MARCH, 1927. Vol. LII., No. 612

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, February 2nd, 1927, Mr. E. Richards Bolton, F.I.C., President, being in the chair.

Certificates were read for the first time in favour of Messrs. William Gordon Carey, F.I.C., William Farrand Elvidge, B.Sc., A.I.C., Lewis Sidney Fraser, B.Sc., A.R.C.Sc., A.I.C., Frederick Percival Hornby, B.Sc., A.I.C., Gerald Roche Lynch, O.B.E., M.B., B.S., D.P.H., Eric C. Martin, George Gilmour Philip.

Certificates were read for the second time in favour of Messrs. Solomon Greenberg, F.I.C., Frank Crafer Ray, M.A., F.I.C., and Geoffrey Charles Matthews, B.Sc., A.I.C.

Mr. Stanley Grove Burgess, B.Sc., A.I.C., was elected a Member of the Society.

The following papers were read and discussed:—"Arsenic in Printing Inks," by T. Hedley Barry; "The Immersion Refractometer and its Value in the Analysis of Milk," by G. D. Elsdon, B.Sc., F.I.C., and J. R. Stubbs, M.Sc., F.I.C.; "Irish Moss Mucilage and a Method for its Determination," by Paul Haas, D.Sc., and Barbara Russell-Wells.

Obituary.

SIR WILLIAM TILDEN, F.R.S.

THE death of Sir William Tilden, on 11th December last, removes a notable and honoured figure from the ranks of British chemists. Born as long ago as 1842, he witnessed during his lifetime the advance of chemical science from the ideas of the mid-Victorian period to the remarkable and almost revolutionary developments of the last quarter of a century. The present generation regarded him with affection as an eminent and courtly representative of the older school, and yet,

at the same time, he showed a keen appreciation of the significance of the new physics and the new chemistry.

Tilden early developed a taste for chemistry, and his aspirations in this direction were, in the opinion of his family, adequately met by his becoming apprenticed, at the age of fifteen, to a pharmacist in Barnsbury. Fortunately, his liberal-minded master gave him opportunities of attending classes at the Pharmaceutical Society's laboratories and Hofmann's lectures at the Royal College of Chemistry, and this contact with the wider world of science made the young pharmacist keener than ever to become a chemist. Ultimately, in 1863, he was appointed Demonstrator in the laboratories of the Pharmaceutical Society, and five years of struggle and hard study saw him gain the award of the B.Sc. degree of London University.

Three years later Tilden obtained the degree of Doctor of Science by examination, and in 1872 he was appointed Senior Science Master at Clifton College under the headmastership of Dr. Perceval, afterwards Bishop of Hereford. Eight arduous years were spent in Clifton in lecturing, demonstrating and coaching, the intervals being filled with experimental investigations on various subjects, to some extent in collaboration with W. A. Shenstone.

From Clifton, Tilden was called, in 1880, to occupy the Chair of Chemistry in the new Mason College at Birmingham, and ourteen years later he was appointed Professor of Chemistry at the Royal College of Science, South Kensington, in succession to T. E. Thorpe. Tilden's tenure of the latter post covered the period of the design and erection of the present laboratories of the Imperial College of Science and Technology. These laboratories were first occupied in 1906, and three years later Tilden retired from the Chair and received the honour of knighthood.

In addition to the academic duties and the research work with which he was mainly occupied, Tilden was closely associated with the activities of various chemical organisations, more especially the Chemical Society. Elected a Fellow in 1865, while still a Demonstrator in the Pharmaceutical Society's laboratories, he served on the Council of the Chemical Society in various capacities, notably as Treasurer from 1899 to 1903, and as President from 1903 to 1905. It was owing to his initiative as President that the well-known and valuable Annual Reports of the Chemical Society were begun. Tilden acted as President also of the Institute of Chemistry for the period 1891–1894, and he was President of Section B (Chemistry) of the British Association at its Bath meeting in 1888.

Tilden's standing as an original investigator led to his election in 1880 as a Fellow of the Royal Society, and from this same body he later received the Davy Medal in recognition of his contributions to chemical knowledge. The Universities of Dublin, Manchester and Birmingham conferred honorary degrees upon him; he was a Fellow of the University of London, a corresponding member of the Russian Academy of Sciences, and an Honorary Member of the Pharmaceutical Society and of the Society of Public Analysts.

The character of Tilden's earlier research work was largely determined by his pharmaceutical associations. This remark applies to his study of the periodides of various alkaloids, and to a lengthy investigation of aloins from different sources and of their oxidation products. Soon after his removal to Clifton he initiated a research on *aqua regia* and nitrosyl chloride, and on the reactions of the latter compound with organic substances, such as phenol and turpentine oil. The employment of nitrosyl chloride as a reagent for terpenes was an early feature of a long series of researches aiming at the elucidation of the constitution of these hydrocarbons—researches in which Tilden enlisted the assistance of numerous pupils, such as Sidney Williamson, J. J. Sudborough and M. O. Forster. It was in connection with his studies of the action of heat on the terpenes, and more especially of the isoprene obtained in this way, that Tilden first observed the polymerisation of this latter substance to caoutchouc, so preparing the ground for later work by others on the production of artificial rubber.

Besides his researches in the organic field, Tilden carried out a number of investigations on physico-chemical lines. These included a study of the effect of temperature on the heat of solution of salts, and notably an extensive research on the relation of specific heat to atomic weight, and on the variation of the former quantity with temperature. His measurements of the heat capacity of metals—more particularly cobalt and nickel—down to – 182·5° C. provided the first instance of the remarkable decrease in the atomic heat of a metal which occurs at low temperatures. This work formed the basis of the Bakerian Lecture of 1900 on "The Specific Heats of Metals, and the Relation of Specific Heat to Atomic Weight."

Tilden's output as a writer also was considerable. One of the best-known of his books was the *Introduction to the Study of Chemical Philosophy*, a presentation of the principles of chemistry on broad and philosophic lines. It appeared first in 1876, and passed through eleven editions. A small volume on *Practical Chemistry* likewise ran through numerous editions. In later years Tilden's literary labours were more concerned with historical and biographical topics, as, for example, in *The Progress of Scientific Chemistry in our own Times*, *Chemical Discovery and Invention in the Twentieth Century*, *Sir William Ramsay: Memorials of his Life and Work*, and *Famous Chemists: the Men and their Work*. Noteworthy also in this connection were the Memorial Lectures on Mendeléeff and Cannizzaro delivered by Tilden before the Chemical Society.

To Lady Tilden, who in her husband's enfeeblement of the last year or two, was his devoted and constant support, the sympathy of all British chemists is extended. But the passing of a veteran, full of years and honour, need not cause any regret, and those who knew Tilden as a considerate and kindly colleague or as a respected and dignified leader in the chemical community will only desire, in honouring his memory, that they may serve science as worthily as he did.

JAMES C. PHILIP.

JOHN WEBSTER.

By the sudden death of John Webster, on January 20, at the early age of 49, there is withdrawn from the ranks of our members a well known and respected figure. Webster, from the humble beginnings as assistant in a Public Analyst's laboratory, was called gradually from position to position, until finally he occupied the responsible post of Senior Official Analyst to the Home Office.

He was born in Birmingham, educated at King Edward's School and Mason's College, Birmingham, and, though migrating to London, was always a Birmingham man; the writer well remembers his pride in his native city and its most distinguished citizen.

On coming to London, Webster, an Associate of the Institute of Chemistry in 1901 and a Fellow in 1904, spent a short time in Mr. Kear Colwell's laboratory, and in 1901 became assistant to Dr. Stevenson at Guy's Hospital, where he was brought into touch with toxicology under the then master of the art. In 1908, on the death of Sir T. Stevenson, Webster decided to forsake the Food and Drugs Act and to cleave to toxicology by becoming assistant analyst to Dr. Willcox, now Sir W. Willcox, who had succeeded Stevenson. In 1919, when Sir William Willcox retired, Webster succeeded him as Senior Official Analyst to the Home Office. He became also Pathological Chemist to St. Mary's Hospital, where he had his laboratory. It is not necessary here to name the various trials in which Webster played a part ; suffice it to say that for a period of twenty-five years he bore, at first a subsidiary, and, for the last eight years, the sole responsibility for the scientific work in connection with most of the medico-legal cases coming to trial in this country, for the solution of which chemistry was called in aid. It is to be feared that the fierce light which from time to time beats upon the holders of his office, and the attendant anxieties, acting upon a sensitive and retiring nature, contributed to the premature wearing out of a constitution which few of us suspected as being other than sound.

Webster was a member of many scientific societies, and served upon the Council of this Society in 1917–1918. His contributions to the ANALYST and other Journals bore testimony to his careful analytical work.

A few words as to the man apart from his work—a quiet man, a man of music, a devotee of Wagner, naturally gifted for violin, piano and organ, a lover of the Welsh mountains, for some years living at Balliol House in Whitechapel, and in that cheerless district dispensing hospitality and music, a Freemason, a member of the Society of Friends—that was Webster. To his memory a tribute and to his widow and son sympathy in their loss.

E. HINKS.

Recent Advances in the Bacteriological Examination of Food and Water.

By WILLIAM G. SAVAGE, B.Sc., M.D. (Lond.), D.P.H.

(*Read at the Meeting, December* 1, 1926.)

My appreciation of the honour you have done me by asking that I should address you upon the subject set out in the title of this paper became mixed with apprehension when I considered the magnitude of the task. The field is so wide, and the term "recent" so elastic, that I am at once confronted with the difficulty and necessity of making a selection of my material. To try and cover the whole field must inevitably lead to incompleteness of presentation and loss of value. After consideration I decided that I can best serve you by restricting this contribution, in part to developments which seem to me of essential importance, and in part to those with which I have been more intimately concerned, and upon which I can speak from personal experience. Restrictions of time and space must be my excuse for the obvious *lacunæ* between my remarks and the comprehensive title. I propose to confine my observations to water, milk, food poisoning, and canned foods.

WATER.—The enumeration of *B. coli* as a guide to the purity of a water supply was firmly established so long ago as 1901–1903, and mainly by experimental research in this country. Difficulties of interpreting the characters of *B. coli* and the significance of aberrant strains vexed us then, and although time has cleared up some of the difficulties, the problem cannot be regarded as completely solved. It soon became apparent that the ability to break down lactose with gas formation was a fundamental characteristic, but while the fermentation of other sugars, alcohols and other substances enabled differentiations to be established and labels attached, such distinctions were of no practical value. By practical value, I mean assistance to differentiate a good from a bad water. The position resolved itself into a conclusion that organisms possessing certain characters could be classed as typical *B. coli*, or, as I called them, *excretal B. coli*, and had full significance as evidence of contamination, whilst the greater the deviation from this typical type, in the way of loss of characters, such as coagulation of milk or indol production, the less the significance as indicators of recent excretal contamination.

High and Low Ratio Types of Coliform Organisms.—The only recent advance of importance on this conception seems to me to be the discrimination between high and low ratio types of coliform organisms, a differentiation which we owe mainly to American workers. It is desirable to consider the practical value of this differentiation test and its later developments. Grouping together studies extending over the last 10 years or so, this differentiation includes the use of four distinct tests. These are:

(*a*) The gas ratio test, *i.e.* the relative proportion of CO_2 to H in the gases evolved; (*b*) the hydrogen ion concentration as determined by the use of methyl red; (*c*) the Voges and Proskauer reaction; and (*d*) the Koser reaction.

The determination of gas ratios is troublesome and not readily adaptable for routine work. Clark and Lubs (1915) and other American workers have shown that there is a definite correlation between the hydrogen ion concentration and the ratio of gases evolved. The troublesome gas ratio determination is therefore rarely made, and the simple methyl red test with a synthetic medium is substituted. The low ratio organisms (CO_2: H_2 = 1) in this medium soon reach the limits of acidity, which inhibits further growth and dissociation of the sugar, and leaves the medium acid to methyl red. These are conveniently called M.R+. Usually they give a negative Voges Proskauer reaction. The high ratio organisms (CO_2:H_2 = 1·5 to 2·0 or more) produce less acid, and fermentation continues until the sugar is used up and the medium becomes alkaline to methyl red (MR−). They usually are +V.P.

Koser (1918) found that the *B. lactis aerogenes* type can use the combined nitrogen of certain organic substances, whilst true *B. coli* cannot, and will not grow in a uric acid medium unless some other source of nitrogen is available.

Much work has been done upon the correlation of these three tests (excluding actual gas ratio determinations), and upon the distribution of the different types in different substances, such as faeces, soil, grain, sewage, milk, and waters. Broadly considered, there seems to be a general correlation between the findings, and an establishment that the three things go together, but numerous exceptions occur, especially as regards Koser's reaction. What the significance is of those organisms which do not agree in all respects it is as yet impossible to state.

In general, a study of the voluminous work done suggests that the division of these lactose-fermenting bacilli into two groups is one corresponding with primary and, possibly, fundamental differences. On the one hand, we have the true *B. coli* type which predominates in excreta and is characterised by a low gas ratio, a + reaction with methyl red (MR+), a negative V.P. reaction, and probably an inability to utilise combined nitrogen in certain organic substances (K−). The other group, which includes *B. lactis aerogenes*, has a high gas ratio, is MR−, V.P.+ and in many cases is K+.

The significance of this differentiation in connection with the bacteriological examination of water must rest upon evidence that the high ratio type does not indicate excretal contamination, and therefore its presence is of comparatively little moment. A further point is whether these newer tests supply information which the older tests, *i.e.* coagulation of milk and indol production, failed to give. If these two points can be established, then one or more of these differentiation tests should be carried out before we give an opinion on a water sample.

In general, it may be said that the examination of excreta, soil, grain, etc., has shown, broadly considered, a differentiation in their distribution. English results are not so definite as the American, largely because, in my opinion, the latter often include strains which deviate so far from our conceptions of *B. coli*

(*i.e.* even non-lactose fermenters and gelatin liquefiers are sometimes included), that no English bacteriologist would include them. Their inclusion flatters the differential distribution of the two types.

We have used these newer tests in the Somerset County Laboratory since 1918, and Mr. D. R. Wood, the county bacteriologist, has spent much time investigating their value. Summing up our experience, it is to the effect that the differentiating tests should be employed, and that when high ratio organisms only are present much less significance can be attached to their presence as indicating possible contamination. One practical point, important to remember, is that not uncommonly both types may be present. In ordinary practice the positive gas tube containing the smallest amount of water is plated out to isolate the organisms. If the result is a high ratio strain, it does not follow that the tubes containing more water may not contain low ratio organisms, and the laboratory practice in such cases is to plate out these lower dilutions and to settle the point. They are always retained for just such a contingency. While the results in our laboratory have not been so decided as others, we have occasionally had findings which strikingly demonstrate the value of the method. For example, we have found a water supply with as many as 1000–10,000 lactose fermenters per litre but without any streptococci, when all these organisms have been high ratio, and detailed inquiry has demonstrated heavy soil but no evidence of excretal contamination.

It would take too much time to discuss the results of correlation of the three different variants. Miss D. A. Bardsley (*J. Hyg.*, 1926, **25**, 11) has recently published a valuable paper on the subject, to which I would refer you.

MILK.—It is probable, in view of recent legislation, that bacteriology will be more and more utilised in milk control. There has not been a great deal of recent work in this field, but certain procedures merit some discussion.

Detection of Tubercle Bacilli in Milk.—While existing methods are far from satisfactory, there have been few recent developments. The direct microscopic examination of mixed milk samples for tubercle bacilli is still too unreliable to be other than worthless. For samples of milk withdrawn direct from an udder suspected to be tuberculous it is not possible to speak so definitely. In well-marked cases the bacilli are so numerous that a positive result is reliable and, probably, conclusive. In the early stages, when the bacilli are comparatively scanty, it is easy to miss them, even in well-centrifuged deposits. I do not think that a negative direct examination, however carefully done, is adequate to exclude the possibility of udder tuberculosis. Mr. Wood and I are carefully comparing our results, but they are too few yet to be worth recording, while we are studying improved methods.

Douglas and Meanwell, in 1925, suggested an improved method of detection, based upon centrifuging after treatment with trypsin and subsequently mixing with ether, shaking well, and re-centrifuging. The bacilli are concentrated in the gelatinous layer between the fat-holding ether layer and the clear fluid beneath, and can be detected by microscopic examination in the usual way.

For some time we have used trypsin digestion with great advantage to obtain a less bulky deposit, but in our hands the ether treatment has not been found of assistance.

Streptococci in Milk.—The problems which arise in connection with these organisms in milk remain very thorny, and I do not know of recent work which gives us assistance towards adequate differentiation. Streptococci are extremely common in milk, even from quite satisfactory sources. What we need are reliable methods to indicate easily the types associated with bovine disease and, in particular, those potentially pathogenic to man.

In the more restricted field of the detection of types associated with mastitis in the cow and the detection of the type or types concerned with outbreaks of sore throat, comparatively recent American work has added some valuable tests. My pioneer investigations for the Local Government Board in 1906–1909 led me to the conclusion that the ordinary streptococcus of bovine mastilis, which I called *Streptococcus mastitidis*, and described carefully, was not pathogenic to man, but that very occasionally another type allied to *Streptococcus anginosus* was found in these cases, and that it was this type which caused human disease in the form of outbreaks of sore throat, etc. American work, undertaken many years subsequently, has strikingly confirmed this conclusion, and has added several differentiating tests which the results show to be of considerable value.

In my work the cultural tests used were not very helpful. The essential differences upon which I relied were that *S. mastitidis* possessed low virulence for mice and guinea pigs, possessed in marked degree the power of setting up mastitis in goats when introduced by the teat canal, and was non-pathogenic to man by direct inoculation on to the healthy throat. The other variety, which U.S.A. workers now call *S. epidemicus*, possessed considerable virulence for mice and guinea pigs, and usually failed to set up mastitis in goats when introduced by the teat-canal without any superficial injury being caused.

The recent U.S.A. tests, referred to above, are the following:—

Haemolytic Power.—The simple presence or absence of haemolytic power does not help much, but J. H. Brown and others have shown that the amount of haemolysis, with a rigidly defined technique, is of considerable differentiating value, the human strains producing much more haemolysis than the true bovine strains.

Limiting Hydrogen ion Concentration.—Avery and Cullen, using one per cent. glucose broth, found that after 48 to 68 hours incubation the P_H value of the bovine strains was 4·5 to 5·0, that of the human strains over 5·0.

Hydrolysis of Sodium Hippurate.—Ayers and Rupp showed that haemolytic streptococci of bovine origin decomposed sodium hippurate into benzoic acid and glycocoll, but that human haemolytic streptococci failed to do so.

Not one of these tests is distinctive, but when all are used it is said to be possible to differentiate between the human and bovine types. I have not, up to the

present, found time to test the validity of these tests with streptococci from English sources.

Laboratory Methods for Judging the Extent of Clean Milk Production.—In my opinion, this subject merits detailed re-investigation, as a quick, simple and reliable method is required. None of the ten methods with which I am acquainted really meets our requirements. The two commonest are the ordinary bacterial enumerations on Petri plates, and *B. coli* determinations. Both in their present form are really too time-consuming, if we take into consideration the large amount of glassware which has to be cleaned and sterilised, for the routine examination of very large numbers of samples.

Certain chemical tests based upon the results of bacterial action are employed to a considerable extent. These include acidity determinations, the onset of clotting when incubated at a definite temperature, the reductase test and the reductase fermentation test. All are very simple, but they have the great drawback that they do not reliably measure anything, and certainly not the initial bacterial contamination of the milk. I have spent much time experimenting with them, using bacterial counts as the control. They do differentiate the grossly contaminated from the lightly contaminated samples, and so, in restricted circumstances, they have their use, but that use does not carry us very far.

The determination of the amount of sediment, by gravitation methods, by pressure force, or by centrifugalisation, is a procedure much employed, since it is so easy. Unfortunately, except for demonstration purposes for the farmer, it is worthless. Its fundamental defect is that it is a measure of straining efficiency, and not a measure of bacterial contamination. Two other methods may be mentioned with which perhaps you are less familiar.

One is the direct microscopic count of bacteria in the milk (Breed's method). An accurate pipette delivers 0·01 c.c. of milk on to a microscopic slide, and this is spread uniformly over a previously marked-out square of 1 sq. cm. The film is air dried (within 5 to 10 minutes), protected from dust, treated with xylol, and then with alcohol, then stained by methylene blue, and the bacteria counted after standardising the microscope field of vision. Of course, its accuracy depends upon minute attention to details, which space compels me to omit. It cannot differentiate between dead and living bacteria. I had the advantage of having the technique demonstrated to me in Dr. Breed's laboratory in Geneva, U.S.A. Whilst it is used in a number of laboratories, it does not seem to have come into general use. Many series of comparative tests of samples examined by this method and by the plate method have been published, which indicate its value. I have only used it experimentally on a few occasions, and so am not in a position to assess its value.

The last method I will mention is the use of microscopic colony counts (Frost's method). In this method 0·05 c.c. of milk is placed on an area of 4 sq. cm. on an ordinary microscopic slide. An equal amount of liquefied agar (at 42 to 45° C.) is added, and the two drops thoroughly mixed and spread equally over the whole area. Rapid solidification takes place, and the "little plate" culture is incubated

for about 8 hours at 37° C. The slide is then dried at just below 100° C., treated with 10 per cent. glacial acetic acid in 95 per cent. alcohol, and stained with methylene blue. The colonies are counted under the microscope, and from the count it is possible to calculate the number of viable bacteria in the milk. About a year ago I started testing this method experimentally, and the results suggested great possibilities. Unfortunately pressure of other work prevented my carrying the matter further, and so, again, I am not in a position to give an opinion as to its value. I believe this test is worth extended study, as it is on the right lines, gives quick results, and uses scarcely any glassware, whilst, although it requires a very exact technique, it is not a difficult one.

