Antineuritic Vitamin by Yeast. G. L. Peskett. (*Biochem. J.*, 1927, **21**, 1102–1103.)—It was recently shown by Hawking (*Biochem. J.*, 1927, **21**, 728) that yeast can synthesise the factor which cures the symptoms of head retraction in pigeons fed upon polished rice. The experiments were not quite conclusive, since (1) the amount of " bios extract " used in growing the yeast contained so much of the antineuritic factor that the amount of the latter which was synthesised appeared to be small ; and (2) there was some contamination of the yeast by bacteria. An experiment is recorded in which the cane sugar, salts and " bios extract " were precisely the same as Hawking used, and the yeast was the same strain of *Saccharomyces cerevisiae*. In a medium which did not contain more than 0·7 day doses of antineuritic vitamin 5·7 grms. of yeast were produced. Bacterial contamination was avoided, and experiments on pigeons showed that the yeast contained at least 6 day doses of the antineuritic vitamin of which 5·3 day doses were presumably synthesised. Therefore considerable synthesis can occur. P. H. P.

The Antiscorbutic Fraction of Lemon Juice. VI. E. Hoyle and S. S. Zilva. (*Biochem. J.*, 1927, **21**, 1121–1127.)—During a previous investigation on the behaviour of some of the inorganic constituents of lemon juice in the process of fractionation the study of manganese and of iron was found difficult, owing to the presence of traces of these elements in the reagents used in the various manipulations. All the necessary reagents have now been prepared entirely free from interfering impurities, and manganese, iron, phosphorus and sulphur have been investigated. The concentrated antiscorbutic fraction from lemon juice does not contain any manganese, but contains traces of iron, phosphorus and sulphur. The

active fraction from lemon juice has thus been examined for iron, phosphorus, sulphur, iodine, manganese, nickel, cobalt and boron. See Daubney and Zilva (*Biochem. J.*, 1926, **20**, 1055) for the tests for the presence of these other elements. The last four named elements were found to be absent, and so, if associated with the antiscorbutic activity, they must be so in quantities not detectable by the authors' methods. Iodine may also be excluded, since it did not diffuse through a collodion thimble with a 95 per cent. alcohol index. Antiscorbutic solutions dialysed under the same conditions lose their activity. The iron, phosphorus and sulphur of the active fraction behave on dialysis in the same way as the active principle; however, one must be cautious in postulating a connection between them and the antiscorbutic activity. P. H. P.

Reaction of Fatty Extracts of Certain Organs with the Antimony Trichloride Test for "Vitamin *A*." W. H. Wilson. (*Biochem. J.*, 1927, **21**, 1054–1058.)—An examination has been made of the comparative amount of the substance, which gives a blue colour with antimony trichloride, contained in the fatty mixture extracted from the organs, in particular the liver, of man and other mammals, without necessarily assuming it to be vitamin *A*. The blue colour was matched against solutions of indigo-carmine of known concentration. The standard taken was a sample of cod-liver oil which, when tested in 20 per cent. solution, gave a colour matched by a $\frac{1}{5,000}$ solution of indigo-carmine. This was given unit, value and the content in vitamin *A* was expressed in multiples or fractions of the unit. If the substance which gives the reaction is regarded as being identical with vitamin *A*, and this seems probable, then the observations on the livers of animals used for food would indicate the value of liver as a foodstuff. The following conclusions are given:—The fatty extract from the human liver contains a substance which gives the same colour reactions as vitamin *A* in an amount which may be equal to 25 times that found in a good sample of cod-liver oil. The amount of vitamin *A* found in the liver varies within wide limits, and may, under some conditions of disease, be no more than $\frac{1}{250}$ of the maximum amount observed to occur. The substance is present in considerable amount in the liver at birth, but not, in an amount detectable by the test used, in the placenta. The extract of livers of healthy animals slaughtered for food was found to contain from 6 to 12·5 times the amount of vitamin *A* present in cod-liver oil. It was found that fuming nitric acid on a glass rod gave, when drawn across the extract, a rich purple colour which rapidly turned pink and then disappeared. The depth of colour was roughly proportional to the intensity of the reaction with antimony trichloride, and was used as a preliminary test to determine the probable richness of different extracts in vitamin *A*. However, in spite of the apparent correspondence of the results of the two tests, when applied to the fatty extracts examined, the reactions appear to be due to the presence of two different substances. Both are found in the unsaponifiable residue, but the substance which reacts with antimony trichloride is very much more soluble in petroleum spirit than is the substance which gives a purple colour with nitric acid. P. H. P.

Feeding of Rats Exclusively with Various Kinds of Bread. E. Friedberger and E. Seidenberg. (*Münch. Med. Wochensch.*, 1927, No. 37, 1573.)—The " primary increment value " is defined as the percentage increase in weight in 30 days (or other suitable period) of a growing rat of initial weight 40 grms., when food is given *ad lib.* The " secondary increment value " expresses the percentage increase in weight in relation to various methods of preparation of the same food. The " decrement value " expresses in a similar fashion the decrease in body weight. Oats are usually taken as standard, and the conditions of the experiments are kept as comparable as possible. Such methods take no account of the composition of a food or of its assimilation, but yet give useful information concerning foods (*e.g.* white bread), which, though satisfactory in these respects, react badly on the animal. Normally, 8 female rats (of the same breed) 50 to 60 grms. in weight, were supplied daily with weighed quantities of food *ad lib.*, and the uneaten portion weighed back before feeding time on the following day. An abundance of water was provided, and this served to counterbalance the varying moisture contents of the samples of bread. The amount of food eaten was determined every day, and the body-weight every five days for a period of five months. For the secondary increment value experiments, however, the food was supplied so that a small amount of raw food was left over, whilst the cooked food was given in quantities insufficient to mask the change in increment value due to the cooking process. This was necessary when the rats were inclined to eat more of the food which had been cooked longest. Wholemeal bread (Klopfer) gave the highest increment value in these experiments, and was followed in order by grey and white breads. The increment value of white bread was high at first and then decreased, whilst that of Simons' bread was low at first, but increased gradually, and after 4 months proved superior to that of white bread. The rats fed on white bread died after 4 to 4½ months. In experiments on 14 animals (mixed sexes) of two breeds, with pure crust as food, the increment values were lower, but followed the same relative order. White bread crust produced a decrement value, and the Berlin roll (French or water roll) produced no increment value, and in the latter case death occurred after 1 month. Milk-loaf, however, had a higher increment value than the rolls. Graham and Schlüter breads (wholemeal) were intermediate in effect between the Klopfer and grey breads. From these experiments, and from the fact that rusks made from milk and sugar produced a steady decrement value and a relatively early death, it is concluded that the falls in the secondary increment values are due, entirely or principally, to the high degree of heating during the double baking. This inferiority of cooked food, unaccompanied by indications of the lack of vitamins, is confirmed by results obtained for other types of food by the authors and by Usuelli. The contradictory results of Scheunert and Wagner may be due to differences in experimental methods. J. G.

Nutritive Value of Bread, with Special Reference to its Content in Vitamin *B*. W. Cramer and J. C. Mottram. (*Lancet*, 1927, 1090–1094.)—The milling products of wheat have been subjected to a detailed investigation of

their vitamin *B* content by means of feeding experiments on rats. Wheat germ is very rich in vitamin *B* and equal in that respect to yeast ; 0·5 to 1 grm. of sterilised wheat germ per rat, added to 10 grm. of the basal vitamin *B*-free ration, ensured complete recovery from vitamin *B* deficiency. Vitamin *B* is contained in the various milling products of wheat grain in the following proportions, the vitamin *B* content of yeast being taken as 100:—Germ, 100 ; sharps or middlings, 50 ; bran, 33 ; and patent flour, 0. The vitamin *B* content of different kinds of bread depends upon the milling products used in the making of the bread. The vitamin *B* content of the amount of yeast added in the baking is too small to be responsible for a significant addition of the vitamin to the bread. The vitamin *B* content of different kinds of bread, as calculated from the amounts and the vitamin *B* content of the various milling products and yeast used in the making of the breads, corresponds closely to their vitamin *B* content, determined experimentally. Thus white bread made from white flour and yeast is so deficient in vitamin *B* that it has ceased to be a supply of this vitamin in our dietary. Wholemeal bread is rich in vitamin *B*, and bread made of one part of wheat germ and three parts of white flour is even richer. The latter differs from wholemeal bread in being free from bran, so that, contrary to what has been stated, richness in vitamin *B* is not necessarily dependent upon the presence of indigestible material. Mottram has published the erroneous and widely accepted statement that white bread contains an abundant supply of vitamin *B* and is not greatly inferior in this respect to wholemeal bread or germ bread. This statement was based on faulty experiments by Hartwell (*Biochem. J.*, 1924, **18**, 120), in which vitamin *B* was really supplied by butter. The " brownness " of bread is not a measure of its content in vitamin *B*. The significance of these results in relation to dietetics is discussed. The nutritive value of different kinds of bread, if treated as a practical problem of dietetics, must be considered in relation to the diet as a whole. Where the constituents of the diet, other than bread, are poor in vitamin *B* the nutritive value of white bread is definitely inferior to that of bread rich in vitamin *B*.

P. H. P.

Priority for the Antimony Trichloride Test for Vitamins. In the abstract on p. 652 of the November issue of THE ANALYST it should have been stated that the antimony trichloride test was devised by Carr and Price (*Biochem. J.*, 1926, **20**, 497), being based on observations originally made by Rosenheim and Drummond.—EDITOR.

Bacteriological.

Wood Preservation. Preservative Properties of Chlorinated Coal Tar Derivatives. L. P. Curtain and M. T. Bogert. (*Ind. Eng. Chem.*, 1927, **19**, 1231–1234.)—Chlorination of the hydrocarbon portion of coal tar creosote reduces its toxicity towards fungi which cause rotting in wood, but the mono- and di-chloro derivatives of certain coal tar phenolic substances are less volatile, less soluble in water, more resistant to oxidation, and much more toxic towards

the fungi than are the unchlorinated substances. The greatest increase in toxicity occurs when the cresols and xylenols are chlorinated, dichloroxylenol being the most toxic of all the substances tested. In the case of coal tar acids boiling above 270° C., chlorination results in only a slight increase in toxicity, but this is apparently due to the fact that the effect of chlorination is more than counterbalanced by a decrease in solubility in water. W. P. S.

The Pigment Produced by *Chromobacterium Violaceum*. J. Reilly and G. Pyne. (*Biochem. J.*, 1927, **21**, 1059–1064.)—Bacterial pigments have, in general, received very little attention, owing, no doubt, to the small amounts formed in most cases. The pigment produced by *Chromobacterium violaceum* has been isolated and examined. In the earliest stage of the work sterilised skim-milk was used as a medium for growth, but, owing to difficulties in filtering and in isolating the pigment in pure form from such a complex mixture, the milk was replaced by the simpler nutrient lactose broth. The total yield of pigment from 6 litres of lactose broth was about 60 mgrms. The solid pigment is a purple-black amorphous substance which decomposes, without melting, on heating. It is insoluble in water, chloroform and other chlorinated solvents, petroleum spirit, carbon disulphide and acetic acid. It dissolves in ethyl alcohol to a beautiful bluish-violet solution, in ether and in acetone to give purple solutions. Pyridine—the best organic solvent observed—contained, when saturated at ordinary temperature, only about 0·5 per cent. of the pigment. In alkaline solution it dissolves readily, and is very unstable, and some structural change seems to occur, since the addition of acid does not restore the original colour. Determinations have been made of the empirical formula, molecular weight, and nitrogen removable by the action of nitrous acid. The formula $C_{50}H_{59}O_{15}N_5$ is provisionally suggested for the pigment. P. H. P.

Bank Notes and Cholera. H. M. Jettmar. (*National Med. J., China*, 1927, **13**, 254 ; *Lancet*, 1927, **213**, 1112.)—As paper money is used almost exclusively as currency in North China experiments were made to determine how long bank notes contaminated with the cholera vibrio would remain infective. For this purpose a series of bank slips were contaminated by fingers which had been dipped in cholera stool. After varying periods the slips were put into tubes of peptone water, and from these further cultures were made. The serological characteristics of the vibrios cultivated from the infected bank notes were then compared with that of organisms from the original stool. It was found that they remained alive on bank notes for four hours, and that they retained the same cultural and serological characteristics as the original strain. Their lives were shortened by lack of moisture on the notes and by exposure to sunlight.

Toxicological and Forensic.

Carbon Monoxide as a Tissue Poison. J. B. S. Haldane. (*Biochem. J.*, 1927, **21**, 1068–1075.)—Warburg (*Biochem. Z.*, 1926, **177**, 471) found that when

the proportion of carbon monoxide to oxygen in a gas mixture was raised to about 5 the oxygen consumption of yeast and of a coccus was diminished. He concluded that carbon monoxide combined with a catalyst in the cells, with which oxygen must combine before it can oxidise other substances, and that this catalyst was probably an iron compound analogous to haemoglobin. Warburg's observations have been extended to moths, seeds and rats. The movements of a moth and the germination of cress seed are inhibited by carbon monoxide. The greater the partial pressure of oxygen, the more carbon monoxide is required. The general behaviour of the organism, and not its oxygen consumption, was observed. The symptoms were the same qualitatively, whether due to lack of oxygen or combination of this with carbon monoxide poisoning. In the absence of carbon monoxide the moths are motionless in mixtures containing less than 1·0 per cent. of oxygen, normal in over 2 per cent. In presence of 80 per cent. of carbon monoxide about 8·4 per cent. of oxygen is needed for mobility, and 14 per cent. for normal behaviour, whilst in intermediate concentrations of carbon monoxide intermediate amounts of oxygen are required. Rats living on oxygen dissolved in their blood in presence of sufficient carbon monoxide to combine with almost all their haemoglobin are killed by the addition of more carbon monoxide, which must affect some substance in their tissues. It is concluded that cells contain a catalyst of oxidation which is poisoned by carbon monoxide. Its affinity for carbon monoxide is determined in the case of the moth. Its affinities differ in different species, and perhaps in different tissues. As such catalysts have now been found in bacteria, yeasts, higher plants, insects and mammals, they are presumably present in the majority, if not all, of aerobic organisms. P. H. P.