Perhaps, though not strictly germane to the paper, I might indicate why I emphasise the need of a simple quick method. We ought to be able, for example, to sample every milk supply coming to a particular depot on a given day, repeat the tests on another day, and then list all those which are unsatisfactory, sending the list to the inspector to visit the farms and deal with defects (for there must be defects) under the Milk and Dairies Order, 1926. Only in this way can we quickly pick out the worst offenders.

FOOD POISONING.—Modern work has enabled us to arrive at conclusions which, while they make this subject much more definite, have in other directions increased our difficulties. With some confidence we can classify the outbreaks, as regards their causation, into three groups:—

(1) Outbreaks due to *B. botulinus*. These are extremely rare in this country. Usually the history of the attack and the symptomatology at once put us on the track.

(2) Outbreaks due to a Salmonella group strain or to the toxins. The majority of outbreaks fall into this group. For convenience the *B. dysenteriæ* strains may be included here.

(3) The residuum of outbreaks without any ascertainable causation. A number of indefinite outbreaks occur, usually mild and almost always without a fatality, for which no cause can be found. Quite certainly ptomaines play no part, while tainted food does not cause food poisoning. The evidence that *B. proteus*, *B. coli* and other organisms of decomposition are etiologically concerned, when carefully examined, is extremely weak, and, in my opinion, there is no evidence that these organisms have ever caused an outbreak of food poisoning.

Since there are these unexplained outbreaks, it is necessary to keep an open mind on causation and to be prepared to regard any organism found as a possible etiological factor until the evidence negatives it.

Since the time allowance makes it impossible to cover all the ground, I must, perforce, select a few salient points as important in relation to bacteriological investigations.

(*a*) A most valuable clue to the type and scope of the laboratory investigation is yielded by the history, particularly the incubation period. The toxin group is characterised by a short incubation period (2 to 4 hours usually), the brunt

of the attack is upon the alimentary tract, the symptoms are very acute in onset and often severe, but recovery is rapid and a fatal case a rarity. The outbreaks due to living bacilli are of slower onset, but of more protracted duration, and with a greater liability to a fatal ending. The botulism outbreaks have a long incubation period (18 to 36 hours) and a characteristic group of symptoms.

(*b*) The materials to select for investigation are an important consideration. They include any portions of the supposed peccant food, *post-mortem* material from fatal cases, especially the spleen, liver, pieces of small intestine, pieces of large intestine, and kidney, voided material from the cases (vomit and faeces), and samples of blood from sufferers for serological examination. The blood samples should not be collected until at least a week after the onset.

(*c*) For the laboratory investigation ordinary agar plates are useful to get an idea of the general bacteriology, L. B. A. plates, with or without brilliant green broth, for Salmonella strains, and a good nutrient medium such as egg-meat broth grown under anaerobic conditions for *B. botulinus* and other anaerobes.

(*d*) The serological blood examinations must include a sufficient series of test strains, so as not to miss anything. Fortunately most of the food poisoning types share the type agglutinin of *B. Aertrycke, i.e.* Stanley, Newport, Reading, and *B. suipestifer*, so that it is sufficient to use *B. Aertrycke, B. enteritidis*, and also *B. dysenteriae*. Usually, also, I include Derby and *B. suipestifer*.

(*e*) When an organism is isolated and it is a question of establishing its identity, much more comprehensive work must be done, including inoculating animals and obtaining sera and the use of absorption tests. One must establish the double test, *i.e.* the absorption of *x* serum by known strains and the absorption of sera derived from the inoculation of known strains by the *x* bacillus. This work can now be facilitated by the use of specific sera, *i.e.* by the use of sera derived from a strain containing only specific, and not type, agglutinins.

(*f*) The detection of toxin outbreaks is extremely difficult, since there is very little in the way of laboratory tests on which to base a diagnosis. We have built up our evidence upon a series of possible tests which have given results in some instances but not in others, but which, taken together, enable, in the opinion of Bruce White and myself, a definite opinion to be given. These tests include the demonstration of an irritant effect on the stomach wall by feeding, occasional demonstration of agglutinins in the blood of sufferers, and the occasional demonstration of agglutinins in the blood of animals injected with the peccant food.

(*g*) Animal-feeding experiments with the food are worth trying, but often yield no results and, in general, are very unsatisfactory. Injection experiments are valuable in cases of botulism poisoning.

CANNED FOODS.—While much work has been done in U.S.A., mainly during the last few years, on the bacteriological examination of canned foods, but little has been published in this country, apart from my own investigations with Mr. Hunwicke. For the following short account I shall mainly rely upon my own work, with a few points drawn from American studies.

We must distinguish between work carried out for research purposes and work of a practical utilitarian nature. Dealing only with the latter, it has three objectives on the bacteriological side. These are to determine if spoilage has occurred, and, if so, what caused it, to disprove alleged spoilage, and to look for pathogenic bacteria. The only pathogenic bacilli likely to be present and worth looking for are those associated with food poisoning, and these have already been discussed. The other two objectives can be discussed together.

In the examination of canned foods I would emphasise the importance of carrying out the examination in a systematic manner as a regular routine. My own procedure in order is as follows:—

1. Examination of the unopened tin. Points to note are:—The type of tin (hole and cap, or sanitary), particulars on label, evidence of ill-usage, such as dents or rust, number of vent holes, evidence of leaks, physical properties, including bulge, flatness, abnormal sound when shaken or tapped, etc.

2. Sterilisation of top of the tin before opening.

3. Opening of the tin and initiation of the bacteriological tests. Great care must be taken to avoid contamination. This is best avoided by working in a quiet room, making only a small opening (a bit and brace hole is very useful), speed in making the cultures, and having everything quite ready before the tin is opened.

4. A record of the condition of the contents including gas escape, appearance of the food and odour.

5. Direct microscopic examination of the contents. For this, direct smears, stained by methylene blue and Gram's method respectively, are satisfactory. With liquid contents films from the centrifugalised deposit should be made.

6. Initiation of any chemical tests such as titratable acidity, examination for tin or other heavy metal. In rare cases chemical tests for evidence of decomposition are indicated.

7. An examination of the emptied tin. Note signs of corrosion, action upon seams and their condition, if lacquered, etc.

Points arising from most of these examinations are outside the scope of this paper, but the bacteriological tests demand more detailed consideration.

The examination of direct smears is a simple procedure always worth doing. Although it makes no discrimination between living and dead bacteria, it throws light upon the degree of bacterial contamination before sterilisation. Its value is greatest after a long series of examinations has established a rough standard as to the probable numbers of organisms to be anticipated with each of the different types of product.

For cultural tests our own experience and recent American work alike show that there is no need either to employ a very large series of media or to utilise special media for special foods. For example, it is no help to employ fish media for canned fish. The range of culture media and incubation temperatures must

be sufficient to include all possible types of spoilage organisms. The minimum to include are the following:—

Egg-meat medium (or an equally nutrient food) incubated anaerobically. To detect the obligate decomposing anaerobes. Three tubes of glucose nutrient broth (in double tubes) incubated, respectively, at 21° C., 37° C. and 55° C.

Yeast water or other suitable medium for yeasts.

Inoculation of poured glucose agar plates. Such plates give a quantitative idea of the aerobes present, but it is difficult to avoid completely air contamination.

With some such range of media it is possible to detect and isolate any anaerobes, sporing aerobic bacilli, non-sporing bacilli (gas-forming and non-gas forming), thermophilic bacteria, micrococci, yeasts and moulds.

It is important to realise that different foods have their own types of spoilage organisms. Also, that there are different kinds of spoilage, and these are usually associated with different groups of organisms.

A "blown" tin illustrates the commonest type of spoilage. In certain types of fruit this may be due to gases produced by chemical action, but in most instances it is caused by gas formed by bacterial decomposition. The types of gas-forming bacteria found vary to a considerable extent with the nature of the food contents. With sweetened milk, for example, the gas development is usually due to a fermenting yeast, whilst with unsweetened milk micrococci may play an important part. With meat and marine products proteolytic sporing anaerobes are the usual cause. In fruit a special coccoidal bacillus, hitherto not identified, and which we named *B. pleofructi*, was found by us to play an important part, together with yeasts and micrococci.

Spoilage shown by springy or flat tins with unsound contents is usually due to bacteria, but the exact cause is much harder to determine. Probably a wide series of organisms may be concerned, including thermophilic bacteria. Corn spoilage of the type which American workers call "flat sour spoilage," was found, for example, by Cameron and Esty to be caused by members of two thermophilic groups. The same conditions were found in tins of peas, beans, spinach, and other vegetables.

With spoilage associated with the "blown" type of tin it is nearly always possible from a comprehensive examination to arrive at the bacterial cause, but with other forms of spoilage it is often a matter of extreme difficulty to reach a correct judgment as to the precise organisms implicated.

In important cases the final proof of spoilage must not be omitted, *i.e.* the inoculation with the isolated organism in pure culture of previously incubated samples of sound tinned food with similar contents. The inoculation can be made through a small hole (*i.e.* made with a sterile bradawl), and the tin at once soldered up. It needs to be kept in mind that canned foods are frequently not sterile, and not only must a control tin be incubated (best also with puncture and sealing up), but the inoculated organism must be recovered from the tin which becomes blown and shown to have multiplied. If it is recovered in pure culture the proof is complete.

The problem of having to disprove spoilage comes our way from time to time. A consignment of canned goods is seized by a food inspector and condemned by him as unsound on an examination based upon external abnormalities of the tins. The owner demurs and contends that the contents are sound and should be passed. The parcel of tins may be 1000 tins or more, and the financial interests involved considerable. I have found some of these problems very difficult to solve. Usually by opening a number of tins, and taking into consideration both the bacteriological and physical examinations, it is possible to reach a decision. The scientist, however, is not concerned to know whether the tins are marketable, but whether they are sound from the health standpoint.

DISCUSSION.

The PRESIDENT remarked that it was very valuable to have this paper presented from the medical point of view by a man who had once been a Public Analyst. A wide subject had been dealt with in an admirable way, and it had been clearly stated which tests were to be relied on. He had been particularly struck by the comments on the recognition of tubercle bacilli in milk and on the reductase test. He asked whether light could be thrown on the statement that it was more difficult to sterilise condensed milk in sanitary tins than in soldered tins, complaints having been made that the milk in sanitary tins was not always sterile.

Dr. L. H. LAMPITT was particularly interested in the question of testing milk from the bacteriological point of view, having, for five years, been trying to get a satisfactory method of judging milk coming in to a certain factory for manufacturing purposes. The reductase test, he contended, bore no relation to the bacterial content. He, also, had tried numerous dyes, as, owing to the difficulty of re-oxidation, the standard reductase test was not entirely satisfactory, but he had failed to find a dye which was more satisfactory. In his opinion the Frost method might be useful when the bacteria were present as isolated units, but in so many cases they were already present in colonies. He had tried digesting the milk with trypsin and counting, but obtained a greater count than when trypsin had not been used, the reagent having had the effect of releasing bacteria. He was sorry to hear the author's condemnation of the sediment test, because he had found that milk which gave a bad sediment test also gave a bad reductase test. The great advantage of the sediment test was that, unlike bacteria, it could be shown to the farmer. He asked whether the author could persuade the medical profession to refrain from putting every disease down to ptomaine poisoning.

Finally, he asked what was the significance of *B. coli* in oysters, for he had recently condemned a batch on this score, which was subsequently passed by a well-known bacteriologist.

Dr. G. ROCHE LYNCH thought that all the nonsense about ptomaine poisoning and such-like alleged compounds as ptomaine alkaloids ought to be dropped. In most cases poisoning was due to toxins of bacterial origin. The difficulty was that the organisms were soon killed, and by the time the coroner had decided to hold a *post-mortem* there was no evidence left. He also inquired whether Dr. Savage had been able to make any use of the complement fixation reaction in the diagnosis of alleged cases of food poisoning.

Dr. CASEY, referring to the question of oysters mentioned by a previous speaker, said that he was surprised that a definite answer could be put forward within twenty-four hours, as had sometimes been the case. He asked whether there was anything in the contention that lactic acid bacilli in milk indicated dirty vessels.

Dr. H. E. Cox raised the question of the susceptibility of mice to toxins, and asked what value Dr. Savage ascribed to negative results. He instanced cases in which he had inoculated extracts from suspected potted meats into mice and also inoculated cultures of bacteria from such samples, yet nothing happened. The only certificate which would be given was "not proven," which was not altogether satisfactory. How far could the absence of symptoms in experimental animals be taken as evidence?

Mr. W. PARTRIDGE thought that if the milk from smaller groups of cows were examined, instead of bulk samples, it might be possible to detect tubercle bacilli in the sediment. He asked the author's opinion on the significance of long-chain streptococci in milk.

Dr. SAVAGE, replying, said that, as regards milk, the fact that some of the bacteria were not separate but were in groups, as mentioned by Dr. Lampitt, was an objection, but it was not peculiar to the Frost method, since it applied equally to the ordinary enumeration procedure. He agreed that the "sediment test" did weed out many bad milks, but, if it were made a basic test, farmers would simply concentrate on straining without really improving the quality of the milk. Personally, he would rather forbid straining, so that the dirt could be seen. The experience of Dr. Lampitt with the reductase test was evidently similar to his own. He regarded the presence of long-chain streptococci in large numbers in milk as a suspicious character which would lead him to investigate the source of supply. He did not attach very much importance to the acidity in milk; it gave a rough and ready indication, but was not valuable. He entirely disagreed with the view that the microscopic sediment examination of mixed milk for tubercle bacilli was any good; six films, at least, would have to be examined, and there was always the liability of acid-fast bacilli from dung in the milk being found, which would lead to confusion. The number of lactic acid bacilli could not be taken as a direct indication of dirty vessels.

The value of the bacteriological examination of oysters depended upon the setting up of differentiating standards which experience showed had a considerable value. Personally, he was not prepared to give a definite opinion upon any batch of oysters in so short a time as 24 hours.

As regards the problems of food poisoning the author admitted and deplored the frequent misuse of the term "ptomaine poisoning" by the medical profession. All he could say in mitigation was that its use was gradually diminishing. Unfortunately quite modern text books still introduced the same views on ptomaine poisoning, and these were still being taught to the rising generation of medical men. He agreed with Dr. Roche Lynch as to the need for reform in the methods used in the investigation of food poisoning outbreaks. The trouble was that the usual practice was for the Coroner to ask the local practitioner who had treated the case to undertake the *post-mortem*, and he, knowing nothing of the pathology of food poisoning, was incapable of doing it properly, and usually confined his examination to a naked eye examination and sending the stomach contents for a chemical examination. Actually the investigation was a specialist's job, and a bacteriological examination was essential. Dr. Cox raised an interesting point in regard to animal feeding or inoculation in food poisoning. One of the difficulties in food poisoning investigations was that no laboratory animal reacted like man. When mice were fed with the suspected food, or even if inoculated, and nothing happened, it did not prove anything; very likely the mice would not be affected even if the food was infected. He recommended that all experiments on mice should be accompanied by controls.

The author concluded by an appreciation of the excellent discussion evoked by his paper.

A Critical Review of the Methods of Analysing Waters, Sewages and Effluents, with Suggestions for their Improvement.

By J. W. HAIGH JOHNSON, M.Sc., F.I.C.

(*Read at the Meeting, November* 3, 1926.)

The two short notes which have already appeared in The Analyst (**1926, 51**, 345, 405) on the analytical procedure dealing with this subject entirely fail to convey any adequate idea of the proposed revision, and I am therefore glad to have an opportunity of bringing this suggested revision to the notice of the Society.

At first sight there does not appear much ground for fundamental criticism, but the repeated efforts which are now being made to revise and standardise these analytical methods may, perhaps, be the subconscious expression that something is radically wrong with our present procedure.

In the first place, the present methods suffer from the serious disadvantage of being a piecemeal collection of individual tests of varying importance, accuracy and age. The result is that we are now burdened with a number of individual and disconnected tests having little or no uniformity either in method or object, but not entirely free from the disadvantages associated with overlapping.

A moment's consideration of even the physical conditions under which the present examinations are made is sufficient to prove a regrettable lack of uniformity.

The reaction of a liquid is of the utmost importance and is intimately associated with the amount of soluble solids which that liquid may contain. Since the reaction of all large masses of water is slightly alkaline, it does not seem reasonable to determine the amount of suspended solids in an acid liquid without first neutralising the acidity.

It will be readily realised that an acid discharge must finally attain this natural reaction, and in the process probably deposit large amounts of suspended matter.

This suspended matter has to be dealt with during purification and often involves much trouble and expense. The usual analysis gives no idea of this costly contingency; rationally such a liquid should be condemned either on its acidity *per se*, or the suspended matter should be determined after efficient neutralisation and precipitation has occurred.

Similarly, excessive amounts of alkalinity, especially if caustic in character, may tend to stabilise putrescible liquids, even so far as to permit the formation of nitrites and nitrates. Such liquids may present no danger of becoming self-putrescent, but dilution with natural water may give rise to serious pollution effects.

Although the exact procedure which should be adopted in these cases may be debatable, I think that sufficient has already been said to show that even such an elementary question as the adjustment of reaction to natural conditions has been overlooked in our present methods.

If, now, our attention is directed to the more purely chemical aspect of this question, a similarly unsatisfactory state of affairs is found to exist.

The tests individually may be radically sound and commendable, but collectively they tend to confusion, giving a mass of figures which often affords no reliable basis for comparison, and in critical cases often fails to provide the necessary convincing evidence.

The present methods are chiefly the result of the activities of the Royal Commission on Sewage Disposal; they examined and modified many existing methods, and even introduced new ideas into analytical practice. In this they were not entirely successful, as the one test bearing the Commission's name has proved. Recently, official or semi-official opinion has again supported the Commission's attitude by retaining the present methods. This is much to be regretted, as it tends to crystallise opinions around inadequate and erroneous ideas. It is even pernicious, as it impedes progress and research by suggesting that the desired finality has already been attained.

Recent modifications in sewage treatment have enormously increased the frequency of occurrence and amount of nitrogen oxidation products in effluents and thus further added to the difficulties of accurately applying methods which were available a decade or two ago. To-day these methods present the interestingly anomalous condition of affording reliable results when the liquid is obviously polluted, but of being often unreliable when the liquid has attained a certain desired degree of purity.

It is obvious that, since the principles of the present methods are so well-founded, serious revision is only possible on account of the grossness of existing crudities. Future efforts should therefore secure increased accuracy and discrimination, together with combination of partial processes and their correlation with natural requirements. This incidentally involves a better and more detailed knowledge of existing processes.

There are only two means of determining direct organic pollution, *viz.* the organic nitrogen content and the amount of oxidisable organic matter. For this purposef our well-known processes are available—two for each, viz. the Wanklyn and Kjeldahl processes; the acid chemical process—usually the 4 hours' oxygen absorbed test at 80° F.,* and the biological oxidation processes.

* The results of the 4 hours' oxygen absorbed test at 80° F., used throughout this work for comparative purposes, are given in parts of oxygen per 100,000 volumes of the liquid.

As various modifications of this process are in vogue, yielding comparative, but not identical, results, it should be stated that the sample (10 to 100 c.c.) was always diluted with 150 c.c. of distilled water, acidified with 10 c.c. of 1 in 4 sulphuric acid. Ten c.c. amounts of *N*/80 permanganate were added, care being taken that the active excess did not fall below 5 or 6 c.c. during the 4 hours' incubation. The determination was made colorimetrically, and blank absorptions for the reagents used were deducted.

It is a matter of prime importance that these four processes should be accurate and comparable. In its present form the Wanklyn process is too vague to be really reliable. The oxidation occurs during a constantly increasing concentration of the active reagents, and the end-point is often unsatisfactory. On the other hand, the chemical oxidation process—the 4 hours' oxygen absorbed test—yields only one-fifth of the result obtained biologically. Efforts have been made to increase the chemical result by operating at boiling temperature, but this is unsatisfactory, as the action of chlorides on the hot acid liquid yields free chlorine which splits up the ammonia molecule and renders the process unavailable for correlation with the Wanklyn result. Acid processes must therefore be discarded.

It is remarkable that, although Wanklyn used alkaline permanganate to oxidise the organic matter in his albuminoid test, yet its availability for accurately determining the oxygen consumption has not yet been realised.

The two processes can be effectively combined by treating the sample with alkaline permanganate under a *glass* reflux condenser. Under these conditions the presence of chlorides does not interfere with the production of albuminoid ammonia, which under the proposed conditions is not only increased, but has the additional advantage of distilling off both quickly and completely. The results are remarkably concordant and establish a definite correlation between the two most important tests from a sanitary chemist's point of view.

In order to make the correlation accurate and the process as simple as possible it is proposed to distil off the free ammonia before adding the permanganate. This initial procedure does not affect the oxygen absorbed in well-aerated and purified liquids. In the cases of sewages, however, considerable differences may occur, owing to the volatility of reducing substances. If it is desired to determine the effect of these substances, a total determination may be made by omitting the preliminary distillation.