Action of Carbon Monoxide on Certain Oxidising Enzymes. M. Dixon. (*Biochem. J.*, 1927, **21**, 1211–1215.)—Various workers have shown that carbon monoxide in its inhibitory action is analogous to the cyanides, yet the action of cyanide is usually the more marked, and fairly large amounts of carbon monoxide are necessary to produce a large inhibition. The enzymes of the aldehyde and oxidase type (the dehydrases) had not been tested with carbon monoxide, and, since the aerobic oxidation by certain of these systems is inhibited by cyanide, whilst that of others is completely unaffected, it was thought to be of interest to determine whether carbon monoxide would show a similar distinction in behaviour towards these systems. The aldehyde (and xanthine) oxidase of milk and the succinoxidase of muscle were chosen for investigation. Carbon monoxide has no inhibiting effect on the aerobic oxidation of aldehyde or hypoxanthine by the milk oxidase, as is the case with cyanide, or of succinic acid by the succinoxidase of muscle. Its action thus differs from that of cyanide, which strongly inhibits the latter reaction. If it is assumed that cyanide inhibits the oxidation of succinic acid by inactivating an oxygen-transporting substance, and that carbon monoxide inhibits tissue respiration by acting in the same manner, it follows from these results that different oxygen-transporters must be concerned in the two cases.
P. H. P.

Organic Analysis.

Identification and Determination of Aldehydes and Ketones. S. Veibel. (*Bull. Soc. Chim.*, 1927, **41–42**, 1410–1416.)—The method involves the determination of one of the three nitrogen atoms introduced into the molecule on the formation of the semicarbazone. Between 1 and 2 grms. of the semicarbazone are boiled under a reflux condenser for half an hour with 100 c.c. of water and 10 c.c. of concentrated sulphuric acid, followed by the addition of 0·4–0·5 grm. of potassium iodate, and the mixture is boiled until the colour of the iodine has disappeared. (In the case of only slightly soluble semicarbazones a more concentrated sulphuric acid may be used.) The mixture is diluted with 200 c.c. of water, 50 c.c. of about 10 *N* sodium hydroxide added; distillation as for a Kjeldahl determination is carried out, and 150 c.c. of distillate collected. This method was found to be accurate within 0·6 per cent. for 11 semicarbazones, except in the case of *o*-nitrobenzaldehyde, which decomposes. Details are given for the preparation of the semicarbazones, together with a table of m.pts. obtained by a quick heating method for the recrystallised substances. D. G. H.

Determination of Cyanogenetic Glucosides. L. R. Bishop. (*Biochem. J.*, 1927, **21**, 1162–1167.)—As a preliminary to a study of the behaviour of the cyanogenetic glucoside (prulaurasin) in the leaves of cherry laurel (*Prunus laurocerasus*) an attempt was made to find a method for the determination of the glucoside in a single leaf or half leaf. Various methods are discussed, and most of them considered unsatisfactory. In particular, the distillation method has been found to give untrustworthy results. Two new methods are described in which the hydrogen cyanide of the glucoside is liberated by enzymic action and carried over into potassium hydroxide solution by a current of air. The hydrogen cyanide absorbed is titrated in the alkaline liquid with silver nitrate (Liebig's method). This method is considered satisfactory if certain details of procedure are adopted. The results by it agree with those by a simpler "water" method which was adopted for use in plant physiological investigation. The details of apparatus and procedure used in this are described. Readings may be obtained with accuracy which correspond to 0·00001 grm. of hydrogen cyanide; thus the quantities in a single leaf (0·001–0·005 grm.) can be determined with sufficient accuracy. With amygdalin in amounts which correspond to those of prulaurasin in a cherry laurel leaf the error is 1 to 3 per cent. The hydrogen cyanide contents per 100 grm. of fresh weight of the corresponding halves of leaves also agree within these limits. It is open to question whether the hydrogen cyanide, as measured in this way, is representative of the cyanogenetic glucoside in the plant, but it seems probable. It is hoped to publish elsewhere data obtained by this method on cherry laurel leaves. P. H. P.

Method for Direct Methylthiolation and its Application in the Preparation of some Substituted Thioanisoles. H. H. Hodgson and F. W. Handley. (*J. Soc. Chem. Ind.*, 1927, **46**, 435–436T.)—Although sodium disulphide reacts

with both the chlorine atoms in 2:4-dichloronitrobenzene to form 1:1′-dinitro-2:2′:4:4′-diphenyl tetrasulphide, only one chlorine atom is replaced by methyl mercaptan in presence of alkalis, to give 3-chloro-6-nitrothioanisole. The latter change is of general application to chloronitrobenzenes with labile chloro- or nitro-groups, and may be effected by passing the gases evolved from a warm mixture of methyl sulphate and 10 per cent. aqueous sodium hydrosulphide solution into an alcoholic solution or suspension of the chloronitrobenzene in presence of sodium hydroxide or, in some cases, of potassium carbonate. In this way excellent yields were obtained of 3-chloro-6-nitrothioanisole from 1-chloro-3:4-dinitrobenzene, and of 2-nitro-, 4-chloro-2-nitro-, 2-nitro- and 4-nitro-methoxy-, and 2:4-dinitro-thioanisoles from 2-chloro- and 2:5-dichloro-nitrobenzenes, 3-chloro-4- and-6-nitroanisoles, and 1-chloro-2:4-dinitrobenzene, respectively. An exception is furnished by 4-chloronitrobenzene, which is converted mainly into 4:4-dichloroazoxybenzene ; as the latter results also when the methylthiolating agent is absent, the velocity of reduction is evidently much greater than that of replacement. T. H. P.