It is of extreme importance that these tests should be carried out under strict conditions relatively to the concentration of the reacting substances. It is therefore proposed that the reacting volume be about 300 c.c. This volume permits the use of a maximum amount of 250 c.c. of a sample; smaller volumes of stronger liquids are made up to this volume with ammonia-free water. The water and reagents used for this purpose and throughout this test should not only be ammonia-free, but also albuminoid nitrogen free.

In making the test it is advisable that the whole of the reactions should be carried out in the same vessel, a round-bottomed flask of 1 litre capacity being recommended.

Thirty minutes is the time suggested for this reaction; this provides a well stabilised end-point, as the oxidation occurs chiefly in the first half of this period.

COMBINED OXYGEN ABSORBED AND ALBUMINOID NITROGEN DETERMINATION ("OXY-ALBUMINOID TEST"): *Reagents.*—Ammonia-free water; sulphuric acid (1 in 4); sodium hydroxide (20 per cent. solution); potassium permanganate ($N/8$ solution); sodium carbonate solution; potassium iodide solution (10 per cent.); and sodium thiosulphate ($N/80$ solution).

Procedure.—A suitable quantity of sample is taken and, if necessary, a measured amount of ammonia-free water added. This is made slightly alkaline with sodium carbonate, and the free ammonia distilled off. This should be done in the same flask in which the test is to be made. The ammonia in the distillate may be determined, if desired.

To the remaining liquid, which should approximate 250 c.c. in volume, 25 c.c. of the sodium hydroxide solution and 20 c.c. of the *N*/8 permanganate solution are now added, and the mixture "refluxed" for 30 minutes. (It is at times of advantage to turn the flask gently during the refluxing process in order that any deposited matter may come under the influence of the reagents). The amount of permanganate solution may be reduced to 10 c.c. in the case of waters of good quality; whilst occasionally an extra 10 c.c. may be required for exceptionally polluted liquids.

At the end of this time the flask and contents are cooled, and the volume made up to 500 c.c. with ammonia-free water in a graduated flask. (If the flask contains deposited manganese dioxide it is advisable to wash out with a few c.c. of the sulphuric acid solution; a little warming assists solution of the brown deposit.)

From the 500 c.c. flask, two volumes of 50 c.c. each are pipetted and acidified with 10 c.c. of the acid. Two c.c. of the potassium iodide solution are added, and the liberated iodine is titrated against *N*/80 sodium thiosulphate solution.

A blank determination should be made with the ammonia-free water. The titration figure for the sample, deducted from the blank, gives the oxygen consumed by one-tenth of the original volume of sample taken. The usual corrections should be made for reducing substances, such as nitrites, etc. If nitrites are troublesome, they may be removed as in the Kjeldahl process by acidification, etc., after distillation of the free and saline ammonia.

The remaining 400 c.c., which should still be alkaline, is returned to the original flask. The ammonia is distilled off and nesslerised. This gives the "Oxy-Albuminoid Nitrogen."

The 400 c.c. remaining from the blank experiment afford a useful check on the purity of the reagents used.

It will be readily realised that the results obtained by this method do not agree with either the 4 hours' oxygen consumed or the Wanklyn albuminoid figures. The oxygen-consumed amount is four times that obtained by the 4 hours' test, and a slightly larger amount of albuminoid nitrogen is obtained than by the Wanklyn test.

These increased values are of considerable advantage, particularly in the case of the purer liquids, as the effect of the blank is less pronounced and the amount to be determined is much larger, so that the final result is more convincing.

ORGANIC NITROGEN—KJELDAHL'S PROCESS.—This process is fundamentally an excellent one, but it has recently been seriously overburdened, and, in the presence of nitrites and nitrates, has given unsatisfactory results.

Several suggestions with a view to remedying this defect have been made, but few, if any, of them really achieve the desired result, whilst the complications introduced are often most undesirable.

In its ideal form this process should only be applied to the direct determination of the albuminoid or organic nitrogen, that is, after the elimination of the ammonia, nitrous and nitric nitrogen.

The removal of the first may be readily accomplished by boiling the slightly alkaline solution until all ammonia is evolved; the second may be removed by continuing the distillation after making the liquid slightly acid to the extent of 2 c.c. of 1 in 4 sulphuric acid.

The elimination of the nitric nitrogen is not so readily accomplished, and hitherto no satisfactory method has been discovered for the removal of this substance, which in some instances may be preponderatingly large, and therefore undesirable.

In the suggested modification the ammonia and the nitrous nitrogen are first removed, as already described. They may be determined in the distillates in the usual manner.

When nitrites cease to appear in the last distillate, 0·5 grm. of zinc dust in the form of an ammonia-free suspension is added, together with the usual quantity (10 c.c.) of sulphuric acid required to complete the ordinary Kjeldahl process. The flask is attached to a *glass* reflux condenser and its contents boiled for 15 minutes. By this time the whole of the nitric nitrogen will have been reduced to ammonia, and the process may now be completed in the usual manner. (As much as 0·01 grm. of nitrogen has been reduced in 15 minutes.)

After deduction of the necessary blank amounts the results may be relied upon to give an accurate determination of the organic and nitric nitrogen present.

The accuracy of the final organic nitrogen determination is naturally influenced by the relative amounts of these two forms of nitrogen, a low nitric value being desirable, as pointed out above.

The occasional failure of somewhat similar reduction processes is attributable to the fact that, although the reagents employed do eventually produce ammonia, some loss of nitrogen is occasioned in the intermediate nitrous stage, in which form it readily escapes from the heated acid liquid. By means of the reflux condenser used in this modification the intermediate forms are returned and finally reduced to ammonia without loss. The amount of acid necessary for the reduction stage of this process is not more than 2 c.c. of the dilute acid. It might obviously be suggested that after reduction the nitric nitrogen could be determined as ammonia by distilling the liquid after it had been made slightly alkaline. Experience, however, proves that by this time some albuminoid ammonia may have been liberated and would, in consequence, distil over with that from the reduced nitric nitrogen.

THE ROYAL COMMISSION TEST.—The biological estimation of the oxygen absorbed forms the basis of the Royal Commission test, which in its present form is unreliable. There is much misapprehension and confusion regarding this test, and the present results are chiefly the outcome of unnatural conditions of experiment, as the following observations demonstrate.

Dilution plays a very important part in this test, and without some clear ideas of the effects of dilution the value of the results must inevitably suffer. Much of the effect of the dilution may be attributed to the presence of the oxygen introduced with the dilution water, for it has been found that the addition of oxygen gas to a polluted liquid tends to produce a temporary stability.

This is doubtless the condition which the Royal Commission experimentally attained when they recommended that a sufficiently diluted sewage could be admitted to a watercourse without apparent ill-effect.

Experimentally, this is quite sound, for the suspended matter, which is the all-important factor, is maintained in a highly diluted condition, but in nature secondary concentrations of this suspended matter are liable to occur, with undesirable results.

The effect of the concentration of oxygen during incubation, however, presents a very interesting study. When the oxygen concentration is above 7·0 parts per million the reaction seems to be fully maintained, but with good effluents the rate of absorption rapidly decreases in concentrations below this amount, and nitrates present in the sample are reduced. American workers have actually introduced nitrates as a source of oxygen, but obviously this reaction is not consonant with that of the dissolved gaseous element. This decreased absorption is particularly associated with the better quality of the effluent. It will be realised that the numerous partial results which have been obtained under such conditions are unreliable.

On the other hand, it has been found that the oxygen concentration may be increased to 30 or 40 parts per million without any appreciable acceleration.

Similar results were obtained during experiments on the activated sludge process, carried out in special apparatus which permitted the use of normal and oxygen-enriched air devoid of carbon dioxide.

These results seem to indicate the presence of some restricting agent in the passage of the oxygen to the polluting substance. A study of the effect of concentration of the polluting substance on the absorption of oxygen is somewhat difficult, as the range of reaction is strictly limited.

It is generally accepted that the rate of oxidation in this process is directly proportional to the concentration of the reacting substances, and this, broadly speaking, must be the case for the polluting substance.

However, certain experiments in this direction have proved that in some circumstances an increase of the polluting substance does not lead to a materially increased absorption, or, in other words, dilution may increase the oxygen-absorbing capacity of the polluting substance. On the other hand, it has already been shown that high dilutions tend to stabilise the composition.

These results all lead us to the conclusion that the oxygen-consuming capacity of a liquid can only be satisfactorily determined over a small range of experimental conditions, and that these conditions need careful attention.

The primary essentials before satisfactory results can be anticipated with this test are:—(1) The incubated liquid shall have and maintain a suitable reaction—

slightly alkaline to litmus—throughout the period of incubation. (2) The incubated liquid shall contain the necessary living organisms. (3) The mixture shall be maintained at a temperature suitable for the development of the organised entities. (4) There shall be an adequate supply of oxygen—during the period of incubation—for the requirements of the living organisms.

The absorption itself exhibits three distinct reactions or phases:—(1) Preparatory or primary phase, (2) carbonaceous oxidation phase, (3) nitrification phase. Under present conditions one or more of these phases may contribute to the final result. The preparatory phase usually terminates within 24 hours, the second or carbonaceous phase generally lasts several days and forms the real basis of this test. The termination of this phase is indicated chemically by the increase of nitrogen oxidation products, which characterise the third stage.

It therefore follows that when nitrites appear in the incubated liquids the process should be discontinued. This statement does not necessarily preclude the examination of a liquid by this test, if such a liquid contains nitrates or nitrites as the result of an admixture of more or less purified liquids. The real criterion of successful application is the unaltered concentration of nitrification products during the incubation period.

Nitrites appear to develop more rapidly in high dilutions, and it is also possible that the presence of carbon dioxide has some bearing on this reaction. It will therefore be seen that the use of fairly concentrated liquids is preferable. This is only possible when high oxygen concentrations are employed. The use of these high concentrations does not appear to encourage nitrification unduly.

One very important factor, which appears to have been overlooked in this test, is the retarding effect of small amounts of carbon dioxide (2 up to 8 c.c. per litre). (See Chart.) The presence of large quantities of dissolved oxygen counteracts to a large extent the effect of carbon dioxide. It is, however, desirable to extract the dissolved gases both from the sample and the dilution water before saturation.

The reaction of the liquid undergoing incubation is of the utmost importance. The proper reaction should be maintained throughout the whole period of incubation, and for this purpose it is desirable to use a prepared or standard water. Distilled water, being relatively free from organic and other impurities, forms a suitable basis for the preparation of this standard water, but even distilled water does not always possess the necessary reaction. Acidity, however slight, is to be stringently avoided. It is proposed to ensure the correct reaction by adding an excess of pure re-precipitated chalk to the distilled water, which dissolves about 18 parts per million. A mixture of re-precipitated chalk containing about 10 per cent. of pure calcium phosphate has often been used with success.

In the case of well purified liquids it may not be desirable to use dilution water, in which case a sufficiency of this mixture should be added to the sample before incubation.

It may happen that the polluting liquid possesses a marked alkaline reaction. This should be suitably adjusted before incubation, care being taken to remove

any carbon dioxide liberated during the correction. The final alkalinity should depend upon that of the chalk introduced.

Presence of Living Organisms.—The samples usually contain sufficient bacteria to dispense with inoculation, but the addition of a small quantity of previously inoculated material would ensure the test working even with a sterile sample. It may eventually be necessary to introduce definite bacterial flora into all samples, but at present this is not deemed necessary.

Temperature of Incubation.—The temperature of 65° F. was probably selected by the Royal Commission as being the mean summer temperature of the streams in Great Britain. It is a temperature which is difficult to maintain and does not appear to be the optimum temperature for this reaction. A temperature of 80° F. would in all probability be more suitable, and if not exactly the optimum, it is one already in vogue for other purposes in the sewage analyst's laboratory.

In the past it has been difficult, owing to overlapping conditions, etc., to determine the effect of temperature increase on the absorption rate. Apparently 1° C. rise in temperature causes an increase of 10 per cent. in the absorption rate.

Blank Absorption of Dilution Water.—It is desirable that a blank determination should be made of the dilution water used, particularly in the case of high dilutions. The following figures serve to illustrate the influence of dilution water, the samples being so arranged as to give an absorption of 20 parts per million, after 5 days' incubation; it is also presumed that the dilution water itself absorbs 1·0 part per million of oxygen, but this effect is ignored at first, and corrected later.

Dilution of sample.	5 Days' R.C.	Total amount of oxygen absorbed.	Amount absorbed by water.	Amount absorbed by sample.	Correct R.C. absorption.
Nil	20·0	20·0	0·0	20·0	20·0 p.p.m.
1 in 5	20·0	4·0	0·8	3·2	16·0 ,,
1 in 10	20·0	2·0	0·9	1·1	11·0 ,,
1 in 20	20·0	1·0	0·95	·05	1·0 ,,

The above table serves to illustrate the very important influence of dilution upon the resulting figures. The last column gives the correct absorption, which varies from 20·0 to 1·0 parts per million, whereas the recorded result would be 20·0 in all instances. It may be argued that the first *nil* dilution is impracticable, and that the last is too high for a sample having such a low absorption. These widely different results, however, are strikingly impressive, while those obtained at dilutions of 5 and 10, which are in general use, differ by more than 30 per cent. Such results cannot fail to impress upon one the absolute necessity for making: (1) A blank absorption determination of the dilution water, and (2) the great importance of using as low a dilution as possible.

Unfortunately, in ordinary circumstances, one is limited by the initial oxygen saturation to 10 parts per million.

It might be advisable to consider the use of enriched air for saturation purposes; thus, if air containing about 70·0 per cent. of oxygen were used, the *nil*

dilution test in the above table would become practicable. The volume of gas required for such purposes would be only small in amount.

Gas Bubbles.—During the incubation period there is a tendency for gas bubbles to appear in the bottles. "De-gassing" before saturation considerably reduces this inconvenience. As the concentration of oxygen in these bubbles may be thirty times that of the liquid, it is important that it should be determined. If the bubble is of convenient size, the determination may be completed with the bubble entrapped.

Sedimentation.—During the incubation period there is an undoubted tendency for sediment to form on the bottom of the bottle. This sediment may contain a considerable amount of organic matter and bacteria. In such circumstances it might, therefore, be an advantage to distribute the sediment occasionally by agitation during the incubation.

Chemical Details.—I should like to warn operators against the use of excessive amounts of potassium oxalate when discharging the potassium permanganate tint. It is only fair to point out that the Commission had evidently foreseen this difficulty, as their notes on the test indicate. Unfortunately, the use of 2 c.c. of a 2 per cent. potassium oxalate solution, and even the addition of crystals of this substance, have been both recommended and used. Under such conditions the titration amount is too small. It might, however, be contended that if a given amount of oxalate be used in all cases, the results would still be comparable. It appears, however, that the effect of this excess varies with the amount of oxygen in solution. Whenever possible, the "direct" method is to be preferred, *i.e.* omit the use of the first three reagents, and in other cases the use of a minimum of oxalate solution is imperative.

The advantages of using high oxygen concentration in these determinations are fairly obvious. These concentrations permit the use of a more concentrated liquid in the test, giving a higher titration figure. They also tend to delay the formation of nitrites, making possible a longer period of absorption.

The disadvantages are: the use of oxygen, although the amount of gas used for this purpose is comparatively small, and the process of super-saturation, relatively simple and easy to perform. In the case of small absorptions it may happen that the titration figure is exceedingly large compared with the amount of oxygen absorbed. An effort should be made to avoid excessive concentration when dealing with well purified liquids.

One now passes on to consider the actual absorption. This feature of the test has not received the treatment which scientific insight and acumen would demand. In the first place, the character of the absorption is not uniform for the same liquid throughout the incubation period. Excluding the nitrification phase which eventually supervenes, detailed experiments have shown that the absorption prior to this phase may exhibit considerable irregularity. There are, in fact, two phases or stages in this absorption—an abnormal primary one, usually completed within the first 24 hours, followed by a secondary and regular

one extending over a period of some days. During the primary stage the absorption is usually sub-normal or hyper-normal. In the former case this may be attributed to a certain amount of delay or lag in the bacterial activity and appears to be associated with well-purified and well-aerated liquids of considerable stability; in good effluents it usually amounts to eight hours.

The hyper-normal absorption is associated with putrescible liquids and may be largely due to the direct chemical absorption of oxygen by the products of decomposition.

Whether in the first stage there be lag or acceleration, after the first 24 hours or so the absorption continues at a definite and uniform rate.

This rate, under suitable conditions, is a measure of the oxidisable organic matter, and is characteristic of the polluting nature of the liquid.

It is proposed to use this rate as the primary basis for the comparison of pollution, and for that purpose the straight line graphically expressing this uniform rate is continued backwards until it cuts the axis of time. It will now be seen that in the case of a primary sub-normal absorption this line intersects the axis of time at a point some eight hours or so later than the zero time point; whereas in the case of a hyper-normal absorption the intersection may occur 30 or 40 hours prior to the zero time point. (See Chart.)

In these oxidation experiments it was noticed that the oxidation was very largely selective, and that one reaction appeared to occur almost to the exclusion of any other. It therefore follows that the oxidation is almost specific for the substance oxidised. This important fact accounts for the hyper-normal primary rate usually observed in polluting liquids.

The uniform secondary rate of absorption therefore points to the oxidation of relatively stabilised organic matters.

The period usually taken for this oxidation is a relatively lengthy one which provides suitable conditions for accurate observation.

The fact that it is graphically represented by a straight line of considerable length provides us with a starting point for mathematical consideration.

In co-ordinate terms the line may be expressed generally as $y = x \tan \alpha \mp b$; where b = the amount absorbed in the primary phase. In terms of $\tan \alpha$ and lag time $l : b = l \tan \alpha$. The original expression therefore becomes $y = (x \mp l) \tan \alpha$. The effect of this lag would be expressed as $x \mp l = y/\tan \alpha$. This brings us to an entirely new conception of the effects of unstable and semi-stable elements of pollution on the absorption amounts. It will be seen that a determines the rate of absorption, whilst l determines the point of time from which this absorption is measured. It follows that variations in l may therefore exert a marked effect on the total absorption without necessarily involving any change in the rate of absorption; as usually recorded, this varies from $(x+l)$ to $(c-l)$.

The causes for the variation in values of l have already been outlined. α therefore indicates the amount of this polluting substance; as α increases, the amount of this substance increases, and it therefore follows that equal values of α

represent equal values of this substance and therefore are represented by identical or parallel lines; this is clearly indicated in the above equation.

The idea of a parallelism gives us a new conception of pollution identity which does not depend upon the total absorption in a given experimental period of time. It may also afford some explanation of the difficulty usually experienced in obtaining concordant results with the same liquid when using different dilutions. (See Chart.)

Further, it will be seen that if $x=y/\tan \alpha$, the line will pass through the origin or zero time point; under this condition all absorption amounts obtained after a given period of time—say, 5 days—will be strictly comparable. This result is obtained by deducting the lag amount in all cases of primary acceleration and adding it in other instances; by this simple means additional information and more rational results are obtained.

The use of this rate of absorption line to cover the total absorption does not detract from previous methods of recording the total absorption, but makes the lag element stand out in proper perspective.

In well-stabilised liquids this lag element is almost negligible, but in liquids which exhibit some putrefactive changes it may amount to a considerable portion of the total absorption; appears to be a measure of the unstabilised condition of the liquid and is only secondary in importance to that of the rate itself.

THE GRAPH STANDARD METHOD.—The basis of this method has been briefly outlined in the preceding paragraph. It attempts to determine the period of absorption, as measured by the regular rate of absorption during the secondary phase. It does not interfere with the older idea of a total absorption over a given number of days, but by equating the irregular primary absorption in terms of the secondary rate of absorption we obtain a better and more correct idea of the magnitude of the primary absorption in terms of time required to complete this absorption at the absorption rate of the secondary phase.

It is proposed to call this method the "Graph Standard," the reason for this being that the results are more readily compared by graphical representation. It is suggested that the results obtained by this method be set out in the following form:—

(1). (×5) 5(+0·12) days' absorption at 65° F. =40·0 parts per million (original sat. 20·0 p.p.m.).

(2). (×50) 5(−1·5) days' absorption at 65° F. =280·0 parts per million (original sat. 25·0 p.p.m.).

The above represent (1) a hypothetical sewage effluent in which it will be noticed there is a positive lag of 0·12 day. The first bracket indicates the dilution; the second bracket indicates the nature and amount of lag observed—which in the second example, it will be noticed, is negative in character and large in amount, indicating an unstable liquid. The other figures are self-explanatory. It seems desirable, for some time at any rate, that the original saturation should be stated.

The 5 days' absorption results obtained by this method will naturally be much lower than those usually associated with grossly polluting liquids, owing to the fact that the negative lag amounts are eliminated, but considerably increased in the case of purer liquids, as the biological activities are fully maintained by an adequate oxygen supply and a reduced carbon dioxide concentration.

The five days' absorption of a satisfactory effluent on the Royal Commission basis would now be 4·0 parts per 100,000.

SUGGESTED METHOD:—Although the details already enumerated in connection with this test appear somewhat forbidding, the whole process can be fairly efficiently performed in a relatively simple manner.

The sample, diluted to the desired extent with standard water, is placed in a strong bottle fitted with cork and tubing. This is attached to a vacuum pump and evacuated until "water hammer" is well established. The bottle is then sealed by means of a screw clip and allowed to stand, with occasional agitation. The necessary degree of aeration may be attained by saturation with normal or oxygen-enriched air at atmospheric pressure; saturation under partial pressure with pure oxygen has, however, afforded satisfactory results. After saturation the mixture is syphoned off into 7 or more bottles of about 180 c.c. capacity. Two of these are titrated at once, the others incubated in an inverted position with sufficient water to seal the stoppers. The remaining bottles are titrated after 24, 48, 72, and 96 hours, respectively—unless active nitrification is taking place; a spare bottle should always be avaiable for this examination.

Under normal conditions and after a little experience the 24 hours' and one later determination (40 to 80 hours) usually suffice, the five days' absorption amount being determined graphically, as previously explained.

After the first 24 hour period the other determinations may be made when convenient and results plotted for the actual hours of incubation. Stagnant samples may require special treatment before dilution to remove the dissolved gases. Small quantities of suspended chalk mixture in the dilution water may be tolerated, but larger amounts, giving rise to gas formation in the final stages, should be avoided.

The nature of the liquid and the type of curve form additional checks on the results obtained, which should exceed the 4 hours' oxygen absorbed in all cases of sewage pollution.

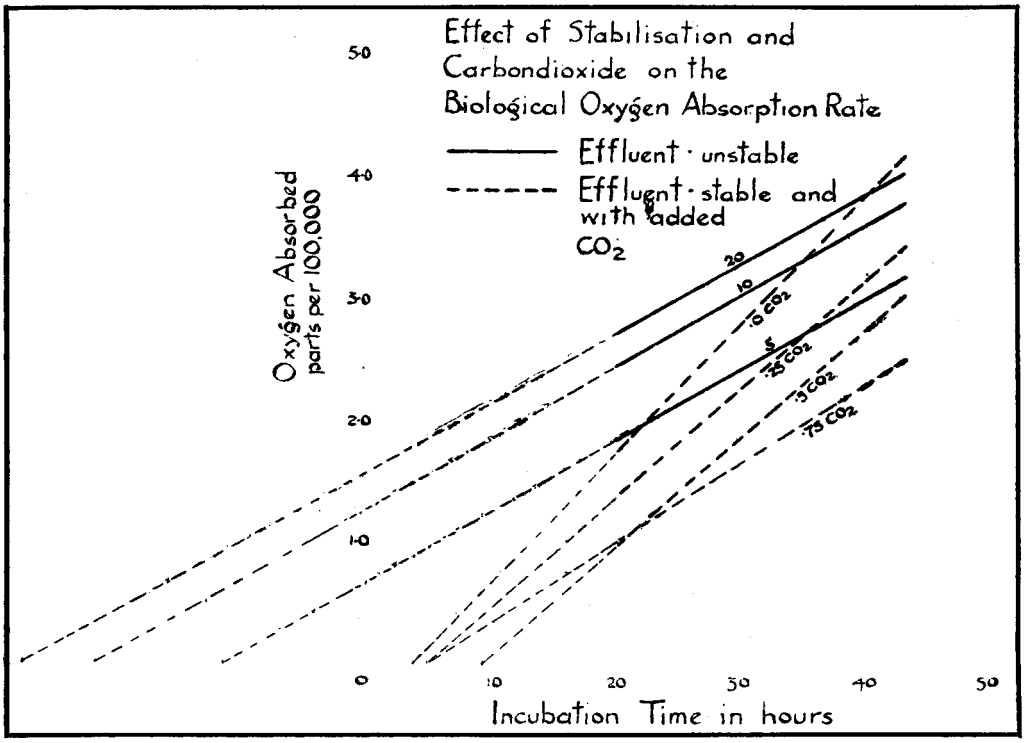
EXPLANATION OF CHART SHOWING THE EFFECT OF (1) STABILISATION AND (2) CARBON DIOXIDE ON THE BIOLOGICAL OXYGEN ABSORPTION RATE.

The liquid used in these experiments was well purified domestic sewage which had been allowed to stand over the activated mud until it acquired a disagreeable odour. It was then decanted, and the absorption measured at 20, 10 and 5 dilutions, as shown in continuous lines in the chart.

The remainder of the liquid, after standing in an open vessel for two days, was odourless; the absorptions now obtained at 10 dilutions and with percentage

volumes of added carbon dioxide are indicated by the interrupted lines between 20 and 43 hours,

It will be observed that the three continuous lines representing the original absorptions at 20, 10 and 5 dilutions are parallel, and therefore by the "Graph" method give an identical result of 6·8 for the 5 days' absorption. Further, on producing these lines backwards it will be observed that they all intersect the



base line to the left of the zero time-point; they all exhibit acceleration in the early period—indicative of putrescibility or instability. The amount of lag varies, being 1·16, 0·91 and 0·5 days, and would be represented thus:—

20×5 (−1·16) days' absorption at 65° F. = 6·8 parts per 100,000.
10×5 (−0·91) ,, ,, ,, ,, = 6·8 ,, ,, ,,
5×5 (−0·5) ,, ,, ,, ,, = 6·8 ,, ,, ,,

It will be noticed that the intersection by interrupted line (no added carbon dioxide) is at an entirely different and larger angle; also that the intersection is 3 or 4 hours to the right of zero time. The former indicates an increased absorption = 12·6 for five days, and the positive lag indicates a stabilised liquid.

The increasing quantities of carbon dioxide reduce the 5 days' absorption, until, with 0·75 per cent. by volume of added gas, it falls to 7·9. Normally this reaction ceases when the concentration reaches 1 per cent.

The effect of stabilisation on the absorption rate is, perhaps, somewhat surprising, but it has an apparent parallel in the results obtained on dilution of the concentrated solution, already mentioned in the text.

SUMMARY.—The present methods of water and sewage analysis demand radical revision for the following reasons:—(*a*) Marked discrepancy between analytical and practical requirements. (*b*) Existing methods are inadequate, as they often lack precision, discrimination and scientific insight. (*c*) Inherent difficulties; *e.g.* nitrification products have considerably increased in modern purification processes leading to further vitiation of results. (*d*) The biological oxygen absorbed test involves reactions depending on obscure biochemical conditions not hitherto fully determined. (*e*) The uncertainty of the results thus obtained is a most serious handicap to present and future progress.

The proposed methods deal only with tests of fundamental importance, *i.e.* organic nitrogen and oxygen absorbed.

The organic nitrogen methods include:—(1) A modified Kjeldahl process, affording accurate results in the presence of nitrites or nitrates.

(2) An albuminoid ammonia process carried out under strictly controlled conditions of time, temperature and concentration of reagents, yielding a definite and easily distilled amount of ammonia. In addition to this, the process simultaneously affords an accurate method for the determination of the oxygen absorbed chemically. These results are in relatively close agreement with practical requirements, and are intimately correlated with the albuminoid nitrogen determination. The use of relatively large amounts of sample tends further to increase their accuracy.

It is proposed to dispense entirely with all acid oxidation processes, as they are unsatisfactory.

The Royal Commission Test, or the determination of the oxygen consumed biologically is a most difficult problem. The asymptotic curves usually associated with this reaction are untenable; they are the result of various unnatural conditions of experiment, and should be strictly rectilinear.

The suggested modifications produce results which are definite and strictly comparable *inter se*, and their relation to previous determinations is sufficiently well maintained to obviate serious difficulties, so that they reasonably offer a reliable basis for the future development of our analytical processes.

DISCUSSION.

Mr. E. HINKS agreed that if the indications of pollution could be magnified, so much the better, but there was likely to be immense difficulty in changing over to a new process, especially after so many data had been collected. It was a great tragedy that the Sewage Commission ever ceased to sit, or rather that its recommendations had been allowed to lapse. The complications arising from the addition of excess of oxalate after permanganate destruction of nitrites could, he said, be short-circuited by adding magnesium carbonate at the end of the

five days and before the titration was carried out. There were difficulties associated with maintaining a temperature of 65° F. for five days.

Mr. E. H. NURSE asked whether the author had tried the dissolved-oxygen-absorbed test, using a higher temperature for a shorter period. He had seen some results in which incubation at 27° C. for 2 days was stated to give figures in close agreement with the usual 5 days' test. The Wanklyn albuminoid ammonia test often gave useful information in conjunction with the oxygen absorbed from permanganate in 4 hours at 80° F. In the case of waters contaminated with animal organic matter, the ratio of the albuminoid ammonia to oxygen absorbed in 4 hours at 80° F. was generally of the order of 1 to 5, whereas when the organic matter present was mainly of vegetable origin, the ratio would be of the order of 1 to 20 or 30.

Mr. E. M. HAWKINS said that when only small amounts of nitrite were present a slight excess of chalk gave good results and precluded oxalate complications.

Dr. E. C. JEE, commenting on the work on self-purification in sewage polluted rivers carried out by the Royal Commission on Sewage Disposal, interpreted the results as defining the replacement of the nitrogen phase of analytical methods by an oxygen phase in which an attempt had been made to evolve the biochemical dissolved-oxygen-absorbed test. The proposed reversion to nitrogen tests was, he thought, retrogressive. A further step had been taken by the Standing Committee on Rivers Pollution, who advocated concentrating on the examination of the rivers themselves by sub-surface dissolved oxygen estimations along the axis of the river.

Mr. HAIGH JOHNSON replied that he was in general agreement with Dr. Gee regarding the value of oxygen in solution; but he instanced the case of one river where the water below the point of pollution was frequently supersaturated. Unfortunately, this test involved too many factors—beyond the control of the observer—which affected the result; thus temperature and topography alone might cause a grossly polluted stream to exhibit a fully saturated condition. Control could only be obtained in the laboratory; hence the necessity for these other tests. He justified his attempts at correlation on the grounds that the oxygen absorption and organic nitrogen content were the only means of determining direct organic pollution, and should constitute the minimum of any chemical examination. The investigation dealt with the whole of this question, and had incidentally attempted to improve both processes. The difficulty of changing over to the new processes was relatively simple and fully justified by the advantages which would accrue.

He had never used magnesium carbonate; and calcium carbonate only in the preparation of the standard water. He agreed that the five days' period could be reduced if the temperature were increased, but many other obscure factors also needed consideration before comparisons were possible. The method now suggested did not usually involve more than a 3 days' period, even at the lower temperature, and afforded additional information as to stability, etc.

The Analysis of Egg Yolk Preserved with Glycerin.

By T. COCKBURN, F.I.C., and M. McF. LOVE, A.I.C.

The operation of the Public Health (Preservatives, etc., in Food) Regulations has caused an increasing use of glycerin in place of boron compounds, as a preservative for egg yolk.

Glycerined egg yolk does not appear to possess the same keeping properties as that preserved with boron compounds, though we are informed that for baking purposes it is quite satisfactory, sponge cakes, etc., retaining their softness longer than those prepared with borated egg yolk.

During the past few months we have examined a large number of samples of glycerined egg yolk and have made a comparative study of the methods for the determination of moisture, fat and glycerin. We prefer to determine moisture by drying *in vacuo* over sulphuric acid, though after 48 hours' desiccation this method gives results slightly lower than those obtained by drying at 100° C. in a flat porcelain dish, either with or without sand.

In the determination of fat or oil, care is necessary in the choice of a solvent. Thus we have seen it stated that, whilst extraction of the desiccated residue with hot chloroform is inadmissible on account of the simultaneous solution of some glycerin, extraction with cold chloroform is free from this objection and yields a satisfactory determination of the fat or oil. We have invariably found that extraction with cold chloroform dissolves an amount of glycerin sufficient to affect the result. When this chloroform extract was treated with petroleum spirit, washed with water in a separating funnel, and the residue weighed after distillation of the ether, an improvement was effected.

In our hands the Gottlieb process has proved a satisfactory and rapid method for the determination of the fat or oil. The accuracy of this method was confirmed by adding to egg yolk (unglycerined) in which the fat had been previously determined by maceration with chloroform and by the Gottlieb method, after the addition of known amounts of glycerin, the fat being again determined.

From the results shown below it will be seen that the Gottlieb method gives a close approximation to the true percentage of fat.

Fat by Calculation.

	1.	2.	3.
From chloroform extraction of original yolk ..	25·45	24·50	23·95
From result of Gottlieb method on original yolk	25·12	24·29	23·61

FAT FOUND.

	Hot chloroform.	Subsequent extraction with petroleum spirit.	Cold chloroform.	Subsequent extraction with petroleum spirit.	Gottlieb method.	Contains glycerin. Per Cent.
(1) ..	28·04	27·08	27·87	27·05	25·56	12·69
(2) ..	28·92	26·91	27·43	25·37	24·11	20·91
(3) ..	29·45	28·16	28·87	27·52	24·09	21·04

The following method gives fairly satisfactory results for the determination of the glycerin.

Ten grms. of the sample are made into a thin paste with water at 45° C. to 50° C. Dialysed iron (B.D.H.) is then added, drop by drop, until the proteins, etc., are precipitated; usually about 5 c.c. are required. The solution is filtered, the filtrate and washings made up to 250 c.c. One hundred c.c. are evaporated to a syrupy consistence in a flask and the glycerol determined by the usual acetin method.

We have examined many samples by this method, and the following examples, obtained by adding known quantities of pure glycerol (checked by actual determination) to yolk of egg, will indicate the accuracy which may be expected.

Glycerol added, per cent.	12·69	20·91	21·04
Glycerol found (acetin method), per cent.	12·98	21·26	21·29

If the addition of dialysed iron has been carried out carefully an approximation to the amount of glycerin is obtained as follows:

One hundred c.c. of the above filtrate are shaken twice in a separating funnel with 10 c.c. of ether to remove any fat present. The ethereal solutions are mixed, washed with water, and the washings added to the original aqueous layer. The aqueous solution is then transferred to a weighed platinum basin, evaporated in a water bath, dried in a steam oven for 1 hour and weighed.

The glycerol is driven off by ignition at as low a temperature as possible, the basin again weighed, and the difference being taken as glycerin. This method gives results which are about 1 per cent. too high.

We have to thank Mr. F. W. Harris for permission to publish these results.

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A Note on Technique in Testing for Vitamin B.

By A. L. BACHARACH, B.A., A.I.C., and
GLADYS ANNIE HARTWELL, D.Sc.

In tests for the presence or absence of vitamin *B* (water-soluble growth-promoting) carried out on rats, as recommended by Drummond and Watson (1922), their technique has generally been followed, though certain modifications have from time to time been made, as, for example, the omission of lemon juice from the standard basal diet (Bacharach, 1925).

Furthermore, one of us (Hartwell, 1925) has shown that considerable differences in condition of experimental rats can be observed according to the actual method of preparation of the synthetic diet used, and it seemed of interest to discover whether an analogous state of affairs existed with a diet deficient in certain respects.

We thought that it might also be of interest to find what variations would occur in the hands of different observers, using the same stock of rats and different techniques or using a different stock of rats and the same techniques. Accordingly the experiments described in these notes were carried out.

Animals.—We shall refer to the two investigators as B and H, to the two laboratories where their work was conducted as I and II, and to the two strains of rats as A (Albino) and P (Piebald). The rats ordinarily used by B in laboratory I are those described in a recent note (Bacharach, 1926). These are rats A. The rats used by H in laboratory II are animals of the pied variety, the result of 4½ to 5 years' inbreeding of an "intra-colonial" kind. These are rats P.

Food.—All the basal diet used in these experiments was prepared in laboratory I, and consisted of:

	Parts.
Caseinogen (free from Vitamin B)	20
Rice Starch	60
Cod Liver Oil	2
Cottonseed Oil	8
Salt mixture (McCollum's, plus trace KI)	5

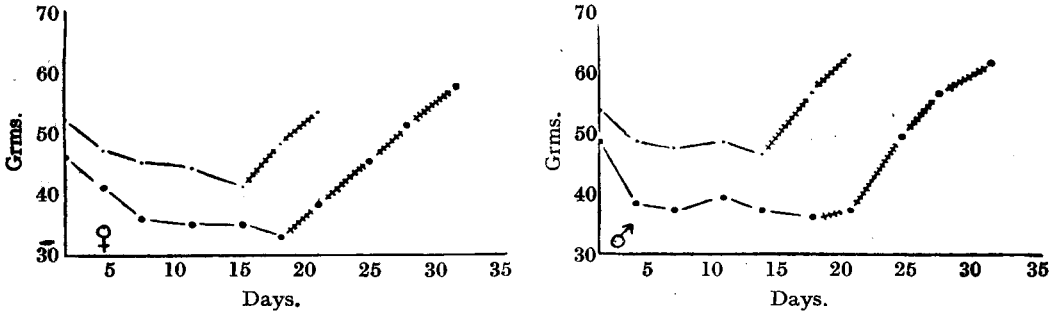
Two methods were used for preparing the food. Method 1 (B's technique) was identical with that laid down by Drummond and Watson (1922). The solid diet was mixed with about three-quarters of its weight of water to a stiff dough. Method 2 (H's technique) consisted in mixing a given weight of the dry food with three times its weight of water; it was then "cooked," as described by one of us in a previous communication (Hartwell, 1925).

Method of Experiment, Weighing, etc.—In laboratory I the animals were given almost exactly 10 grms. dry weight each per day, but no estimate was made of the amount consumed; tap-water was given *ad lib.* In laboratory II the animals were given food and distilled water *ad lib*; the approximate amount consumed was known. B weighed the animals twice a week, and H every day.

In all the experiments conducted by B on his own stock of animals (A rats), "litter control" was maintained. Whenever two groups were compared, every animal in one group was controlled by an animal from the same litter and of the same sex in the other group.

During the "curative" period, when marmite was administered in addition to the basal B-free diet, H incorporated the marmite in the diet, using a 5 per cent. solution of marmite instead of water to make up the moist food mixture. B administered separately 0·4 grm. of marmite per day to each animal.

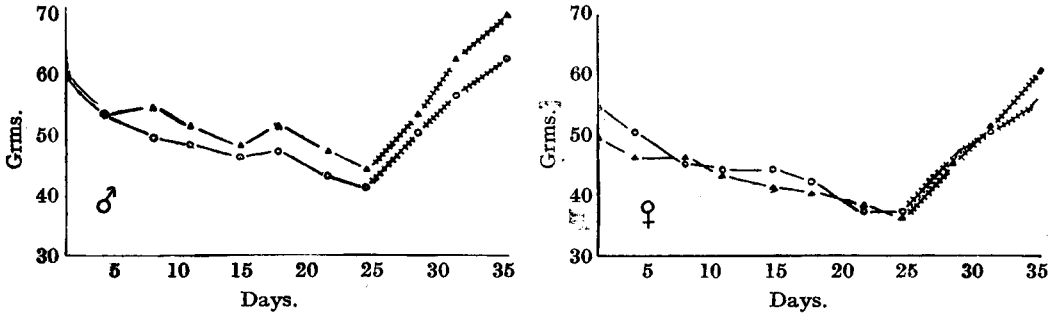
FIG. I.



Food prepared by the "raw" method. Piebald rats bred by H.

•——• Growth curves obtained by B (average 3 rats).
·——· ,, ,, ,, ,, H (,, 6 ,,).
Marmite added to the diet.

FIG. II.



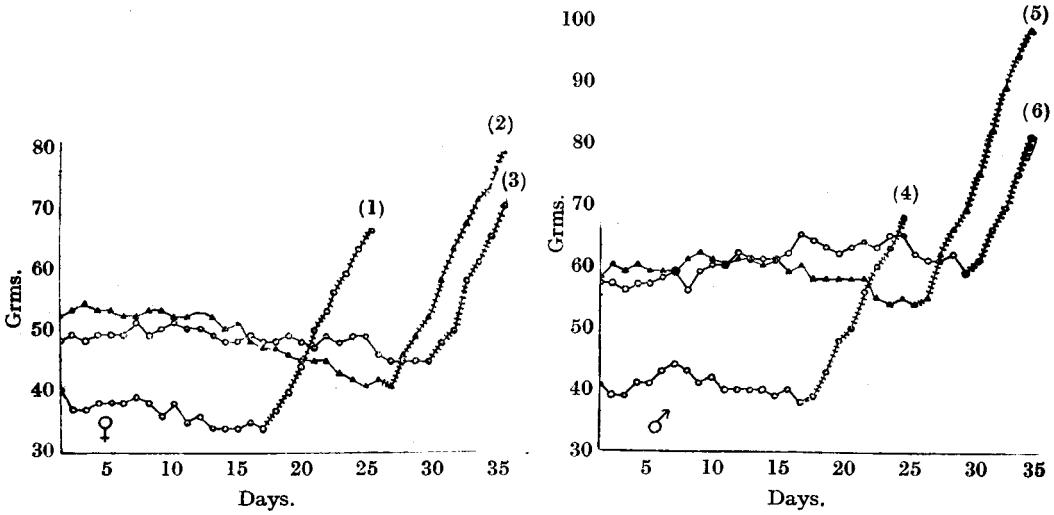
Food prepared by the "raw" method. Curves obtained by B.

▲——▲ Rats bred by B (Albino).
○——○ ,, ,, ,, H (Piebald).
Marmite added to the diet.

EXPERIMENT 1.—P rats were used by both B and H. The food in both cases was prepared by the first method ("raw"). During the latter part of the experiment a supplementary ration of marmite was given, B and H using his or her own method of administration. Very closely similar curves were obtained. It did not appear, therefore, that there were any unknown factors of importance

in the conditions under which B and H worked which were likely to vitiate the comparisons made by them when certain known factors were varied. The results of Experiment 1 are shown in Figure I.