The α-Elaeostearic Acid of China Wood Oil (Tung Oil). J. Böeseken. (*Rec. Trav. Chim.*, 1927, **46**, 619–634 ; *cf. id.*, 1926, **46**, 161.)—Substances with isolated and conjugated double bonds may be distinguished by their rates of reaction with Wijs' reagent. In the latter case two of the carbon atoms become saturated in less than 15 minutes, and the remaining two after more than 5 hours, whilst in the former both linkages are saturated in less than an hour. Since the rate of reaction depends on the concentration, the Wijs iodine value is found to vary with the amount of substance taken. A brown coloration which appears rapidly in the reagent owing to the liberation of iodine, is characteristic of the presence of a conjugated linkage. Hydrogenation under pressure at 115° C., with a nickel catalyst, halogenation by the above method, and oxidation (*vide infra*) of tung oil, or of α-elaeostearic acid, or its esters have shown that the acid contains three conjugated double bonds between the carbon atoms 9 and 14, and that these react according to Thiele's principle, *i.e.* the carbon atoms 9 and 14 are first saturated, then the atoms 10 and 13, and finally the atoms 11 and 12. The acid rapidly forms a tetrabromide with bromine, even below 0° C. ; a third molecule of bromine does not normally enter into combination, but may be induced to do so with the aid of ultra-violet radiation. Double linkages may be studied quantitatively by oxidation in stages with increasing quantities of peracetic acid (a mixture of commercial (60 per cent.) perhydrol and glacial acetic acid), free from acetyl peroxide, and the method has been applied to unsaturated substances of known structure, and to α-elaeostearic acid and its tetrabromide (tetrabromoleic acid). J. G.

Determination of Sulphur and Sulphur Derivatives of Hydrocarbons in Naphtha Solutions and in Petroleum Distillates. W. F. Faragher, J. C. Morrell and G. S. Monroe. (*Ind. Eng. Chem.*, 1927, **19**, 1281–1284.)—A method is given for the determination of sulphur present as hydrogen sulphide,

elementary sulphur, mercaptans, sulphides, disulphides, and (by difference) residual sulphur. *Hydrogen sulphide:*—This is determined as cadmium sulphide by precipitation with a 10 per cent. cadmium chloride solution containing 0·3 per cent. of free hydrochloric acid (HCl). *Elementary sulphur:*—The sample is shaken with metallic mercury, and the mercury sulphide is separated by filtration; lamp determinations before and after this treatment give, by difference, the percentage of elementary sulphur. *Mercaptans:*—After the removal of hydrogen sulphide and sulphur the sample is dissolved in benzene and shaken with alcoholic sodium plumbite solution; the lead mercaptides dissolve in the alcohol, and a lamp determination on the oil gives, by difference, the sulphur in the mercaptans. An alternative method consists in treating the benzene solution of the sample with basic lead acetate and separating the lead sulphate by filtration. The acid remaining in the solution, and that in the washings, are titrated, and the mercaptans equivalent to the acid which combined with the lead mercaptides are calculated. *Disulphides:*—The sample, free from hydrogen sulphide and sulphur, is heated under a reflux condenser with dilute hydrochloric acid and zinc; the disulphides are reduced to mercaptans, and these are removed by treatment with alcoholic sodium plumbite solution. A lamp determination gives the sulphite and residual sulphur in the reduced oil. The sulphur present as disulphides is found by subtracting the mercaptan sulphur from the combined disulphide and mercaptan sulphur. *Sulphides:*—The oil at this stage is treated with mercurous nitrate, which precipitates the sulphides. A lamp determination gives the sulphur present as residual sulphur, and the sulphide sulphur is found by difference.

W. P. S.

Separation of the Components of Petroleum. VI. The Action of Acetic Acid. P. F. Gordon and J. Merry. (*J. Soc. Chem. Ind.*, 1927, **46**, 429–432T.)—In order to avoid loss by volatilisation during the fractional precipitation of constituents of crude petroleum, it is necessary to remove light components beforehand by fractional solution in certain solvents. The behaviour of untopped crude Persian petroleum, of d^{16}, 0·839, towards glacial acetic acid is here described. The portion soluble in this acid contains both the lightest components, which mostly volatilised during the further fractionation, and the heaviest oils, but no wax. The acid-insoluble oils are of intermediate specific gravity and contain all the wax other than that contained in the asphaltic portion, which is also insoluble in the acetic acid; the relatively low specific gravity may, therefore, be attributed to the presence of the wax. This suggestion appears to be confirmed by the distinctly higher flash-points of the acid-insoluble oils. T. H. P.

Modified Method for Determination of the Copper Number of Paper. B. W. Scribner and W. R. Brode. (*U.S.A. Dept. of Commerce, Techn. Paper No.* 354.)—With high-grade rag-fibre bond papers, the heavy glue surface sizing and the hard finish render it impossible to obtain accurate values for the copper number unless the papers are finely ground before testing. The grinder recommended is similar to that used by G. Hall and consists of a 12-inch rotor, fitted with

six serrated steel lugs and revolving within a steel housing serrated inside; the rotor teeth clear the stator teeth by one-sixteenth of an inch. The rotor makes 1700 revolutions per minute, the speed of the rotor teeth being over a mile per minute. The strips of dry paper are passed through a slot into the rotor space near its centre, and are carried by centrifugal force into the space between the teeth, where they are disintegrated and ejected through a screen into a receiver below. Such treatment does not heat the paper, this being an essential point. When the disintegrated paper is titrated by the molybdenum method, the blue colour of the molybdenum is readily removed from the paper, whereas this is almost impossible if cut pieces are used.

The Fehling solution used is prepared by the careful addition of a solution of 277·3 grms. of crystallised copper sulphate in 4 litres of water to an equal volume of a solution of 150 grms. of sodium hydroxide and 500 grms. of sodium potassium tartrate in 1·5 litres of water. If kept mixed, the Fehling solution should be protected from air and light, and if used some time after being mixed, a little should first be diluted and heated to boiling for a few minutes to see if it remains clear. The molybdophosphoric solution is made by dissolving 100 grms. of sodium molybdate (43 per cent. of Mo) and 75 ml. of phosphoric acid (83 per cent.) in a solution of 275 ml. of concentrated sulphuric acid in 1750 ml. of water. To prepare the potassium permanganate solution, 6·25 grms. of the salt are dissolved in 1 litre of water, and the solution filtered through asbestos in a Gooch crucible and diluted to 4 litres. This is standardised by dissolving 1·5 to 2·0 grms. of sodium oxalate in 150–200 ml. of water at 80–90° C., adding 6·5 ml. of sulphuric acid (1 : 1), and, with constant and vigorous stirring, adding the permanganate, at first not faster than 10–15 ml. per minute and finally dropwise; the temperature of the liquid should not fall below 60° C.

In the determination of the copper value, a mixture of 25 ml. of the copper sulphate solution, 25 ml. of the alkaline tartrate solution, and 125 ml. of hot water is heated to 100° C., and 1·5 grms. of the ground paper introduced. The liquid is heated for about 30 minutes, with gentle stirring, in a 500 ml. wide-mouthed Erlenmeyer flask in a bath of 30 per cent. calcium chloride solution at 110–115° C., so that the liquid is kept boiling gently. The hot solution is filtered through a 5·5 cm. filter-paper (No. 40 Whatman paper is suitable) in a Büchner funnel, and the residue washed with 250 ml. of hot water. The paper fibre and filter-paper are placed in a 250 ml. beaker with 25 ml. of the molybdate solution, 25 ml. of water being added after a short time, and the mixture stirred to disintegrate the paper. The solution is then filtered as before, and the residue washed with about 200 ml. of cold water to remove the blue molybdenum colour. The blue solution is titrated with the permanganate solution, the number of ml. of this solution used is multiplied by the calculated factor, and the result divided by the number of grms. of sample used. The factor represents the number of cgrms. of copper in the cuprous oxide, equivalent in reducing power to the oxidising power of 1 ml. of the permanganate solution, the copper number being the number of cgrms. of copper in the cuprous oxide reduced from the cupric sulphate by 1 grm. of the paper. T. H. P.