FIG. III.

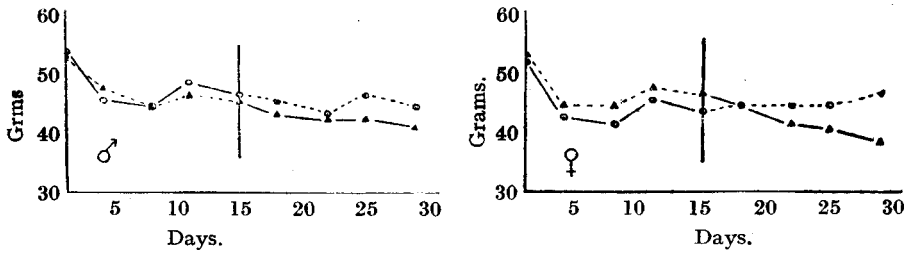


Food prepared by the "cooked" method. Curves obtained by H.

▲——▲ Rats bred by B (Albino).
○——○ ,, ,, ,, H (Piebald).
Marmite added to the diet.

(1) Average of 6 rats. (3) Average of 4 rats. (5) Average of 5 rats.
(2) ,, ,, 4 ,, (4) ,, ,, 6 ,, (6) ,, ,, 6 ,,

FIG. IV.



Curves obtained, and rats bred by B.
——— Food prepared by the "raw" method.
----- ,, ,, ,, ,, "cooked" ,,

EXPERIMENT 2.—In this experiment both B and H used both A and P rats, each worker still keeping to his or her own feeding technique. It appears as if each breed of rats did slightly better in a familiar laboratory than in strange surroundings, where they arrived after a journey, albeit a short one, and were subsequently handled by complete strangers. In spite of this very small difference,

an examination of the individual curves, which show very good agreement within each group, confirms our belief that each breed of rats was of a fairly uniform genetic constitution. Comparisons of B's experiments on A rats with the other series also shows that sex differences and small variations in pre-natal nutrition have no appreciable influence in this kind of test. The results of Experiment 2 are shown in Figures II and III.

EXPERIMENT 3.—In this experiment both B and H used their own rats, that is, A and P respectively. Each made two experiments; in one the animals were first fed on the "raw" diet and then changed over to the "cooked" diet, and in the other the process was reversed. Little difference was observed, either as between one worker and the other, or as between one feeding technique and the other. In the case of the experiments carried out by B, a slight rise in weight was observed when one group of rats was changed from the "raw" to the "cooked" basal diet. The results as a whole, however, are in agreement with the earlier work of one of us (Hartwell, 1925), who has shown that no appreciable difference was found during the first few weeks in the weights of similar animals fed by the two different methods. The results of B's experiments are shown in Figure IV, and in the following table:—

AVERAGE CHANGE IN WEIGHT.

(Grm. per animal per day.)

	Preliminary Period.			Subsequent Period.		
Diet.	♂	♀	Average.	♂	♀	Average.
"Cooked"	−0·54	−0·43	−0·48	−0·17	+0·19	+0·03
"Raw"	−0·52	−0·58	−0·55	−0·29	−0·56	−0·42

The experiments summarised in Figures II and III suggest distinctly that the loss of weight consequent upon a B-free diet shows more rapidly with the "raw" than with the "cooked" food. In earlier experiments one of us (Hartwell, 1925) noted no difference in effect for the first two weeks with a diet that was physiologically complete. Here the diet is inadequate; although the duration of Experiment 3 was somewhat short, at the time when the method of preparing the diet was changed the animals were obviously somewhat enfeebled, and therefore more susceptible than at the beginning both to the evil effect of a deficient diet and to any slight advantages due to an improved feeding technique.

It would appear, then, that the effects of vitamin *B* deficiency are somewhat more rapidly obtained when feeding with the "raw" than when feeding with the "cooked" food, and this suggests that the "raw" method is the more expeditious for testing for the absence of vitamin *B* (for example in determining the suitability of caseinogen for a basal diet) and the "cooked" method more sensitive in testing for the presence of vitamin *B*. In certain other experiments, not described in detail here, one of us (H) found that twelve animals fed on a B-free diet prepared by the "cooked" method all showed typical symptoms of B-deficiency, whereas twelve other rats given the same diet in the "raw" condition all died, though ten of them had shown none of the typical symptoms of B-deficiency.

Finally it should be added that certain other factors were found during the above experiments to be of comparative unimportance in this kind of work. Our results indicate fairly clearly that the exact age at which the animals are subjected to experiment is unimportant; the youngest group of animals used was 23 days old and the oldest 36. Similarly, the precise initial weight of the animals seems unimportant. The heaviest animal used weighed 61 grms. at the beginning of the experiment, and the lightest 43 grms. These figures are obtained solely from the experiments of B, but similar observations were made in the case of H's animals.

SUMMARY.—(1) Rats from two separate inbred stocks (albino and piebald) showed similar weight curves on a B-free diet.

(2) Similar weight curves were obtained by two workers in different laboratories, with both their own and each other's animals.

(3) On a diet lacking vitamin *B* the loss in weight shows more quickly when the food is given "raw" than when it is "cooked"; but the former method is not so specific a test for vitamin *B*, because the rats do not exhibit typical symptoms of B-deficiency before death.

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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 ANALYSIS OF SODIUM SALICYLATE AND SODIUM BENZOATE.

THE method of analysis of sodium salicylate and sodium benzoate given in the British Pharmacopoeia entails ignition, extraction with water, and titration with acid.

A quicker method of arriving at the same result is as follows:—Direct titration with standard acid, with methyl orange as indicator, is not possible, as the indicator is affected by the liberated acid. In the presence of neutral ether direct titration to a point just short of the true end-point (methyl orange as indicator) can be obtained. By removing the ether when this point is reached, and adding more neutral ether, the titration may be continued to the correct end-point.

A weighed quantity (2 grms.) of sodium salicylate is transferred with water to a cylindrical separator, and methyl orange and about 30 c.c. of neutral ether

added. Semi-normal hydrochloric acid is run in with careful shaking (usually the greater part of the standard acid which should be required can be added at once) until the methyl orange turns a distinct red; the colour is best seen while shaking horizontally over a sheet of white paper after each addition of acid.

At this stage the greater part of the salicylic acid has been liberated and is in the ethereal layer. The aqueous layer is run off into another separator, and water washings of the ether added; then neutral ether is added, and, on shaking, the indicator will revert to yellow. The titration is now continued (generally only a few more drops are required) until the end-point, which is quite sharp, is reached. The solution can be titrated back if the addition of acid be overdone. A third shaking with neutral ether is not necessary.

The same method is used in the analysis of sodium benzoate, 1 grm. of the sample being used and titrated with 0·5 *N* hydrochloric acid. The first end-point is sharp and is within a few drops of the final figure.

The quantities given above for the determinations are those laid down in the British Pharmacopoeia, but smaller quantities may conveniently be taken and 0·1 *N* hydrochloric acid used for neutralisation. This method can usefully be employed when sodium salicylate is mixed with other salts. In the presence of neutral salts the proportion of sodium salicylate can usually be estimated as has been described.

In the presence of sodium or potassium carbonates or bicarbonates the sodium salicylate may be determined as follows:—

(1) Titrate by the method described. The figure obtained represents the total combined alkali.

(2) *A* Remove carbonates by precipitation as insoluble carbonate, filter, wash and titrate filtrate as in (1). The figure obtained is calculated to sodium salicylate. The proportion of carbonate is obtained by difference from the total titration figure, or the precipitate of carbonate may be titrated. (If bicarbonate is to be determined by the titration of the precipitate of insoluble carbonate, ammonia must be added with the precipitating agents.)

B Use phenolphthalein as indicator and titrate the solution with standard acid while boiling until the colour remains discharged. This figure is calculated to carbonate or bicarbonate, whichever is present, and the difference from the total titration figure is calculated to sodium salicylate. Further, the sodium salicylate may be obtained by titrating the cold neutral solution as in (1).

Medicinal mixtures, such as sodium salicylate and potassium bicarbonate, etc., may be quickly analysed, except in cases where the solution is so strongly coloured that the indicator change is masked.

If required, the salicylic acid in the mixed ethereal portions may be determined by distillation at a low temperature in the usual way. The acid can also be determined by direct titration, but where the ether extractions have been obtained from a carbonate and salicylate mixture the phenolphthalein indicator is acted on by the carbon dioxide present. In these circumstances the acid may be determined by extraction from the ether with a slight excess of standard sodium hydroxide. The dissolved ether is gently expelled, and the excess of sodium hydroxide neutralised by the addition of standard acid, phenolphthalein being used as indicator and the liquid boiled until the colour remains discharged. It is essential that the ether used should have been thoroughly purified over sodium hydroxide.

DOUGLAS HENVILLE.

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.

FAT IN CHESHIRE CHEESE.

On January 14 a Burslem tradesman was summoned at Stoke-on-Trent for having sold Cheshire cheese deficient in fat. On analysis the sample was found to contain, on the moisture-free substance 37·68 per cent. of fat instead of 45 per per cent. as recognised by the Cheshire Farmers' Union as the standard for fat, calculated on the moisture-free Cheshire cheese.

The solicitor for the defence asked whether the prosecution intended to call expert evidence as to the minimum amount of fat permissible in Cheshire; if not, in his submission, the statement regarding the supposed recognised standard was inadmissible.

The stipendiary pointed out that, as there was no statutory standard for cheese, the standard was fixed by the magistrates in accordance with previous decisions.

To this the solicitor replied that the stipendiary had no option but to dismiss the case, in view of the fact that the Public Analyst was not competent to fix the standard for fat at 45 per cent., and that this standard was not generally known among grocers.

The stipendiary said that whilst expert evidence was sometimes necessary, it imposed a hardship on a small trader, who had to bear the costs when there was a conviction. According to the secretary of the Cheshire Farmers' Union, Cheshire cheese was a full cream cheese, and should contain from 47 to 50 per cent. of fat, calculated on the dry basis. Having regard to special circumstances in this case, he would impose a nominal penalty of £1.

ADULTERATION AND MISBRANDING OF VINEGAR.*

On August 6, 1925, the U.S. Attorney for the Western District of Arkansas alleged shipment by the defendant company, in violation of the Food and Drugs Act, from the State of Arkansas into the State of Oklahoma, of a quantity of vinegar which was adulterated and misbranded.

Adulteration of the article was alleged in the information, for the reason that a compound, consisting of distilled vinegar and sugar vinegar and containing a mere trace of apple vinegar, if any, had been substituted for apple vinegar, which the article purported to be; for the further reason that distilled vinegar and sugar vinegar had been mixed and packed therewith so as to reduce and lower and injuriously affect its quality; and for the further reason that it had been coloured with sugar vinegar so as to simulate apple vinegar, in a manner whereby damage and inferiority were concealed.

Misbranding was alleged for the reason that the statement, to wit, "Apple Vinegar," in large prominent type, together with the pictorial design of an apple, borne on the label of the bottle containing the article, was false and misleading,

* U.S.A. Dept. Agric. Service and Regulatory Announcements, Bureau of Chemistry. Supplement 215 (1926). No. 14,244.

in that they represented that the said article was apple vinegar, and for the further reason that it was labelled as aforesaid so as to deceive and mislead the purchaser into the belief that it was apple vinegar, whereas it was not apple vinegar but was a compound consisting of distilled vinegar and containing but a trace of apple vinegar, if any, and the said article was not labelled so as to indicate plainly that it was a compound, in that the words "Compound," "And Sugar" appeared in small inconspicuous type. Misbranding was alleged for the further reason that the article was an imitation of, and was offered for sale under the distinctive name of another article, to wit, apple vinegar.

On March 5, 1926, the defendant entered a plea of guilty to the information, and the court imposed a fine of $20.

ADULTERATION AND MISBRANDING OF EGG SUBSTITUTE.*

ON May 18, 1921, the U.S. Attorney for the District of Nebraska, acting upon a report by the Secretary of Agriculture, asked for the seizure and condemnation of 418 lbs. of alleged egg substitute, shipped in two consignments from the State of Maryland into the State of Nebraska, and charging adulteration and misbranding in violation of the Food and Drugs Act.

It was alleged that the article was adulterated within the meaning of Sec. 7 of the Act, par. 1 and 2, under Food, in that it was a mixture of skimmed milk, corn starch and sugar, coloured with coal-tar dye. Adulteration was further alleged in that the article was mixed and coloured in a manner whereby inferiority was concealed.

Misbranding was alleged for the reason that the label bore the statement, "Egg Substitute," which was false and misleading and deceived and misled the purchaser, and for the further reason that the article was an imitation of, and offered for sale under the distinctive name of another article.

On December 3, 1925, the defendant company, having withdrawn its claim and all pleadings without admitting the charges of misbranding or adulteration, but expressly denying the same, and having stated that the manufacture of the product covered by the libel had been discontinued and that the question of fact involved in this case would not be conclusive in any future proceeding, judgment was entered, forfeiting the product to the Government and ordering that costs be paid by the claimant.

ADULTERATION AND MISBRANDING OF INSECT POWDER.

THE following is typical of several judgments published by the U.S. Department of Agriculture†:—

On October 18, 1924, the U.S. Attorney for the Northern District of Ohio asked for the condemnation and forfeiture of 220 lbs. of "Insect Powder," shipped from the State of New York into the State of Ohio, alleging that it was an adulterated and misbranded insecticide within the meaning of the Insecticide Act of 1910.

Adulteration of the article was alleged for the reason that the words "Insect Powder," borne on the tags affixed to the cases containing the said article,

* U.S.A. Dept. Agric. Service and Regulatory Announcements, Bureau of Chemistry. Supplement 217 (1926). No. 14,312.

† U.S.A. Dept. Agric. Service and Regulatory Announcements [54], Insecticide and Fungicide Board (1926). No. 1035.

represented that it consisted entirely of insect powder, that is to say, powdered pyrethrum flower heads; whereas the strength and purity of the article fell below the professed standard and quality under which it was sold, in that it did not consist entirely of insect powder, but did consist of powdered pyrethrum stems, which had been substituted in whole or in part for insect powder which the said article purported to be.

Misbranding was alleged for the reason that the words, "Insect Powder," borne on the said tags, were false and misleading, in that they represented that the article consisted entirely of insect powder; whereas, it consisted in whole or in part of powdered pyrethrum stems. Moreover, the name and percentage amount of the said inert substance were not stated plainly and correctly, or at all, on the tags affixed to the cases containing the article.

On November 2, 1925, no claimant having appeared for the property, judgment of condemnation and forfeiture was entered, and it was ordered by the court that the product be destroyed by the United States marshal.

Ministry of Health.

THE DETERMINATION OF BENZOIC ACID IN FOOD STUFFS.*

THIS Report reviews the existing literature with references to papers and abstracts, arranged in a bibliography at the end. Prominence is given to the principles upon which the methods are based, rather than to manipulative details, excepting when exact observance of detail is essential. The Report deals not only with the foodstuffs in which benzoic acid is permitted, but also with other foods (milk, fats and meal products), in which it has, at times, been used as a preservative. Details are given of a new distillation method† which has given good results in the determination of benzoc acid in certain typical fruits.

The Report has not been published with the view of prescribing or imposing any special methods of analysis or testing under the Regulations, nor yet of a standardised technique in regard to benzoic acid; but of providing information which it is hoped may be useful and timely to public analysts, chemical advisers of industrial concerns, and laymen and others charged with duties or responsibilities in this matter.

From a study of the literature the following conclusions are drawn:

1. Removal or destruction of proteins is almost always necessary, as they are liable to cause emulsions on extraction with solvents, and also to retain benzoic acid.

2. Removal of proteins by precipitation may lead to loss of benzoic acid in several ways:—(i) By precipitation of metallic benzoates. (ii) By actual precipitation of the free acid, in solutions containing much salt or mineral acid. (iii) By absorption of benzoic acid by fat or fatty acids.

3. With foods containing much fat it is probably safer to separate the fat, and to extract benzoic acid independently from the fat and from the fat-free residue.

* *Reports on Public Health and Medical Subjects.* No. 39. By G. W. Monier-Williams, O.B.E., M.A., Ph.D., F.I.C. Pp. 57. Ministry of Health. 1927. H.M. Stationery Office. Price 1s. net.

† An abstract of this method will be published in the next issue.—EDITOR.

4. Steam distillation is successful with non-fatty foods if certain precautions are observed.

5. The separation of benzoic acid from volatile fatty acids is a matter of some difficulty.

6. It is only in rare cases that direct extraction yields a product sufficiently pure for titration or weighing without further treatment.

7. Of the various purification methods:—(i) Washing the solvent with water requires care and discrimination, and may lead to loss of benzoic acid from some solvents. Washing with salt solution is preferable in many cases. (ii) Oxidation with alkaline permanganate is effective in removing impurites, with certain exceptions. (iii) Precipitation of insoluble benzoates is of limited application. (iv) Sublimation is the best and most reliable method, but may fail if any considerable amount of impurity is present.

8. The most delicate test for benzoic acid is Mohler's test (formation of ammonium *m*-diamino-benzoate), The ferric chloride test is somewhat less delicate, but more characteristic. The most characteristic test is Jonescu's (conversion into salicyclic acid by hydrogen peroxide), if we except those which depend on the sense of smell.

9. In only one edible fruit (cowberries) does benzoic acid occur naturally to any appreciable extent, but certain substances occasionally met with in foodstuffs may give reactions similar to those of benzoic acid, and care is necessary in applying confirmatory tests.

THE MILK AND DAIRIES ORDER, 1926.

CIRCULAR 757.*

THIS Circular, dated January 20th, 1927, has been sent by the Minister of Health to Clerks of County Councils and Sanitary Authorities in England and Wales, to indicate his views on the construction and administration of the Order, especially in relation to farmers who sell only a small quantity of milk, mainly to their employees and their neighbours.

REGISTRATION UNDER THE ORDER: (2), (3) and (4).—Under the Act a "dairy" includes *inter alia* all farms from which milk is supplied for sale, whether or not the extent of such sale amounts to the carrying on of a trade. If a farm keeps more cows than would normally be required for the needs of his household, there appears to be *prima facie* ground for assuming that he is "carrying on the trade of a dairyman."

APPLICATION OF THE ORDER.—The general requirements of the Order, prescribing the conditions of cowsheds and other registered premises, apply to all places where milk is produced for sale whether in large or small quantities.

ADMINISTRATION: (6), (7), (8), and (9).—The standard to be attained is left to a large extent to the Local Authority, subject, in case of dispute, to the ruling of the Courts. Co-operation with the Agricultural Education Authority is recommended.

CLEAN MILK COURSES FOR SANITARY INSPECTORS.—This is dealt with in paragraphs 10 to 12.

REGISTRATION PROCEDURE.—(Par. 13–17.) The exact form of registers to be kept is left to the decision of Local Authorities. Suggestions are made as to information that would be useful.

INSPECTION OF CATTLE.—This is dealt with in paragraphs 18 and 19.

* H.M. Stationery Office. 1927. Price 1d. net.

Meteorological Office, Air Ministry.

ADVISORY COMMITTEE ON ATMOSPHERIC POLLUTION.*

REPORT ON OBSERVATIONS IN THE YEAR ENDING MARCH 31ST, 1926.

STANDARD GAUGE.—There are now 29 authorities co-operating in the collection of data from 61 deposit gauges, an increase of 12 gauges over the previous year. One of these gauges is at Brighton—the first set up south of Kingston-on-Thames. The deposits recorded are grouped under letters as before, and the highest and lowest deposits, summer (S) and winter (W), expressed as metric tons per hundred sq. kilometres for the year, are as follows: *Tar;* (S) Burnley, 494; London, Golden Lane, 17; (W) Burnley, 491; Huddersfield, Cooper Bridge, 23. *Carbonaceous matter;* (S) St. Helens, 4352; Rothamsted, 313; (W) Newcastle, City Road, 7833; Rothamsted, 181. *Insoluble ash;* (S) Newcastle, City Road, 6900; Marple, 292; (W) Newcastle, City Road, 9358; Marple, 147. *Volatile matter,* (S) Wakefield, 3006; London, Ravenscourt Park, 259; (W) Wakefield, 4820; Birmingham, Southwestern, 254. *Soluble ash;* (S) Burnley, 3774; Rothamsted, 294; (W) Burnley, 6704; Rothamsted, 425. *Total Solids,* (S) St. Helens, 16,621; Leeds, Headingley, 1813; (W) Newcastle, City Road, 21,580; Rothamsted, 1440. *Rainfall,* in mm. (S) Salford, 557; London, Meteorological Office, 235; (W) Burnley, 734; Rothamsted, 262.

AUTOMATIC FILTER.—Records are given for the same number of instruments as last year, and on the same plans. It is noticeable that the double maximum in the Blackburn curves has disappeared, and, if the first maximum is taken as due to industrial smoke and the second to domestic smoke, the proportion of the former has been reduced in relation to the latter. In Victoria Street the percentage of "Z" days (maximum over 1·28 mgrms. per c.c.) is higher than last year, 20 per cent higher for S. Kensington, the same for Kew, less for Blackburn and much less for Stoke-on-Trent.

JET DUST COUNTER.—Continued work in England with this instrument (ANALYST, 1926, 51, 86), has not been possible, but American observations confirm the fact that high wind velocities show a lower dust count, and also suggest that the product of the number of dust particles per c.c., the relative humidity, and the visibility in miles, is a constant.

SUNDRY RESEARCHES.—Dust from furnace ash may contain large numbers of spherical bodies which can be colourless, transparent and polished, or deep ruby or yellow, solid, or hollow, and may travel much greater distances in the air than solid particles. It is established that there is a close relation between the amount of ultra-violet radiation received on the ground and freedom from smoke pollution. The relation between tar and sulphur, respectively to total deposit is discussed.

A NEW ATMOSPHERIC DEPOSIT RECORDER.—The instrument consists of a circular tray (about 10 in. diameter) rotating once in 24 hours by clockwork in a horizontal plane and enclosed in a cylindrical zinc box which has a sector-shaped opening in the lid, 1·8 sq. in. in sectional area, and through which passes a 2 in. tube reaching to within a quarter of an inch of the tray, and to this opening a fresh surface of the tray is continuously exposed. The tray is covered by a piece of perforated zinc allowing rain water to collect underneath, and covered

* M.O. 290. H.M. Stationery Office, Kingsway, W.C.2. Price 6s. 6d. net.

in its turn by filter paper kept moist by water in the tray, and to which the insoluble matter deposited adheres. In order to count the particles, the damp filter paper with the deposit is laid centrally on a piece of white paper with radial lines drawn from the centre, dividing it into 2 equal sectors, making a time scale for the deposit; this paper is soaked in melted paraffin wax. A large number of curves are drawn from data collected with this instrument, which has proved itself useful in enabling a finer analysis to be made of the effect of rain, snow, wind force, and wind direction on the deposition of impurities from the air than is possible with the standard gauge.

D.G.H.

Food Standards for Madras.

A REPORT on the Madras Food Adulteration Act 1918, has been made by Mr. H. Hawley, M.Sc., F.I.C., Public Analyst with the Government of Madras, and his recommendations for food standards (given in outline below) have been followed in the official regulations* and in a Draft Amendment Act which has been published by the Madras Government for criticism.

THE MADRAS PREVENTION OF ADULTERATION ACT, 1918.—This is based on the English Food and Drugs Acts, 1875 and 1899, but an important consideration appears to have been overlooked in drafting the Madras Act. In England no regulations have been made under the Food and Drugs Acts since 1902, as it has been found difficult to use these Acts in making regulations prohibiting or limiting the addition of colouring matters, preservatives or other substances, however undesirable, where such addition has become a trade custom. Such regulations are now always made under the Public Health Acts.

Under the Madras Act the standards to be prescribed are only allowed to "raise a presumption until the contrary is proved" that the sample is not genuine by reason of the fact that it does not comply with the official standards, and a vendor can counter the presumption of adulteration by proving a "custom of the trade" either generally or in a particular locality. As it stands, the Act could not prevent the sale of watered milk with a declaration to the effect that it is not sold as genuine milk, nor could it deal with the sale of "fore-milk" (the calf being allowed to take the last and richest portions of the milk) as "milk," in cases where a vendor establishes a plea of "custom of the trade."

Mr. Hawley suggests that later amendments should be made to the Madras Act by which it might be made to combine the functions of both the English Food and Drugs Acts and the Public Health Acts (so far as they relate to adulteration).

A further amendment is necessary to enable sample-takers to add a preservative to milk samples; otherwise there is a possibility, particularly in the case of samples sent from a distance, that the sample bottle may burst *en route*, or that, on arrival at the analyst's laboratory, it may be too badly decomposed for an analysis to be possible. Powers to make such an addition have been taken by the Government of Bengal in their regulations under their Food Adulteration Act, but under the Madras Act, as it stands at present, any such regulation would probably be *ultra vires*. On the other hand, should a sample taker add a preservative without legal authority, the vendor might plead that the sample, as analysed by the Public Analyst, was not as sold by him, and might get a dismissal of his case on the technical point.

* Government of Madras. Local Self-Government Department (Public Health). G.O. No. 1329, P.H., Aug. 4, 1926.

MILK STANDARDS.—The only official standards in India are those of the Bengal Government, who have adopted standards of 3·5 per cent. of fat, and 8·5 per cent. of solids-not-fat for cows' milk. It is recommended that the English standards of 3 per cent. of fat and 8·5 per cent. of solids-not-fat should be adopted for Madras, since the Bengal standard would be too high for Madras. It would mean the condemnation of a large proportion of milks taken during the monsoon period. The average fat content of the whole of the samples taken from individual cows was 4.16 per cent. The lowest average was for morning milk during the monsoon period (3·41 per cent.), the highest 4.60 per cent. for evening milk during the pre-monsoon period.

It would be a step in the right direction if a definition of milk were given in clause 2 of the Act, such as to prevent fore-milk being sold as "milk," while allowing the sale of after-milk.

It is recommended that a supplementary standard of 0·50 per cent. of nitrogen should be included, to be used by the analyst at his option, for the purpose of calculating the percentage of added water in an adulterated sample, in those cases where he considers that the sample is so far decomposed that a determination of solids-not-fat would be unreliable. The lowest percentage of nitrogen found in an authenticated sample was 0·48 per cent., which would correspond to only 4 per cent. of added water.

Buffalo Milk.—The standards adopted by the Government of Bengal are 6·0 per cent. of fat and 9·0 per cent. of solids-not-fat. The limits recommended by the Health Officer for Bombay (no limits have been officially adopted) are 5·0 per cent. of fat and 9·0 per cent. of solids-not-fat. Mr. Hawley's recommendation for Madras is that the standard minimum limits should be 4·5 per cent. of fat, 9·0 per cent. of solids-not-fat, and a supplementary limit of 0·53 per cent. of nitrogen. Of the 33 samples from individual buffaloes, one (of morning milk) contained only 4·3 per cent. of fat, and another (evening milk) contained only 4.2 per cent.; the maximum amount found was 8.3 per cent. The limit recommended for solids-not-fat is more lenient than that for cows' milk, as only one sample gave a figure below 9·0 per cent. (8·8 per cent.), but the adoption of a limit more stringent than that in force in Bengal might suggest to the Courts that the Madras standards were too stringent, with the result that only trifling penalties might be imposed.

DIRT IN MILK.—Much of the milk in Madras is obtained under very dirty conditions. In England a commonly accepted standard is that of 2 parts of sediment per 100,000 of milk, but this would probably be too stringent for India, and it is suggested that a limit of 5 parts per 100,000 should be adopted. Four samples of milk from the Military Dairy at Bangalore contained 1·5, 5·0, 5·0 and 7·5 parts of sediment per 100,000 respectively, and four samples of Madras milk, as supplied in bulk to one of the hospitals, contained 70, 15, 17 and 30 parts, respectively, particles of cow dung being identified in each case. At the Bangalore Dairy the precautions taken to obtain clean milk consist solely of a proper washing of the cow and of the milker's hands, and of insistance on the milker milking with dry hands.

BUTTER, GHEE AND CHEESE STANDARDS.—It is recommended that the English Preservatives Regulations should be adopted *in toto* under the Madras Act.

A limit of 20 per cent. is suggested for the moisture in butter, since, without the use of ice, it is difficult to make butter with so low a moisture content as 16 per cent. Of the samples purchased casually in Madras, only one contained less than 20 per cent. of moisture (19·8 per cent.), whilst other samples contained

from 20·0 to 27·9 per cent., and one sample from a Madras dairy contained 57·5 per cent.

For ghee it is suggested that a 1 per cent. limit should be allowed for adventitious moisture. The natural standard for the fat in butter or ghee is the adoption of the definition given in the Act, *viz.* that it be exclusively milk fat.

In Bengal, standard Reichert values of 24 for cows' butter fat, and of 30 for buffalo butter fat have been fixed. Probably the animals in Bengal produce milk fat giving very different results from those in Madras, since the Bengal limits would be unworkable in Madras. Of 52 samples of cows' butter fat examined, 25 gave figures below 24, four of these being below 20; the lowest value was 14·7. Of 29 samples of buffalo-milk butter fat, only two gave values which would have been accepted as those of genuine fats in Bengal (30 and 31), whilst the remainder gave values varying from 23·0 to 29·7. The average value for cows' butter fat was 23·1, and for buffalo butter fat 26·7.

As regards cheese the only standard prescribed is that it shall conform to the definition given in the Act, and be made exclusively from milk or cream, unless labelled and sold otherwise in accordance with the regulations to be made under sec. 20 (*d*).

Federated Malay States.

REPORT OF THE CHEMIST, INSTITUTE FOR MEDICAL RESEARCH, FOR THE YEAR 1925.

THE report of the Chemist, Mr. R. W. Blair, F.I.C., gives an outline of the work undertaken by the Chemical Laboratory for different Government Departments. The total number of samples examined during the year was 6021, as compared with 7368 in the previous year.

MILK.—According to the Sale of Food and Drugs Enactment, 1913, milk must contain not less than 3.25 per cent. of fat, and not less than 8·5 per cent. of solids-not-fat. Of the 741 samples examined, 19 were deficient in fat, and 126 in solids-not-fat.

TODDY.—The standard for acidity (as acetic acid) is 0·8 per cent. One of the 262 samples examined showed acidity in excess of that amount.

TOXICOLOGICAL ANALYSIS.—Twenty-four exhibits were examined for the medical authorities. Arsenic was found in one of the six cases of human poisoning and in one of the four cases of animal poisoning.

VITAMIN B EXTRACT.—The preparation of this extract from rice polishings, for the treatment of beri-beri, was continued during the year, 6056 fluid ounces being prepared. The extract, which is issued free or at cost price, is now made by a modification of the original method. Fresh rice polishings (2 kilos) are sifted and extracted four times during a week with four times their weight of 20 per cent. alcohol containing 0·1 per cent. of hydrochloric acid. The liquid is filtered through paper, with the aid of suction, and concentrated under reduced pressure (about 40 m.m.), to about 800 c.c., which is then made up to 900 c.c., and mixed with 100 c.c. of distilled alcohol.

CHANDU.—The Chandu Enactment prohibits the importation of chandu of other than Government manufacture, and also makes it an offence to be in possession of chandu re-prepared from dross, or more than 7½ tahils (1 tahil = about 37·8 grms.) of Government Chandu. Of the 400 samples examined for the

Trade and Customs Department, 209 were Government chandu, 167 illicit chandu, 5 chandu prepared from Government dross, and 4 imitation chandu.

POLICE DEPARTMENT. *Coins and Coining Materials.*—Of the 495 exhibits, 236 were counterfeit coins, 5 were genuine coins, and 16 moulds, the remainder consisting of pieces of metals and chemicals used in the manufacture of counterfeit coins.

Blood Stains.—Thirty-nine of the 107 exhibits gave reactions for blood, and 35 of these showed the precipitin reaction for human blood.

Toxicological.—Poison was found in 6 of 16 exhibits of human viscera, opium being present in 2, morphine in 1, acetic acid in 1, arsenic in 1 and strychnine in 1. Other exhibits included vomits, urine and four medicines. The poisons identified were morphine in two exhibits, opium in one, arsenic one, strychnine one, and the alkaloids of *Datura stramomium* in one.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs Analysis.

Edible Viscera. A. M. Wright and J. C. Forsyth. (*J. Soc. Chem. Ind.*, 1927, **46**, 36–38.)—Tables are given showing the yield of edible viscera (the edible by-products of slaughtered animals not included in the carcase), and blood from sheep, lambs and steers. The results of other workers on the vitamin contents are summarised and compared with those for other foodstuffs. Liver and kidney are particularly rich in vitamins *A*, *B* and *C*, but deteriorate rapidly soon after the slaughter of the animal. The tables show the chemical composition (in percentages) of edible viscera from the sheep and the ox.

SHEEP.

	Tongue.	Heart.	Kidney.	Liver.	Brain.	Thymus.	Dia-phragm.	Blood.	Pan-creas.	Tripe.
Moisture	69·46	69·80	78·96	71·26	80·24	79·46	73·24	80·66	—	—
Ether extract	14·71	11·54	3·52	3·90	6·92	3·84	2·78	0·58	—	—
Ash	1·21	1·12	1·20	1·22	1·30	1·27	1·02	0·81	—	—
Phosphorus	0·19	0·20	0·22	0·33	0·29	0·22	0·21	0·02	—	—
Total nitrogen	2·32	2·70	2·54	3·10	1·66	2·26	3·54	2·79	—	—
Insoluble ,,	1·66	1·62	1·34	2·23	1·47	1·73	2·37	—	—	—
Amino ,,	0·22	0·24	0·21	0·26	0·08	0·34	0·28	—	—	—
Carbohydrates (dextrose)	—	0·26	—	3·36	—	—	0·19	—	—	—
Acidity (lactic acid)	0·17	0·46	0·22	—	—	—	0·38	—	—	—

OX.

Moisture	68·30	70·32	79·36	69·82	78·04	—	—	79·34	70·92	84·72
Ether extract	11·46	10·86	2·78	3·12	7·26	—	—	0·52	12·16	1·96
Ash	1·18	1·24	1·16	1·32	1·44	—	—	0·90	1·54	0·26
Phosphorus	0·19	0·19	0·23	0·36	0·34	—	—	0·12	0·42	0·05
Total nitrogen	2·90	2·66	2·60	3·26	1·78	—	—	3·07	2·73	2·01
Amino ,,	0·34	0·26	0·38	0·24	—	—	—	—	1·88	1·87
Carbohydrates (dextrose)	0·17	0·51	0·30	—	—	—	—	—	—	—

The compositions and food values of the edible viscera and of the meat extracts prepared from them are also discussed. J. G.

Determination of the Amount of Connective Tissue in Meat. H. H. Mitchell, R. L. Zimmerman and T. S. Hamilton. (*J. Biol. Chem.*, 1927, **71**, 379–387.)—Schepilewsky (*Arch. Hyg.*, 1899, **34**, 348) worked out a chemical method for the determination of the connective tissue in flesh, and this has been followed on a number of samples of meat. It is found, however, that the protein collagen is appreciably soluble in 5 per cent. sodium hydroxide (the solution used to remove the last traces of muscle tissue), and thus results for the connective tissue proteins are low by this method. A new method for the determination is described in detail. The procedure for the separation is as follows :—Most of the muscle tissue is removed by cold water extraction and mechanical separation on a sieve, according to a method equivalent to that of Schepilewsky, and the collagen is converted into gelatin by heating in the autoclave and then removed by exhaustive extraction with hot water. The residue remaining is digested with an alkaline trypsin solution at approximately 40° C., for the muscle proteins are readily digested by trypsin, whilst elastin is quite resistant and remains in the residue. The determination method will not detect small differences in the connective tissue content of different samples of meat, but it is sufficiently accurate for many practical purposes. A table gives the amounts of collagen nitrogen and elastin nitrogen found in various samples of meat. A rib of beef is a tenderer cut than a shank, and shows a smaller content of connective tissue. According to the analysis pork tenderloin is also a tender cut. A composite sample of cockerel flesh is tougher than that of pullet flesh, and the thigh of a chicken is tougher than the breast. Some results by other workers, obtained by other methods of separation, are given for a rough comparison. P. H. P.

Influence of Peptic Digestion in the Determination of Total Carbohydrates in Cereal Products. B. G. Hartmann and F. Hillig. (*J. Assoc. Off. Agric. Chem.*, 1926, **9**, 482–484.)—The difficulty of completely converting the starch present in cereal products by the action of diastase or acid appears to be due to occlusion of starch by the proteins, since preliminary overnight digestion of a flour with pepsin renders it possible to complete diastatic conversion in 30 minutes.

T. H. P.

Detection of Apple in Jams. C. F. Muttelet. (*Ann. Falsif.*, 1926, **19**, 580–585.)—In the jellies, jams and juices of preserved apples, pears and quinces laevulose predominates over glucose, but in the juices of apricots, peaches, greengages, mirabelle plums, and red plums, glucose is in excess, whilst in red and white gooseberries the two sugars are found in practically equivalent proportions. Tables are given showing the proportions of these sugars present in the juices of preserved fruits, and in home-made and commercial jellies and jams. In the case of citric acid fruits the presence of apple may be determined either from the sugar figures or by detection of malic acid, whilst in the presence of other malic acid fruits the proportion of acid may be determined or the sugars. In apple jellies indications of adulteration may be obtained by determining the sugars.

D. G. H.

Iodine Value of Paprika Oil. L. C. Mitchell. (*J. Assoc. Off. Agric. Chem.*, 1926, **9**, 477–482.)—The iodine value of the non-volatile ether extract of paprika was suggested by Doolittle and Ogden (*J. Amer. Chem. Soc.*, 1908, **30**, 1481) as a means of detecting the addition of olive oil to the ground product. Paprika oil readily undergoes oxidation, which lowers the iodine value. This effect may be reduced to a minimum by extracting the paprika with chloroform and using one portion of the extract for the determination of the oil content, and another portion for measuring the iodine absorption. The procedure suggested gives iodine values in close agreement with those yielded by the anhydrous ether extraction method and occupies only one-half to one-third of the time, as the drying of the sample over sulphuric acid and the preparation of anhydrous, alcohol-free ether are avoided. T. H. P.

Detection of Castor Oil in Fatty Mixtures. Vizern and Guillot. (*Ann. Chim. anal.*, 1927, **9**, 1–2.)—Castor oil may be detected in fatty mixtures by the formation of octylic alcohol when about 10 grms. of the sample are saponified, and the dry soap mixed with 7 to 8 grms. of potassium hydroxide and heated in a porcelain dish ; the fused alkali is stirred into the soap, and heating is stopped on the appearance of white fumes, and the dish covered. When cool, the odour of octylic alcohol is perceptible on the under side of the lid if 5 per cent. of castor oil was originally present, and by using a control with the same predominating oil as in the sample as little as 1 per cent. may thus be detected. D. G. H.

Micrographic Detection of Tartaric Acid in Officinal Preparations. M. François and C. Normand. (*Ann. Falsif.*, 1926, **19**, 599–605.)—The crystals formed on precipitating tartaric acid by calcium acetate are characteristic (photomicrographs given), and the presence of sugar does not interfere with their formation. For syrups or lemonades 80 c.c. of water are added to 100 c.c. of the sample, followed by 20 c.c. of a strong calcium acetate solution (*Ann. Falsif.*, 1923, 602), the mixture left for 3 days, and the crystals examined microscopically. Iron in the syrup should be previously eliminated. In the case of wines, elixirs and medicinal vinegars the wine can usually be detected by other means, but the characteristic crystals are formed if 100 c.c. of the sample are treated with 25 c.c. of a lead acetate solution (200 grms. per litre), followed by 25 c.c. of sodium carbonate solution (400 grms. per litre), the liquid filtered after 15 minutes, the filtrate made up to 180 c.c., and 4 c.c. of glacial acetic acid and 20 c.c. of strong calcium acetate solution added. The crystals are examined after 3 days. In the case of certain Malaga wines the solubility of the calcium tartrate must be diminished by adding 50 c.c. of alcohol at the same time as the calcium acetate solution. For such saline compounds as Seidlitz powders magnesium sulphate must first be removed. D. G. H.

Ethyl Phthalate Test. H. Wales. (*J. Assoc. Off. Agric. Chem.*, 1926, **9**, 476–477.)—Fluorescence obtained from drug products containing alcohol by extracting with light petroleum or distillation, followed by treatment with

resorcinol, does not prove the presence of ethyl phthalate unless interfering substances are removed by means of basic lead acetate. Such quantity of the preparation as contains about 10 c.c. of alcohol is treated with excess of basic lead acetate and filtered, the clear liquid being freed from lead by addition of solid sodium carbonate and filtration. The filtrate is extracted with 15 to 20 c.c. of light petroleum and the extract evaporated to dryness with 0·2 c.c. of about 10 per cent. sodium hydroxide solution on a steam bath. The residue is warmed for a few minutes with 5 c.c. of concentrated sulphuric acid and the liquid heated with 0·025 grm. of resorcinol to give a solution, which is transferred to a clean dry test-tube and heated for 10 minutes in a paraffin wax bath at 160–170° C. The cooled liquid is poured into 150 c.c. of water, and this then made alkaline with approximately 10 per cent. sodium hydroxide solution. A greenish-yellow fluorescence appears immediately and persists for 36 to 48 hours if the original liquid contains ethyl phthalate.

All glassware to be used in the test should be washed with soap and rinsed several times with alcohol, and porcelain dishes should be heated to redness. A blank test should be made with the reagents. T. H. P.

Determination of Caffeine. S. Gobert. (*Ann. Falsif.*, 1926, **19**, 586–594.)—Caffeine in roasted or green coffee may be satisfactorily determined by the following method. Five grms. of the finely powdered coffee are weighed into a centrifuge tube, 5 c.c. of ammonium hydroxide (sp. gr. 1·18) left in contact, with occasional stirring, with roasted coffee for 20 mins., and with green coffee for half an hour. Four extractions of 10 minutes each, with constant stirring, are made with 4 quantities of 25 c.c. of ethyl acetate, the liquid centrifuged for 5 to 7 minutes and decanted, the ethyl acetate distilled off after addition of 0·5 grm. of paraffin wax, and the residue dried for half an hour at 100° C. The united extracts from 3 extractions of 50 c.c. each with boiling water are boiled, cooled, filtered, treated for 15 minutes with 20 c.c. of 1 per cent. potassium permanganate solution for roasted, and 10 c.c. for green coffee, and the manganese precipitated with 12 vol. hydrogen peroxide, containing 1 per cent. of glacial acetic acid. The mixture is left for 15 minutes on the water bath, filtered, the residue washed with boiling water, the filtrate evaporated, and the residue, after drying, extracted 3 times with 25 c.c. of chloroform. The united extracts are distilled, and the residue dried and weighed. As the caffeine obtained by this method is sufficiently pure, it is only necessary to determine nitrogen in the case of samples which have been treated to remove caffeine. D. G. H.