Damar Penak. R. W. Blair and F. E. Byron. (*Malay. Forest Rec.*, 1926, **4**, 1–2.)—Damar Penak is the resin produced by *Balanocarpus Heimii*, King, and is one of the most valuable of the resins produced in the Federated Malay States. Analysis of five grades of resin showed little difference between them; they were as follows:—

Grade.	Pale.	Yellow.	Amber.	Dust (coarse).	Dust (fine).
Moisture per cent. ..	0·82	0·86	0·84	1·00	1·05
Insoluble in chloroform, per cent.	0·05	0·02	0·03	1·32	1·65
Resin (by difference), per cent.	99·13	99·12	99·13	97·68	97·30
Ash	0·05	0·02	0·02	1·20	1·25
Melting point, °C. ..	95°–105°	95°–105°	95°–105°	82°–95°	85°–95°
Acid value	37·3	32·1	31·6	36·3	39·9
Saponif. value	51·5	50·4	49·5	52·1	54·9
Iodine value	104·3	103·4	100·7	99·7	98·4

Experiments to determine the effect of storage on the acid value showed that it increases with the age of the sample, but that darkness and light have no apparent effect. *Dead damar* is the opaque white exudation obtained from some of the *Balanocarpus Heimii* trees. The amount varies with the district, but is never high. A sample was sorted into (1) opaque pieces (chiefly tears), (2) clear pieces with opaque centres or striations (chiefly lumps), and (3) clear pieces, chiefly lumps containing a large amount of impurity in the resin itself; and the following figures were obtained:—Acid value: (1) 32·4, (2) 27·8, (3) 26·6; average 29·8; per cent. loss at 105° C.: (1) 0·4, (2) 0·2, (3) 8·0; average 1·5; per cent. insoluble in chloroform: (1) 1·2, (3) 0·02; average 0·5. D. G. H.

Inorganic Analysis.

Ozone in the Atmosphere. J. Levine. (*Compt. rend.*, 1927, **19**, 962–963.)—The author's previous observation that the proportion of ozone in air is connected with the presence of atmospheric depressions, is confirmed by Dobson (*Proc. Royal Soc.*, series A, **110**, 660, and **114**, 521), who had simultaneous observations made at Oxford, Valencia, Lerwick, Abisko, Lindenberg and Arosa, and found that the proportion of ozone is higher in depressions than in anticyclones. It is suggested that the first step in solving the problems involved is the spectroscopic analysis of the higher atmosphere, but a study of nitrogen and hydrogen condensations is also required. D. G. H.

Determination of Copper Oxide and Metallic Copper in Mixtures of the Two. W. D. Bonner and B. D. Kaura. (*Ind. Eng. Chem.*, 1927, **19**, 1288–1289.)—The method described depends on the fact that copper oxides dissolve rapidly in sodium cyanide solution, whilst metallic copper dissolves much more slowly. About 0·5 grm. of the sample is treated with 50 c.c. of 0·5 *M* sodium cyanide solution, and the mixture is shaken occasionally during two hours. The

mixture is then filtered, the filtrate is treated with an excess of nitric acid, boiled, evaporated to dryness, and the residue ignited. This residue is dissolved in nitric acid, the iron is precipitated with ammonia, removed by filtration, and the copper (representing that present as oxides) is determined iodimetrically in the filtrate. The portion of the sample which is insoluble in the cyanide solution is dissolved in nitric acid, and the copper determined in the same way after removal of the iron. W. P. S.

Determination of Bismuth by Means of Oxyquinoline. R. Berg. (*Z. anal. Chem.*, 1927, **72**, 177–179.)—Bismuth is precipitated by oxyquinoline from weakly acid acetate (sensitiveness 1 : 300,000) or ammoniacal tartrate (1 : 217,000) solutions as a crystalline, orange-yellow complex, $Bi(C_9H_6ON)_3.H_2O$, stable at 100° C., becoming anhydrous after some heating at 130° to 140° C. The compound may be weighed after being dried at 100° C. (factor for Bi, 0·3171) or at the higher temperature (factor, 0·3260); or the bismuth is more conveniently determined bromimetrically (*cf.* ANALYST, 1927, **52**, 302, 431, 494, 611) after solution of the precipitate in 10 per cent. hydrochloric acid containing tartaric acid. The nitrate solution, after addition of a sufficiency of tartaric acid, is neutralised against phenolphthalein with ammonia or sodium hydroxide, faintly acidified with acetic acid, and treated with sodium or ammonium acetate (1 to 2 grms. for 0·05 grm. Bi). The prepared solution is precipitated at 60° to 70° C. with a cold saturated alcoholic or acetonic solution of the reagent, which is added all at once; an excess is immaterial. After heating to incipient boiling, filtration takes place through a Jena glass crucible No. 7, for gravimetric or volumetric determination. (1 c.c. 0·1 *N* bromate-bromide solution = 0·001743 grm. Bi.) To precipitate metals at boiling heat with oxyquinoline and titrate the excess of precipitant in the filtrate is not accurate on account of the volatility of the reagent, high results being obtained. W. R. S.

Volumetric Determination of Bismuth. H. Kubina and J. Plichta. (*Z. anal. Chem.*, 1927, **72**, 201–207.)—The weakly acid chloride solution is treated with about 0·100 grm. of aluminium dust and 40 c.c. of 3 *N* potassium hydroxide, left to stand for 20 minutes, with occasional shaking, slightly diluted, and boiled till the sponge is coagulated, the aluminium completely dissolved, and the supernatant liquor quite clear. The liquid is filtered, and the sponge washed and dissolved in 15 c.c. of ferric chloride solution (200 grms. of $FeCl_3$ and 250 c.c. of strong hydrochloric acid per litre) by slight warming. Sulphuric acid (1 : 5 water; 15 to 20 c.c.) is added, as well as phosphoric acid and manganous sulphate, and the liquid titrated with permanganate. A blank should be run with the same quantity of aluminium dust. Other metals precipitated by aluminium (*e.g.* lead) interfere. Reissaus' method (ANALYST, 1927, **52**, 250) was tested, and found to give good results if all the operations are carried out in an atmosphere of carbon dioxide, and the bromate solution is added quickly. (*Cf.* also ANALYST, **1927**, **52**, 659.) W. R. S.