Separation and Determination of Morphine, Pseudomorphine and Related Substances. A. K. Balls. (*J. Biol. Chem.*, 1927, **71**, 543–558.)—The author gives a number of analytical methods, worked out in his laboratory, for a study of systems in which the gradual oxidation of morphine is taking place. The first oxidation product is theoretically pseudomorphine, which may be quantitatively determined in several forms, and so make possible a method for the

chemical separation and analysis of a morphine solution that is undergoing oxidation. The reaction may be abruptly terminated and the composition of such a system determined at any given time with respect to residual morphine, pseudomorphine, and the other morphine-derived substances precipitable by silicotungstic acid, taken together as a class. If desired, five variables can thus be evaluated in the system, when the initial concentration of morphine and the quantity of oxidant used are known. An account of the materials used in testing these methods is given, followed by descriptions of the details in determining the individual bases. The scheme of separation of the mixed silicotungstates is next outlined, and a description is given of the modification adapted to biological material. Morphine and pseudomorphine can be determined in amounts as small as 2 mgrms. in 20 to 30 grms. of biological material, such as ground meat, with an error of about 30 per cent. In the analysis of tissues for morphine alone the method is also useful, because it is rapid. P. H. P.

Determination of Mercury in Mercuric Salicylate. A. F. Murray. (*Amer. J. Pharm.*, 1926, **98**, 639–642.)—A saving of time is effected as compared with the U.S.P. method for determining mercury in mercuric salicylate, with no risk of volatilisation of mercury, by the following method. Half a grm. of mercuric salicylate is dissolved in 10 c.c. of 10 per cent. sodium hydroxide solution, 10 c.c. of 10 per cent. sodium sulphide solution are added, and the whole boiled. While still hot, 10 per cent. hydrochloric acid is added till the solution is acid and then 5 c.c. more, the mixture transferred to a weighed Gooch crucible, and the residue washed until free from chlorides, and then with two 5 c.c. portions of alcohol, followed by 5 c.c. portions of a mixture of equal parts alcohol and ether, until 5 c.c. of washings diluted with 15 c.c. of water fail to react with 1 drop of ferric chloride solution. Washing is completed with three 5 c.c. portions of carbon tetrachloride, and the residue is dried at 110° C. and weighed. D. G. H.

Biochemical, etc.

Cholesteryl Allophanate and its Use in Biochemistry. R. Fabre. (*J. Pharm. Chim.*, 1927, **119**, 21–24.)—If a current of hydrocyanic acid is passed into a 20 per cent. solution of cholesterol for 15 minutes a precipitate is formed; this, after standing for 6 to 8 hours, is treated with ether to get rid of any residual cholesterol, and the allophanate is then filtered off and purified by recrystallisation from amyl alcohol. Cholesteryl allophanate melts at 277–278° C., and at 18° C. its solubility in 100 c.c. of anhydrous ether is 0·063 grm.; of absolute alcohol, 0·061 grm.; of amyl alcohol, 0·19 grm.; and of boiling benzyl alcohol, 5·89 grms. At 18° C. it has $[\alpha]_D$, −33·3°. Cholesterol is readily recovered from the allophanate by hydrolysis with 0·1 *N* aqueous sodium hydroxide solution, and the allophanate can therefore be used for detection of cholesterol in various biochemical tests. D. G. H.

Properties of Cholesterol obtained from Different Sources. R. J. Anderson. (*J. Biol. Chem.*, 1927, **71**, 407–418.)—Attention is called to the fact that cholesterol preparations obtained from different sources show slight differences in physical properties. In order to determine whether different cholesterol preparations are homogeneous it would be necessary to prepare cholesterol esters of various acids or some other derivatives of cholesterol, fractionally recrystallise them, and compare the properties of the different fractions. When apparently pure cholesteryl acetate was fractionally recrystallised from ethyl alcohol, a bottom fraction was separated which possessed a much lower melting point and a lower optical rotation than the top fraction—probably due to a small amount of a substance having a lower rotation than ordinary cholesterol. It is possible that a derivative other than the acetate, and a solvent better than alcohol might be found that would cause a more effective separation. If cholesterol is formed from plant sterols, a number of different, as well as isomeric, cholesterols might be expected to occur in animal fats, corresponding to the various phytosterols contained in the plant material which serves as food. P. H. P.

Distribution of Dihydrositosterol in Plant Fats. R. J. Anderson, F. B. Nabenhauer and R. L. Shriner. (*J. Biol. Chem.*, 1927, **71**, 389–399.)—Previous work on the sterols is discussed. The saturated sterol, dihydrositosterol, $C_{27}H_{47}OH$, appears to be rather widely distributed in plant fats. It occurs in association with unsaturated sterols, not only in the endosperm and bran of maize and wheat, but also in small amounts in the oils obtained from the germ of these grains. Appreciable quantities of the substance have been isolated from maize gluten, maize bran, wheat bran, rice bran, maize oil and wheat germ oil. The dihydrositosterol preparations that have been obtained from different sources show slight variations in physical properties. The melting points vary from 141–142° to 145–146° C., and the specific optical rotations vary from about +23° to +25°. The acetyl derivatives vary in melting points from 137° to 141° C., and in optical rotation from about +13° to +14°. Whether these variations in properties depend upon the degree of purity or are due to the presence of isomeric saturated sterols, cannot be determined from the present data. The dihydrositosterol which occurs in plant fats appears to be identical with the synthetic sitostanol which is obtained when an unsaturated sterol, which possesses the properties usually ascribed to sitosterol, is reduced with hydrogen in the presence of platinum black. The properties and composition of the various dihydrositosterol preparations that have been isolated are summarised in a table in comparison with the synthetic sitostanol. P. H. P.

A Useful Compound of Histidine. H. B. Vickery. (*J. Biol. Chem.*, 1927, **71**, 303–307.)—Efforts have been made to find a compound of histidine which possesses a convenient solubility and a capacity for crystallisation sufficient to prevent the formation of syrupy mother liquors. In view of Kossel's success with 2,4-dinitro-1-naphthol-7-sulphonic acid as a reagent for arginine (Kossel and Gross, *Z. physiol. Chem.*, 1924, **135**, 167; Kossel and Standt, *Z. physiol. Chem.*,

1926, **156**, 270), it was decided to investigate the compounds which this substance forms with histidine. Histidine may be almost quantitatively isolated in pure form as the di-salt of 2,4-dinitro-1-naphthol-7-sulphonic acid by crystallisation from a solution containing excess of the reagent. The presence of small amounts of mineral acid has little, if any, effect upon the composition or yield of the substance. The di-salt separates in microscopic sulphur-yellow needles which appear to contain half a molecule of water of crystallisation, and which decompose at from 251–254° C. (short stem thermometer), depending on the rate of heating. No change in colour occurs below 245°. This salt presents many advantages over other salts of histidine which have been described, not only for the isolation, but for the purification of histidine. Free histidine or its dichloride may be recovered from the di-salt by decomposition with dilute mineral acid and removal of the reagent by extraction with butyl alcohol. The mono-salt crystallises from 66 per cent. alcohol containing an excess of histidine in chrome yellow plates which contain 3 molecules of water of crystallisation. It becomes orange in colour on heating at 100° C., sinters at 190° C., and decomposes at 212–214° C. It is difficult to prepare and thus has little practical value. P. H. P.

On the Nature of the Urine Sugars. H. S. Eagle. (*J. Biol. Chem.*, 1927, **71**, 481–495.)—By the incubation of urine at 37-38° C. with appropriate quantities of yeast, any glucose present, up to 0·4 per cent., may be quantitatively fermented within 40 minutes. The presence of glucose in concentrations as low as 0·010 per cent. may be determined, provided certain substances in urine which obscure the results of fermentation are first removed. Preliminary shaking with Lloyd's alkaloidal reagent, suggested by Folin for use in the determination of urine sugar, is useful in this respect. It is found that glucose is not normally excreted in the urine. What has been considered to be glucose is a group of substances which only gradually decompose under the conditions of fermentation, with the production of reducing substances, possibly due to bacterial decomposition ; such decomposition, however, is a matter of days. Until a certain critical level of blood sugar has been reached, the kidney interposes an absolute barrier against the excretion of glucose, and only when such large quantities as 100 grms. of glucose are taken is this critical level exceeded in a certain proportion of normal individuals, and fermentable sugar appears in the urine. It is doubtful whether the normal individual, on an average diet, ever shows true glucose in the urine. The increase in urine sugar following food (and glucose) intake, discovered by Benedict and termed glycuresis, represents, not glucose, but non-fermentable substances. Probably in the case of food, as has been suggested, these are either foreign non-assimilable carbohydrates originally present in the food, or decomposition products formed in the process of preparing food or of its digestion. The increase in their excretion following the ingestion of glucose is somewhat difficult to understand, and may be due to any of several factors suggested ; variations in the rate of secretion, a trace of impurities in the glucose used, or a mobilisation of these substances in the blood coincident with the rise in blood sugar. P. H. P.

Iron in Nutrition. III. Effects of Diet on the Iron Content of Milk. C. A. Elvehjem, R. C. Herrin and E. B. Hart. (*J. Biol. Chem.*, 1927, **71**, 255–262.)—Previous literature on the effects of diet on the iron content of milk shows divergent results and conclusions. Recently a method was developed by Elvehjem and Hart (*J. Biol. Chem.*, 1926, **67**, 43), by which the amount of iron in milk may be determined very accurately, and this method has been used in a study of the iron content of milk from goats receiving a basal ration plus various additions. The results are tabulated. The iron content of goats' milk cannot be increased by giving the goats ferric oxide (Fe_2O_3) or the very soluble iron salt, ferrous sulphate ($FeSO_4.7H_2O$). No noticeable change in iron content can be detected even if the addition of the iron increases the original iron content of the ration fivefold. Fresh green cabbage given with the ferric oxide also had no effect. The addition of ferric oxide or ferric oxide plus fresh green cabbage to the ration of a goat brought about no changes in the milk which would prevent the development of nutritional anaemia when given to young growing rabbits. There was no difference in the average iron content of milk from cows on such different rations as alfalfa hay (which contained 0·0151 per cent. iron) and Timothy hay (which contained 0·0071 per cent. iron). The percentage of iron in the milk from individual cows varied as much as 100 per cent., which may explain some of the erroneous results obtained by earlier workers. This variation cannot be correlated with the length of the lactation period. P. H. P.

Relation between the Vitamin *C* Content of a Cow's Ration and the Vitamin *C* Content of its Milk. J. S. Hughes, J. B. Fitch, H. W. Cave, and W. H. Riddell. (*J. Biol. Chem.*, 1927, **71**, 309–316.)—Up to the present time all work on this subject, except that of the authors, Hughes, Fitch and Cave (*J. Biol. Chem.*, 1921, **46**, 1), has indicated that the vitamin *C* content of milk varies with the content of this vitamin in the feed from which it is produced. The authors began an experiment in 1919 to study the influence of the vitamin content of a diet on the vitamin content of the milk produced by dairy cows. The sunshine the cows received enabled them to put vitamin *D* into their milk. Their ration was low in vitamins *A*, *B* and *C*, and tests showed that the vitamin *A* and *B* content of the milk depended on the vitamin content of the ration, but that there seemed to be no relation between the vitamin *C* content of the milk and the feed which produced the milk. In 1926 a test was made of the relative vitamin *C* and *D* content of milk from cows on pasture, and thus receiving direct sunshine, as compared with the milk from cows receiving neither direct sunshine nor green food. Only the results on the vitamin *C* phase are given. Curves show the results. The milk was tested on guinea pigs. There was no significant difference in the antiscorbutic potency of the two kinds of milk. These results are in agreement with all previous results by the authors. The synthesis of vitamin *C* by the cow seems the only explanation of these results. The vitamin *C* content of a cow's ration has little, if any, influence on the vitamin *C* content of its milk. P. H. P.

Bacteriological.

Infection by certain Organisms of the Salmonella Group. S. R. Damon and L. W. Leiter. (*Amer. J. Hyg.*, 1927, **7**, 27–39.)—The growth of the following organisms in household foods (tinned meats, fish, vegetables, fruits, and evaporated milk) has been studied for various periods of time at the temperatures of the ice-box (10° to 15° C.), room (20° to 22° C.), and body (37° C.) :—*B. anatum ovum W.*, *B. pestis caviae E9C6*, *B. sanguinarium Cornell*, and *B. suipestifer No.* 17. The results, which are given quantitatively and are fully discussed, indicate that all four bacilli multiply readily in the foods examined (except those which have a strong initial acidity), especially at 20° to 37° C. External evidence of infection must depend on the selection of raw products of good quality, with sanitary methods of preparation and efficient preservation. J. G.

Quantitative Interdependence of Sensitiser and Complement in Haemolysis. R. R. Hyde and E. I. Parsons. (*Amer. J. Hyg.*, 1927, **7**, 11–12.)—Haemolytic sera may be standardised by the determination of the potency of one reagent (the sensitiser) in terms of the potency of the other (the complement), with a selected arbitrary unit of red cells against which the haemolytic activities of the mixtures are measured. The relation is expressed by curves which give the actual quantities of the two sera required to produce haemolysis, and connect these quantities with the number of haemolytic units employed. The *dual optimum reacting point* is that point on the curve at which optimum haemolysis is obtained with the minimum amounts of both reagents. Typical data and curves are given to show the quantitative interdependence of an anti-sheep rabbit sensitiser, and an anti-sheep guinea-pig sensitiser, and an anti-sheep rabbit serum sensitiser (heterophile type) with a guinea pig complement in each case.

J. G.

Toxicological and Forensic.

Pyramidon as a Reagent for Blood. M. Elzas and L. M. Lansberg. (*Pharm. Weekblad*, 1927, **64**, 19–22.)—Blood may be detected to the extent of 1 part in 60,000 by adding one drop of the dilute solution of blood to a mixture of 3 c.c. of a 5 per cent. solution of pyramidon in 96 per cent. alcohol, with 8 drops each of 50 per cent. acetic acid and 3 per cent. hydrogen peroxide. A violet colour develops after 1 minute. The method was tested in the case of blood in the faeces as a result of doses of haematogen, by comparison with the phenolphthalein, spectroscopic, and modifications of the guaiacum and benzidine methods.

J. G.

Alleged Differentiation of Human Sera as to Sex. R. R. Hyde and E. I. Parsons. (*Amer. J. Hyg.*, 1927, **7**, 22–26.)—The conclusion of D'Herelle and Gery that guinea-pigs which have been sensitised with an extract of human placental tissue are subject to shock when infected with the blood serum of a female, but not with that of a male, is suggested as a means of sex-determination of man

which might be of practical use in medico-legal cases. Experiments carried out on the lines of the former trials and by other methods, indicate that the method is unreliable. The toxicity of the human male serum varies according to its source. J. G.

Influence of Insoluble Matter on the Marsh Test. L. Barth and R. Massy. (*Bull. Soc. Pharm. Bordeaux*, 1926, **64**, 66; *J. Pharm. Chim.*, 1927, **119**, 30–31.)—The presence of calcium sulphate in the determination of arsenic by the Marsh apparatus results in low figures being recorded; this is regarded as being due to mechanical obstruction rather than to any chemical reaction. D. G. H.

Organic Analysis.

Method for the Determination of Minute Amounts of Ethyl Iodide in Air, Water and Blood. I. Starr, junr., and C. J. Gamble. (*J. Biol. Chem.*, 1927, **71**, 509–535.)—A method is described for the determination of ethyl iodide in air by means of its reaction with silver nitrate, the average accuracy being 2·3 per cent. for amounts from 2·35 to 0·12 mgrms. Standard silver nitrate in nitric acid is added to sampling tubes which contain ethyl iodide, the reaction is allowed to go to completion, and the excess silver nitrate is titrated by the Volhard method. Adaptations of the process for the determination of similar amounts of ethyl iodide in water and blood are given; satisfactory methods for the collection of samples of ethyl iodide in air and fluids are described. Evidence is submitted that the iodine pentoxide method for the analysis of ethyl iodide is not reliable in the form described. The authors summarise their difficulties with it as follows:—"(1) Different tubes of iodine pentoxide give different yields of iodine under apparently similar conditions. (2) Variations in rate of flow make profound differences in iodine yield. (3) Iodine pentoxide is decomposed by traces of a large number of substances. (4) The spontaneous rate of decomposition of iodine pentoxide is quite variable. The proper correction for this decomposition is often doubtful. (5) While the method usually yields consistent results (within 4 per cent.), occasionally it fails to do this, and we are not yet confident that we have all the factors under control." With the new technique the coefficient of distribution of ethyl iodide in air and water has been determined to be 2·7; in air and normal blood *in vitro* to be 7·6 instead of 2·0, as previously believed. Plasma and anaemic blood have a lower coefficient than normal blood. It is shown that the destruction of ethyl iodide in water and blood *in vitro* is a slow rather than a rapid process, and that ethyl iodide is not completely destroyed in the body, but is eliminated by the breath, and so must occur in the venous blood during its inhalation. That the ethyl iodide method for the determination of blood flow gives figures consistent with other methods appears due to the fact that the errors tend to cancel each other. In its present form the method yields results which cannot be accepted. P. H. P.

The Faught Test for Acetone. H. J. Schaeffer. (*Amer. J. Pharm.*, 1926, **98**, 643–645.)—The great objection to this test is the cost of the ethylenediamine hydrate, and a critical examination shows that 1 drop of a 5 per cent. (instead of 10 per cent.) solution of the reagent is sufficient to indicate the presence of 1 part in 10,000. For practical purposes, the Faught test is the most efficient and delicate for acetone, but the tube should be kept cold. D. G. H.

Phenol Tests. II. Nitrous Acid Tests. The Millon and Similar Tests. Spectrophotometric Investigations. H. D. Gibbs. (*J. Biol. Chem.*, 1927, **71**, 445–459.)—The phenol reagents—nitrous acid, dilute nitric acid and Millon's reagent have been studied and the literature reviewed. To investigate the hypothesis that the primary reaction between phenols and the nitrous acid and nitric acid reagents results in the formation of a nitrosophenol, a study of the development of colour at room temperature was made in various solutions. The reaction with phenol produces *p*-nitrosophenol which may condense to produce other colour compounds. The presence of mercury compounds accelerates the colour formation. The pK_a value of pure *p*-nitrosophenol as determined by the method of Salm (*Z. physik. Chem.*, 1906, **57**, 471) was 6·6, and by the spectrophotometric method 6·4. The latter is believed to be more accurate. The dissociation constant for a colour compound formed by the Millon reaction was found to be 6·6. The spectrophotometric absorption curves in the visible region are recorded for *p*-nitrosophenol at P_H 4, 5, 6, 7, 8 and 0·2 *N* sodium hydroxide at concentration of 0·0019 *M*, and for the colour produced by the Millon reagent acting on phenol. Para-cresol and tyrosine react with Millon's reagent and with nitrous acid. The substitution of groups in the para position to the hydroxyl, does not, in general, seem to interfere with the nitrous acid tests. P. H. P.

Arylamine Salts of the Naphthalene-sulphonic Acids. III. Separation of Crocein, Schäffer, R and G Acids and their Arylamine Salts. R. B. Forster and C. M. Keyworth. (*J. Soc. Chem. Ind.*, 1927, **46**, 25–31.)—The direct sulphonation of β-naphthol yields different products according to the concentration of acid, the temperature and the time of reaction. The primary products are oxy-Tobias, Crocein, Schäffer and F-acids (β-naphthol 1, 8, 6, and 7 sulphonic acids respectively), of which Crocein and Schäffer acids may be further sulphonated to produce G and R acids, respectively (6:8 and 3:6 disulphonic acids, respectively). Both these finally yield β-naphthol-3:6:8-trisulphonic acid, whilst F-acid takes up in succession two, three and four sulphonic acid groups. The conditions governing the production of these acids and the patents in present use are discussed. Mixtures of Crocein and Schäffer acids are separated according to their different solubilities in water or alcohol, or by fractional coupling with a diazo-compound. R and G acids are also separated by the last method, but, if Schäffer acid is present, the difference in solubility of the barium, sodium, and potassium salts must be used. Greater differences in solubility are obtained if the arylamine salts of the sulphonic acids are used. Details are given of the salts of Schäffer, Crocein, R and G acids with 19 common arylamines, prepared according

to the method of Keyworth (*ibid.*, 1924, **43**, 341). These include the appearance, ease of preparation, m.pt., and solubilities in alcohol and water. A few salts of naphthalene sulphonic acids are included. The use of these data in the separation of various mixtures of acids is described in detail. J. G.