Determination of Selenium and Tellurium by the Sesquichloride of Titanium. O. Tomicek. (*Bull. Soc. Chim.*, 1927, **41–42**, 1389–1399.)—Hexavalent tellurium may be determined, with an accuracy of 0·1 per cent., by adding sufficient hydrochloric acid to the tellurate solution (quite free from nitrogen and nitrates) to ensure the acid concentration being about 10–20 per cent. The liquid is well mixed by passing through it a current of carbon dioxide, and a solution of 0·1 *N* titanium chloride added until a sudden drop in potential indicates complete reduction of the tellurium. One c.c. of 0·1 *N* titanium chloride is equivalent to 0·002125 grm. of tellurium. Sulphuric acid may also be used in the reaction, but with tartaric acid reduction is not complete. In the presence of dilute sulphuric acid, two drops in potential occur, the first corresponding approximately to a reduction from hexa- to tetravalent tellurium, and the second to complete reduction. The gravimetric determination of tellurates by means of titanium chloride in a strong acid medium is not quite so accurate as the volumetric method, owing to the easy oxidation of the tellurium. Tetravalent tellurium may be titrated in a similar way to hexavalent, or, as in the case of tetravalent selenium, in the presence of tartrates. In order to complete the reduction of selenites the titration with titanium sesquichloride must be made at 70–80° C., and the concentration of hydrochloric acid at the beginning be about 10 per cent. D. G. H.

Determination of Iodine in Soil, Salt and Water-Concentrates. R. McCarrison, C. Newcomb, B. Viswanath and R. V. Norris. (*Ind. J. Med. Res.*, 1927, **15**, 211–233.)—The existing methods for this determination are criticised, and the following suggested:—(1) The mixed, ground and sieved sample (40 grms.) is dried on a water-bath with 60 c.c. of a 10 per cent. solution of potassium hydroxide, and again powdered and heated at about 400° C. for 1 hour in a nickel dish, which is 10 cm. in diameter and protected by an open iron cylinder, 8 cm. high. The object is to destroy the bulk of the organic matter and to oxidise the iodide with a minimum loss of iodine. The brown-coloured, hot water extract (about 300 c.c.) is evaporated to 125 c.c. clarified with 7 grms. of animal charcoal and re-evaporated to 30 c.c. It is acidified with 5 *N* sulphuric acid, filtered into a separating funnel, and allowed to stand for 30 minutes with 1 c.c. of 0·1 *N* arsenious oxide. The iodine is then liberated with 10 drops of nitrose (prepared by passing nitrous fumes, from the interaction of starch and nitric acid, into sulphuric acid) and separated by the addition of carbon disulphide (2 c.c.), the colour of which may then be matched against a standard prepared under similar conditions. The iodine is recovered to the extent of 70 per cent. The water phase should be tested for substances which inhibit the reaction, by the addition, and subsequent determination, of known amounts of iodine. For salt, 10 grms. are dissolved, acidified, filtered and the residue washed. The filtrate and washings are then treated with arsenious oxide and nitrose in the above manner. Common salt gave no reaction, but the method could detect 2 parts per million, and the iodine recovery was 100 per cent. (2) The water concentrate or prepared sample of soil (50 grms.) is saturated with an 8 per cent. solution of pure sodium hydroxide and dried and

ignited for 30 minutes as in method (1). A slightly coloured extract (250 c.c.) should be obtained which is evaporated and again ignited for 10 minutes, acidified and filtered, and the iodine liberated as in method (1). The non-aqueous layer is separated and washed with water and a saturated solution of sodium acetate, and titrated against $N/1270$ sodium thiosulphate solution. The titration is sensitive to within 0·1 c.c., and 0·2 parts per million of iodine are detectable. Kendall's method was adopted for salt (*J. Biol. Chem.*, 1920, **43**, 149). (3) The Carter method (*loc. cit.*) gives low results for soils rich in iron, and it is preferable to heat the soil (in a nickel boat placed in a silica tube) for 1 hour at 900° C. in a stream of oxygen. The products of combustion are collected in 15 c.c. of a 15 per cent. solution of sodium hydroxide and the iodine determined by method (1). Results are tabulated for 41 Indian soils which contained iodine in quantities varying from 0 to 40 parts per million. (*Cf.* Hercus, Benson and Carter, *J. Hygiene*, 1924, **24**, 321.) J. G.

Physical Methods, Apparatus, etc.

Formation of Nitrite from Nitrate as a Measure of Ultra-Violet Intensity. A. E. Gillam and R. A. Morton. (*J. Soc. Chem. Ind.*, 1927, **46**, 415–417T.)—A 0·1 *N* solution of potassium nitrate buffered to P_H 9·4 may be used to measure the bactericidal efficiency of a source of ultra-violet light, since it is unaffected by visible light, and is most effective for the wave-lengths concerned (275 to 300 $\mu\mu$). The potassium nitrite produced photolytically is determined colorimetrically by the Griess-Ilosvay method. An old mercury-vapour lamp was found to produce the same effect as that of an iron-nickel arc. The limiting ultra-violet wave-lengths transmitted by vitaglass and window glass were 275 $\mu\mu$ and 325 $\mu\mu$, respectively. The vitaglass screen rendered the reaction too slow for general use as a guide to the photo-synthetic formation of vitamin *D* from ergosterol (effective range 270 to 305 $\mu\mu$), but the method is recommended for the detection of the first stages of ageing in mercury lamps. (See following abstract.) J. G.

Comparison of Certain Methods for Determining the Ultra-Violet Intensity of a Light Source. A. E. Gillam and R. A. Morton. (*J. Soc. Chem. Ind.*, 1927, **46**, 417–420T.)—The various chemical methods for the determination of ultra-violet intensity are described and have been studied for various types of mercury lamps by means of glass and vitaglass screens which were used to absorb certain portions of the spectrum. The value of any one chemical method depends entirely on the reaction to be controlled, but such methods may conveniently be used to follow the deterioration of lamps. The success of the hydrogen peroxide method (*J. Amer. Chem. Soc.*, 1923, **45**, 1050) depends on the absence of impurities which may have a stabilising or opposite effect on the rate of decomposition. The acetone and methylene blue reaction is simple and quick, but does not possess a high degree of accuracy. The nitrate method, which gives results in agreement with the methylene blue method, and the carbon tetrachloride method (see preceding abstract and *Compt. rend.*, 1914, **159**, 314, respectively), are most

fitted to measure bactericidal rays, though the presence of carbon disulphide in carbon tetrachloride renders the latter method slow. Variants of the oxalic acid and uranyl salt decomposition method are also described. Such methods are most suitable for the middle ultra-violet region, and to obtain data concerning the deterioration of lamps. Of the lamps tested, the K.B.B. type had the highest efficiency, but this decreased rapidly after usage. J. G.

Analysis of Mixtures of Aliphatic Nitrates by Means of the Refractometer. W. H. Rinkenbach. (*Ind. Eng. Chem.*, 1927, **19**, 1291–1292.)—The refractometric method described is particularly applicable to mixtures of nitroglycerin and ethyleneglycol nitrates. The refractive indices at 15° C. of the pure substances are as follows:—Nitroglycerin, 1·3751; ethyleneglycol dinitrate, 1·4491; diethyleneglycol dinitrate, 1·4536; and a mixture of 70 per cent. of nitroglycerin and 30 per cent. of nitropolyglycerin, 1·4767. Tables are given showing the refractive indices of mixtures of nitroglycerin and ethyleneglycol dinitrate; curves plotted from these values show that the points are uniformly below the straight line joining the values for the pure compounds. Whilst the method yields satisfactory quantitative results with the pure compounds, it cannot be applied to the nitroglycerin, etc., extracted from dynamites, owing to the interference of oils, for the removal of which there appears to be no suitable method. It will, however, serve for the detection of ethyleneglycol nitrates in such mixtures.

W. P. S.

Determination of the "Wettability" of a Solid by a Liquid. E. E. Bartell and H. J. Osterhof. (*Ind. Eng. Chem.*, 1927, **19**, 1277–1280.)—A method for the determination of the degree of wetting of a solid by a liquid is based on the measurement of the pressure with which one liquid will displace another from a powdered material which has been compressed within a cylinder. The apparatus consists of a brass cylinder open at both ends and provided with closely fitting perforated plungers which can be inserted in these ends. At the other end of each plunger a glass capillary tube is sealed, one tube serving as a displacement indicator, the other being connected, by a ground-glass joint, with a manometer. Powdered solid (*e.g.* silica or carbon black), thoroughly wetted by the liquid to be displaced, is packed into the cylinder by applying hydraulic pressure (150 atmospheres) to the plunger at the manometer end of the cylinder. When about 3 inches of material has been packed in the cylinder, some of the powder wetted with the displacing liquid is also packed into the cylinder. While the cylinder is still in the press, the plungers are secured by steel yokes and bolts, the pressure end is then connected with the manometer, and the displacing (or equilibrium) pressure is measured. The adhesion tension between a solid and a liquid phase can be determined from the displacement pressure and expressed in terms of absolute units, since the adhesion tension of such a system gives a measure of the degree of wetting. The adhesion tension for silica in water and in aniline is high, being 82·8 and 82·0 dynes per cm., respectively, indicating a high stability for the suspension in both these liquids. Experiment shows that silica suspensions are stable in these

liquids, there being no tendency to flocculation. Adhesion tensions for silica in carbon tetrachloride, hexane, and benzene are 40·7, 42·1, and 52·3 dynes per cm., and there is a decided tendency to flocculation in each of the cases. Examples are also given showing the relation of adhesion tension to the stability of suspensions in varnish and lacquer systems. W. P. S.

Mercury Oscillating Pump. J. T. Donnelly, C. Hamilton Foott, H. Nielsen, and J. Reilly. (*J. Soc. Chem. Ind.*, 1927, **46**, 437–438T.)—The continuous circulation of relatively large volumes of gas in a closed system over long periods of time may be readily effected by means of a simple mercury oscillating pump, driven by an ordinary laboratory suction pump. The free leg of the U-tube containing the mercury is closed by a chamber connected by a tap to the suction pump. Into the dome of this chamber is ground a glass valve joined by a glass stem to a glass float. At the bottom of a stroke the valve is closed, so that a partial vacuum is created in the chamber, into which the mercury rises. When the level of the mercury reaches a certain height, the upward pressure of the mercury on the float opens the valve and thus breaks the partial vacuum. The mercury falls again, the valve closing as before, and so on. A by-pass across the inlet and outlet valves is found unnecessary. By careful adjustment of the tap and the quantity of mercury run into the U-tube from the reservoir, any speed of gas circulation within the limits of the pump may be maintained. Moreover, if the mercury reservoir is left open to the U-tube, the pump will automatically adjust itself to a positive or negative alteration of half an atmosphere in the pressure in the closed system.

For smooth and reliable working, the ratio of the cross-sectional areas of the valve and the float should not exceed 1: 10. To cause the valve to seat properly and to reduce friction between the float and chamber, the internal diameter of the chamber is made about 0·8 cm. greater than the external diameter of the float, and three small teats are blown on the float. The float-chamber may be made in two parts, furnished with ground edges, and bound together with adhesiv tape. Suitable dimensions are: overall height of U-tube, 50 cm.; bore of U-tube, 0·8 cm.; distance from bottom of float to top of chamber, 22 cm.; bore of chamber, 2·4 cm.; external diameter of float, 1·6 cm. The maximum and minimum circulations of such a pump against a pressure of 9 cm. of water are respectively 66 and 0·15 litres per hour; against 16 cm. of mercury the circulation is 5 litres per hour.

T. H. P.

Reviews.

ELEMENTARY PRACTICAL PHYSICAL CHEMISTRY. By J. F. SPENCER, D.Sc., F.I.C. Pp. viii + 263. London: G. Bell & Co. 1927. Price 5s.

This volume is one contained in Messrs. Bell's Natural Science Series, and is intended as a textbook for the use of junior students. Detailed descriptions are given of over one hundred experiments, both qualitative and quantitative, including a wide range of subjects, of which the principal are the use of thermostats, vapour density, melting points and other thermal determinations, molecular weight, solubility, mass action, electrolysis, electrical conductance, transition temperatures, and spectrographic and polarimetric measurements. The treatment of the subject-matter is excellent, all essential details being given without redundant matter, and, where necessary, brief expositions of the theory underlying the practical work are included. The apparatus described is as simple as is consistent with the production of accurate results, and the use of highly complicated and expensive apparatus is avoided as far as possible.

The general style of the text indicates an extensive knowledge on the part of the author of the requirements of the junior student, but, on the other hand, it seems a somewhat daring procedure to place the adjustment of a Beckmann thermometer in the hands of the average senior schoolboy, as indicated on page 80.

Much care has been expended upon the production of this work, and the few errors noted are of little importance. These include a contraction of the page headings of Chapter IV, in which "Weight of dissolved substances" actually refers to molecular weight; a typographical error on p. 233, where "blend" appears instead of "blind"; and a *converging* beam of light leaving the lens in Fig. 80, whereas in the text this is described as "parallel."

Taken as a whole, the volume is a valuable addition to our academic literature, and its clarity and accuracy will enable the conscientious student to acquire considerable experience in manipulation and a useful knowledge of the fundamental laws of chemistry in an interesting manner.

T. J. WARD.

THE INDUSTRIAL CHEMISTRY OF THE FATS AND WAXES. By T. P. HILDITCH, D.Sc., F.I.C., with an Introduction by E. FRANKLAND ARMSTRONG, D.Sc., LL.D., F.R.S. Pp. xv + 461. London: Baillière, Tindall & Cox. 1927. Price 18s.

The production of a book on fats which has the appearance and qualities of a scientific treatise rather than a collection of abstracts and miscellaneous recipes is a fact which argues well for the future history of this subject, but, possibly, little less could be expected from a writer who is Hilditch, and who is also the Professor of Industrial Chemistry in the University of Liverpool.

The first 146 pages give a short account (methods of analysis, tables of results, composition of natural products) of the material usually found in ordinary text-books on oils. In this short space full details cannot, of course, be given, but the amount of information contained therein is surprising, being quite sufficient for any but the specialist in this branch. It is no exaggeration to say that this is the best short account of the present position of the chemistry of oils that we have; it would be difficult to suggest anything that should be added or deleted.

The remainder of the volume contains a series of discussions upon the refining and treatment of oils, fats and waxes, and their various applications in industry. The range of the subjects included is extremely wide, embracing as it does, operations as far apart as the preparation of synthetic fatty acids, the manufacture of linoleum and the production of lubricants. In dealing with a series of subjects of this nature there is always a great temptation for a writer to give undue prominence to subjects in which he himself is especially interested; there is no evidence of any such sub-conscious prejudice in this volume. The length of treatment of each subject appears to be quite in proportion to its industrial importance, and the book, as a whole, contains the strongest evidence of painstaking preparation and sound judgment going to the preparation of a well-balanced work.

Considering the amount of material, errors are remarkably few; they are also of little importance. The statement on page 246, however, that "This renders the Reichert-Meissl value practically conclusive," shows that Professor Hilditch has not spent many anxious moments in deciding precisely what, if anything, should be done in the case of a butter, the fat of which gave a Reichert value of, say, 23·1, or he would not be so cheerfully optimistic. Like so many other analytical servants, the Reichert process is a most excellent slave, but, even in the British Empire, it must never be given its freedom or even a possibility of escape.

The author draws attention to the, in his opinion, unwarranted prejudice which has been shown against the employment of hydrogenated fats in edible fats. That they have their uses no one will deny—even if it is only to keep in check the prices of natural fats—but it would seem that their choice for edible purposes should not be left entirely to the quite proper commercial instincts of the manufacturers, and that their use in this direction should be suitably controlled.

This book, which is easy to read, and which is attractive in appearance, will be read with the greatest of interest by all those having even a remote connection with the subject, and these, having read, are likely to return again and again, so converting a charming text-book into a valuable work of reference.

G. D. ELSDON.

PHOTOGRAPHY: ITS PRINCIPLES AND PRACTISE. By C. B. NEBLETTE, A.R.P.S. Pp. xviii + 644. London: Chapman & Hall, Ltd. 1927. Price 30s.

To summarise the wide field of general photography, its history, appliances,

optics, theories and processes, with the chemistry and physical chemistry underlying these, within the limits of 644 pages is an undertaking of no mean difficulty, but much care and patience on the part of the author has produced, with conspicuous success, a volume of judiciously selected and well-arranged subject-matter.

This book is primarily intended for the use of the advanced student, but is admirably adapted to the requirements of any serious photographer in whatever branches of the subject he may be interested, the simple style of expression rendering the text easily comprehended by the lay mind. Although the volume has a transatlantic origin, it is pleasing to notice the almost entire absence of Americanisms.

The contents are thoroughly comprehensive in character and include the theory and details of methods generally in use at the present time for emulsion making, colour sensitisation, sensitometry, development, fixing and washing, intensification, printing processes, toning, natural colour photography, and the secondary manipulation of bromoil, ozobrom, gum-bichromate and other processes used in pictorial work. Interspersed among the above are brief summaries of investigations which throw considerable light on the context and add increased value to the book. To each chapter is appended a list of references to scientific papers and text-books, and at the end of the volume is an extensive bibliography, including over 1000 items, giving the titles of reference works, papers in technical journals, patents, etc., in English, French and German. The indexes, both name and subject, show a high degree of accuracy, but the latter might be extended with advantage.

For a volume of this size and nature the number of errors is small, and these are fortunately of minor importance. In a few cases letters are omitted from diagrams, thus rendering reference to the text somewhat difficult, and equations on pages 212 and 538 are not quite balanced, whilst the formula of sodium carbonate is given, instead of that of sodium sulphite, on p. 318. Exception must be taken to the statement on p. 61 that "This ray is refracted *from* the normal on entering a denser medium," and to the converse a few lines below; also to "the eye is a long focus instrument" on p. 67—a statement with which few physiologists would agree. An unexpected omission occurs in Chapter XII dealing with developers, since no description of the ferrous oxalate developer and its properties is to be found therein.

The volume is well up to date, the printing and the 238 illustrations good and clear, and the student who works conscientiously through the text will have acquired a sound knowledge of the principles underlying modern photography. Pictorialists who, in many cases, achieve their results by "control," but whose negatives often indicate weak and faulty technique, will find the volume of inestimable value, for the industry of the author in the compilation of this work has served in a highly satisfactory manner to meet an acute need which has been in evidence for many years.

T. J. WARD.

DICTIONARY OF BACTERIOLOGICAL EQUIVALENTS. FRENCH-ENGLISH, GERMAN-ENGLISH, ITALIAN-ENGLISH, SPANISH-ENGLISH. By WILLIAM PARTRIDGE, F.I.C. Pp. xi + 141. 1927. London: Baillière, Tindall & Cox. Price 10s. 6d. net.

The advances in the technique of bacteriology and serology have introduced into scientific nomenclature a large number of terms for which one may search in vain in the ordinary foreign or English technological or medical dictionaries. English workers in these subjects have therefore been forced to compile their own (usually mental) lists of equivalent words after gaining their meaning from the context in the passages in which they occur. In some instances the connotation of the terms used by foreign authors is not very precise, and hence the exact meaning may only be clear to one who is thoroughly familiar with the subject matter. It will be readily understood, therefore, that this little dictionary, which includes not only the names of chemical substances, apparatus, and laboratory processes, but also the names of the diseases of man, animals and plants, supplies a long-felt want.

The book is divided into four sections, corresponding to the four languages, and each section is arranged in the alphabetical order of the words in the particular foreign language. Where there is a possible doubt as to the corresponding English equivalent an explanatory footnote is added, and where a word has been specially coined by an author the fact is made clear.

Mr. Partridge is to be congratulated upon the successful way in which he has carried out a laborious task of compilation, and chemists and bacteriologists are indebted to him for placing his specialised knowledge at their service in such a practical form. The dictionary may be warmly recommended to all who wish to study the foreign literature in the subjects with which it deals.

EDITOR.

Publications Received.

BIOCHEMICAL LABORATORY METHODS. By C. A. MORROW. New York: Wiley. London: Chapman & Hall. Price 18s. 6d. net.

THE ELEMENTS OF VEGETABLE HISTOLOGY. By C. W. BALLARD. 2nd Edition. New York: Wiley. London: Chapman & Hall. Price 16s. net.

EMULSIONS AND THEIR TECHNICAL TREATMENT. By W. CLAYTON. London: Churchill. Price 15s.

THE LITERATURE OF CHEMISTRY. By E. J. CRANE and A. M. PATTERSON. New York: Wiley. London: Chapman & Hall. Price 25s. net.

THE PHASE RULE AND ITS APPLICATIONS. By A. FINDLAY. 6th Edition. London: Longmans, Green & Co. Price 10s. 6d. net.