Nature of the Reaction between Tannin and Carbohydrates. H. B. Stocks and C. V. Greenwood. (*J. Int. Soc. Leather Trades Chem.*, 1926, **10**, 404.) —A comparison is made of the interaction of tannin with gelatin, with tragasol, and with starch as to the weight of coagulum produced and the amount of tannin absorbed from tannin solutions. Films of gelatin and tragasol were prepared and steeped in tannin solutions. At the end of the period the unabsorbed tannin was determined, and from this the amount of tannin absorbed by the films was found by difference. Twice as much tan was absorbed by the tragasol as by the gelatin. The reaction is slow and not complete in 6 days. In some experiments with tannin and gelatin solutions, in which increasing proportions of tan to gelatin were used (from 1:1 to 3:1), the proportion of tannin in the precipitate reached a maximum at 2:1. In dilute solutions (0·3 to 0·6 per cent. of gelatin) the reaction was incomplete, but on adding sodium chloride, the turbid mixture at once precipitated in flocks. If tannin solution is added to a boiled starch paste, a precipitate is formed which agglomerates into a clot on standing for several hours. The amount of tannin absorbed and the weight of coagulum is less than with gelatin or tragasol. The absorption of tannin by starch films is slightly higher than by gelatin, but much lower than by tragasol. R. F. I.

Reaction between Tannin and Casein. H. B. Stocks. (*J. Int. Soc. Leather Trades Chem.*, 1926, **10**, 409.)—Casein was dissolved in sodium carbonate solution by heating. On the addition of tannin and mixing and then rendering the mixture neutral or faintly acid with hydrochloric acid a precipitate was obtained, a little heavier than the precipitates obtained under similar conditions with gelatin (*cf.* preceding extract). After steeping casein (in the form of a granulated powder) in tannin solution the tannin absorbed in 24 hours was determined. and it was found that more tan was absorbed than by gelatin, the amount approximating more nearly to that absorbed by starch. R. F. I.

New Method for Determining Fineness of Wool. J. A. F. Roberts. (*J. Text. Inst.*, 1927, **18**, T48–T54.) The present methods here reviewed include those involving the magnification of the fibre, those involving the use of accurate callipers (as described by Hill), and those involving the use of the micro-balance (Barker and King's method). The author's method is to measure the number of cm. of fibre which weigh one mgrm. In sampling, one to two sq. in. of fleece are taken. It is of fundamental importance to separate out carefully a complete piece of the fleece and to deal with every fibre contained in it, though it may be only necessary actually to measure every fourth, eighth or sixteenth fibre. The sample is washed three times with at least three changes of water, allowed to dry in the air overnight, and then washed with at least three changes of ether. The fibres

are drawn out, one by one, gently stretched out straight, measured to the nearest mm. and again washed with ether. They are then heated in a current of air till dry and weighed, the minimum weight being 50 mgrms. Typical results obtained were: For merino of 80s. counts, 320 cm. per mgrm.; for Lincolns of 36s. counts, 60 cm. per mgrm. The method is more rapid and more accurate than the older ones. R. F. I.

Inorganic Analysis.

Reaction between Elementary Phosphorus and Potassium Iodate and its Utilisation in the Volumetric Determination of Phosphorus. T. F. Buehrer and O. E. Schupp. (*J. Amer. Chem. Soc.*, 1927, **49**, 9–15.)—The reaction of elementary phosphorus with potassium iodate to form iodine is used as a means to determine the phosphorus. The phosphorus is heated under a reflux condenser in an Erlenmeyer flask with excess of potassium iodate and dilute sulphuric acid for at least three hours, and the iodine then distilled into a well-cooled solution of sodium thiosulphate. The distillate, which should then contain an excess of iodine, is titrated with sodium thiosulphate. The result may be checked by titration of the residual iodate after the iodine has been boiled off. The completion of the reaction depends on the presence of the correct amount of acid, which catalyses the oxidation of the phosphorus according to the equation $5I' + IO'_3 + 6H^{\cdot} = 3I_2 + 3H_2O$. This instantaneous reaction determines the rate of reaction as a whole and requires the presence of at least 0·25 *N*, but less than 6 *N*, sulphuric acid. Carbon tetrachloride is added to the distillation flask, and serves to wash down any iodine from the sides of the condenser during the " refluxing " process. Errors may be due to those involved in weighing and titration (which are rather great for elementary phosphorus), and to loss of iodine. The maximum recorded error is about 0·3 per cent. Since the reaction of iodic acid with the common organic solvents is almost negligible, the method may be used for solubility determinations of phosphorus. J. G.

Gasometric Micro-Kjeldahl Determination of Nitrogen. D. D. Van Slyke. (*J. Biol. Chem.*, 1927, **71**, 235–248.)—The use of the manometric blood gas apparatus of Van Slyke and Neill (*J. Biol. Chem.*, 1924, **61**, 523) has been extended to determinations of substances other than the blood gases. It can be used in any determination in which the final product is a gas, or will enter into a quantitative reaction producing a gas. Thereby the measurement is based on direct observation of the amount of substance obtained, independent of comparison with standard solutions by titration, colorimeter, or otherwise. A method for micro-Kjeldahl determinations of nitrogen is described in which a quick digestion is obtained by the use of a mixture of sulphuric and syrupy phosphoric acids (3:1) and potassium persulphate, and in which the ammonia formed is determined gasometrically. The digest, neutralised with alizarin sulphonate and treated with sodium hydroxide, is washed into the gas apparatus and is there treated with hypobromite (1 c.c. of bromine in 50 c.c. of 40 per cent. sodium hydroxide *freshly made on the same half day*

in which it is used). The nitrogen gas is evolved in 2 minutes. The pressure of 1 mgrm. of nitrogen measured at 2 c.c. volume in the apparatus is about 300 mm., so that accurate readings are easy. Results are reproducible to within 1 per cent. Various catalysts and oxidisers previously used for Kjeldahl determinations were tested before potassium persulphate in great excess was found satisfactory. The acid mixture contains sufficient moisture to act with the persulphate on a dry substance; and 1 c.c. of water is not too much. For samples dissolved in more than 1 c.c. of water the procedure is to boil down with 1 c.c. of the acid mixture till fumes begin to come off, and to add 1 grm. of persulphate, with 1 c.c. of water, to the cooled residue, and then to finish the digestion. The sample to be analysed should contain 0·3 to 1·5 mgrm. of nitrogen. P. H. P.

New Reagent for Cobalt. P. Falciola. (*Giorn. Chim. Ind. Appl.*, 1926, **8**, 612.)—Sodium hydrosulphite serves as a sensitive and specific reagent for cobalt, and, when added either as solid or in solution to a strongly ammoniacal solution of a cobalt salt, produces a yellow, orange, ruby red or dark red coloration or a brownish-black precipitate, according to the concentration of the $Co^{\cdot\cdot}$ ions. If this concentration is 1:100,000, and 50 to 100 c.c. of the solution are taken, the coloration is yellow. With a nickel salt under similar treatment, the blue colour of the liquid remains unchanged. With much cobalt together with nickel, the ammoniacal liquid is not pure blue but has a violet tinge, and when such solution is treated with hydrosulphite, it is decolorised by addition of formaldehyde, which may cause reappearance of the green colour of the nickel ion. Hydrogen peroxide also destroys the characteristic reddish-yellow tint, and tartaric, citric and formic acids attenuate or prevent it. If any of the ordinary metals or molybdenum, uranium, vanadium, tungsten, titanium, etc., as well as cobalt are present, addition of hydrosulphite to the ammoniacal solution, followed by filtration, gives a reddish-yellow liquid, the colour of which is intensified by a fresh quantity of hydrosulphite. T. H. P.

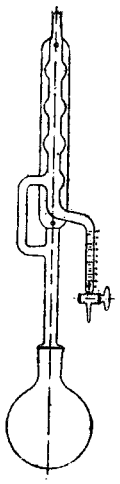
Volumetric Determination of Calcium. F. L. Hahn and G. Weiler. (*Z. anal. Chem.*, 1927, **70**, 1–22.)—The oxalate-permanganate method has been submitted to a critical study. The following conclusions were reached: A satisfactory degree of accuracy is attained if precipitation is effected with a moderate excess of ammonium oxalate in weakly acid acetate solution, and the excess of oxalate measured in an aliquot portion of the filtrate. The precipitate should be allowed to stand overnight unless it is coarsely crystalline, *i.e.* obtained by adding the precipitant, drop by drop; in that case it may be filtered off after the liquid has become quite cold. However precipitated, calcium oxalate undergoes a perceptible alteration on being washed, resulting in a slight deficiency of oxalic acid. Whilst this is immaterial in gravimetric work, residual titration of the excess of precipitant in the filtrate gives the best results. W. R. S.

Reaction of "Aluminon" with Hydroxides of Scandium, Gallium, Indium, Thallium, and Germanium. R. B. Corey and H. W. Rogers. (*J. Amer. Chem. Soc.*, 1927, **49**, 216–217.)—The work of Middleton (ANALYST,

1926, **51**, 537) is extended to the above elements. Red or yellow lakes are produced when to 1 c.c. of solution containing 1 mgrm. of the element are added 5 c.c. of *N* hydrochloric acid, 5 c.c. of 3 *N* ammonium acetate, and 5 c.c. of 0·1 per cent. " Aluminon " solution. If 3 c.c. of 6 *N* ammonium hydroxide solution are then added, the colours are very clear after 24 hours. The behaviour of the precipitates is similar to those previously observed. The absence of precipitates in the control solutions indicates that the lakes are not due to the formation of hydroxides or basic acetates. J. G.

Physical Methods, Apparatus, etc.

Method of Measuring the Size of Particles. P. Lukirsky and M. Kosman. (*J. Soc. Chem. Ind.*, 1927, **46**, 21–25.)—If a vertical tube containing a uniform suspension of a powder in a liquid is connected at different heights with two similar tubes containing the pure liquid, the liquid-levels will be different in each of the latter tubes to an extent depending on the mean density of the suspension in the region of the first tube between the two connections. This density is determined by those particles whose velocity of fall is such that they remain in this region. A formula has thence been deduced which enables the distribution of the particles of a suspension with respect to their velocities to be calculated from the difference in hydrostatic pressures at two points of the falling suspension of powder determined at different moments. From the density of the powder it is possible, by means of Stokes's law, to find the effective radius of the particles, and thus obtain the result in terms of size instead of velocity. The difference in levels is magnified by means of a beam of light reflected on to a scale by a mirror attached to a beam joining two water-tight brass boxes which float on the levels of the liquids. For each powder a characteristic curve is obtained connecting the time and the difference in levels. The method is independent of variations in those conditions which affect all the tubes to the same extent. A higher degree of accuracy (up to 1 micron) is obtainable than with most other methods, and suspensions of weaker concentrations than usual may be measured. J. G.



Automatic Apparatus for the Determination of Water. R. Kattwinkel. (*Chem. Zeit.*, 1926, **50**, 927.)—In this apparatus for the determination of moisture by distillation in presence of benzene, toluene, xylene, petroleum, or the like, the water-measuring tube, divided into tenths of 1 c.c., is connected directly with the condenser. The action of the apparatus may be readily seen from the figure. T. H. P.

References to Scientific Articles not Abstracted.

CASES OF POISONING AND SUSPECTED POISONING. By G. ROCHE LYNCH. *Lancet*, 1927, **212**, 27.

Statistics of homicidal poisoning—What is a poison?—How to investigate a suspected case—The problem after recovery—The cost of analysis—What to reserve and how to reserve it—Withholding the certificate—Chronic poisoning—Sale of poisons—Range of poisons obtainable.

TREATMENT OF POISONING. By E. BOSDIN LEECH. *Lancet*, 1927, **212**, 452.

Acute poisoning—Elimination of poison—Emetics—Neutralisation of the poison—Treatment for individual poisons (carbolic acid, oxalic acid, morphine, strychnine, belladonna, phosphorus, arsenic)—General treatment—Chronic poisoning.

THE CAUSES OF VARIATION IN THE PROPORTION OF BUTTER FAT IN MILK. By J. F. TOCHER. *Scottish J. Agric.*, 1927, X, No. 1.

Water not the only probable adulterant of milk—Reasons why milk may be of poor quality—The analyst's position—Evidence in Court—Do byre samples really help in detecting adulteration?—The rôle and limitations of random samples—Average percentages valueless in Court—Butter fat percentage varies with age of cow—with lactation period—and with breed—The relationship of butter fat to solids-not-fat—Summary.

DORTON SPA AND ITS WATER. By C. A. MITCHELL. *Discovery*, 1927, **8**, 56.

History of the rise and decline of the spa—Local traditions—Recent analysis of the water—Changes since 1912. (*Cf.* ANALYST, 1914, **39**, 210.)

Reviews.

PRACTICAL COLLOID CHEMISTRY. By Prof. Dr. WOLFGANG OSTWALD with the Collaboration of Dr. P. WOLSKI and Dr. A. KUHN. Translated by I. NEWTON KUGELMASS, M.D., Ph.D., Sc.D. and THEODORE K. CLEVELAND, Ph.D. Pp. xvi + 191. 22 figs. London: Methuen & Co. 1926. Price 7s. 6d. net.

This is an English version of the 4th edition of Ostwald's *Kleines Praktikum*. The corresponding French version was reviewed in THE ANALYST, 1924, **49**, 410.

Containing 183 experiments, the book aims at being an elementary experimental development of colloid chemistry suitable for students or practitioners wishing to learn the subject in the laboratory. The chapters begin with a brief account of the theoretical principles involved, and then follow simple illustrative experiments. The subjects treated comprise the preparation of colloidal solutions, diffusion, dialysis, ultra-filtration, viscometry, optical studies, electrical properties, experiments with gels, adsorption, coagulation and protection. A chapter is included on Commercial Colloids, which is purely descriptive, and the final chapter is devoted to a simple scheme of Dispersoid Analysis. There is no index, the author believing that the student should "learn to recognise systematic colloid phenomena in conjunction with the experiments" which follow a systematic arrangement.

The book certainly deserves attention, and as an elementary manual is excellent. Particular treatment is given to optical studies, and the organic

colloid, Congo rubin, figures largely, Ostwald having carried out considerable research thereon. Chapter VIII describes the Ostwald von Hahn flocculationmeter and gives typical results, without, however, showing the student how to convert the readings so as to obtain size-frequency analysis of suspensions, so necessary in technical practice.

A number of errors appear, as, for example, $CaSO_4$ and $CaCl_2$ for the copper salts (p. 8); "decomposed" for "retained" (p. 12, expt. 29); KJ for KI (p. 37, fig. 3); "safronine" for "safranine" (p. 61); "adsorption of light" (p. 65); "gelatin" (p. 88, expt. 108); "dialectric" (p. 105); C for c (p. 124).

The book is well printed and bound, and the price is very reasonable.

WILLIAM CLAYTON.

AN INTRODUCTION TO PHYSICAL CHEMISTRY. By F. B. FINTER. Pp. 275. LONDON: Longmans, Green & Co. 1926. Price 6s.

This book is intended for students who have arrived at the first year of a university course. Prominent features are a historical treatment (but not always historical sequence) and a minimum of mathematics; the latter may be defensible on the ground that mathematics is repugnant to a youthful reader, but one wonders whether this is really sound, in view of the need of mathematical treatment for the proper understanding of the more difficult parts of the subject and of its value as mental training.

There is a bright and interesting style about the book, with a leaven of dogmatism in places, which is perhaps needful for the young. In other parts of the book, for example in the discussion of the dissociation theory, the pros and cons are tabulated in an interesting manner, showing that the thorny question of ions is by no means settled. The main facts and principles of theoretical chemistry are clearly presented, and the need of elementary statements has not led the author into any material errors.

It is a little book which members of this Society may give to their sons, and, when "rusty" on certain points, they may quietly refer to it themselves.

H. E. COX.

TABLES ANNUELLES DE CONSTANTES ET DONNEÉS NUMÉRIQUES.

(1) DONNEÉS NUMÉRIQUES D'ELECTRICITÉ, MAGNÉTISME ET ELECTROCHIMIE. Pp. 52 + 92. Price 56fr.

(2) ART DE L'INGÉNIEUR ET METALLURGIE. Pp. 250. Price 105 fr. Paris: Gauthier-Villais et Cie.

These tables form part of Vol. V of the "Annual Tables of Constants and Numerical Data—Chemical, Physical and Technological," published by the International Committee appointed by the Seventh Congress of Applied Chemistry (London, June 1909). The first volume under consideration deals with electricity,

magnetism, conductivity of electrolytes, electro-chemical equivalents and electromotive forces, and forms a valuable collection of data from a variety of sources fully referenced, arranged and indexed for ready access.

The engineering and metallurgical data, also published separately, comprise mechanical constants for a great variety of materials in common use for structural and other purposes, as well as the thermal constants of refractory materials and fuels. The section dealing with various kinds of wood used in aircraft construction and other non-metallic substances, such as fabric and rubber, should be particularly useful to the aircraft designer.

The metallurgical section gives technical data concerning metals and copper and aluminium alloys, their mechanical constants and electrical and magnetic properties.

This part is particularly full, and the importance of the subjects treated can hardly be over-rated, as Sir Robert Hadfield points out in a special preface.

It is clear that the compilers have spared no pains to make this collection of useful information as complete as possible, and it represents an enormous amount of detail work which has been efficiently and thoroughly carried out.

R. W. SLOLEY.

PHOTOSYNTHESIS. By H. A. SPOEHR. Pp. 393. American Chemical Society Monographs. The Chemical Catalog Company, Inc., New York. 1926. Price $6.50. To Members of the Amer. Chem. Soc. $5.85.

H. A. Spoehr is well known for his numerous publications on photosynthesis as applied to the carbon assimilation by the green plant, and the appearance of his book has been looked forward to for some time. The contradictory results obtained in this country during the last few years, as well as those published by other chemists working in Germany, Switzerland and the United States, have puzzled both chemists and botanists, and it is therefore gratifying to find that Spoehr considers that "unfortunately much of the speculation regarding the manner in which the green plant utilises solar energy has not been restrained by a knowledge of certain facts concerning the process." This practically summarises the position, and we may therefore assume that little or nothing definite is known of the actual mechanism by which carbon dioxide is converted into carbohydrates in the living plant.

The book under review is divided into seven chapters which deal with (1) the cosmical function of the green plant, (2) the nature of photosynthesis, (3) the products of photosynthesis, (4) the methods of measuring photosynthetic activity, (5) the chemistry of photosynthesis, (6) the energy relation of photosynthesis, and (7) chlorophyll and the chloroplasts, respectively. Each chapter is a monograph in itself and complete with its own reference literature. The chapter on the chemistry of photosynthesis (pp. 256–315) is of outstanding merit and will, in due course, become a classic of its kind.

The book is well and attractively written and deserves a place in every scientific library. Chemists and botanists owe a debt to Spoehr for the great trouble he must have taken in compiling this work.

M. NIERENSTEIN.

STARCH MAKING. By FELIX REHWALD. Translated by CHARLES SALTER. Pp. viii + 264 with 93 explanatory illustrations. London: Scott, Greenwood & Son. Price 12s. 6d.

This book goes into details of the manufacture of starch from potatoes, wheat, rice, maize, etc., and it is evident that the author is an expert on the practical side of his subject. His criticism and advice are cogent and convincing. Herein lies the true worth of the book. In a less full degree he treats of the manufacture of dextrin, soluble starch, starch-sugar, syrups and sugar colouring. There has been, he says in the preface, little development since the fourth German edition was published, so that this, the fifth edition, contains little that is new. The practical guidance given should prove of value to manufacturers. It falls short, however, in one particular, for no warning is given as to the possibility of the sulphuric acid, used in the preparation of edible products, being contaminated with arsenic.

The different varieties of machinery used, especially in the production of starch, are illustrated and described in a general way. Tables of output and capacities of these machines are furnished. A manufacturer taking this book as his guide to the acquisition of plant would, it is to be feared, be restricted in his choice, mainly, if not solely, to Germany. Thirty varieties of one brand of machines are prominently displayed.

A certain amount of theory has been included in order to explain the various points. The reasoning is not always quite clear. For instance, the sweet taste in potatoes, we are assured, has nothing to do with frost, and yet at $-6°$ to $-2°$C, which surely *is* frost, sugar accumulates, because the cold prevents or hinders respiration.

Some inaccuracies which appear are perhaps due to translation. Specific gravity is loosely defined as the extent to which a substance is heavier than water, and not as a ratio of weight of substance to weight of an equal volume of water. On page 21, in a description of Reimann's specific gravity balance, it is stated that from the short arm there are suspended "two superimposed baskets." On page 34 mention is made of "automatic weighing machines which record the results automatically." There is looseness of expression, too, in speaking of the *degradation* of starch continuing *up* to the formation of sugar. The term "air-dry" is defined as "looking perfectly dry." Calcium bisulphide is, perhaps, inserted for the sulphite on page 68. When we read on page 214 that the starch molecule is split up in a way which varies with the concentration of the "air" we presume "acid" is meant.

Some terms used would almost suggest an unfamiliarity with current chemical usage—*e.g.* "in the dry," "in the warm," "exposure to high heating," "ten-fold quantity of water," "Aerometer," etc. Calcium sulphate dissolved in water is called "dissolved gypsum."

The above are, after all, comparatively small defects. It is quite otherwise in two instances. The author mentions England once (is it not the only time?) rather disdainfully in regard to an idea, alleged by him to be erroneous, of the superior nutritive value of arrowroot. He then states that "most commercial arrowroots are either extensively mixed with potato starch or else (frequently enough) consist entirely of that substance." The experience of the reviewer is directly opposed to this. Arrowroot rarely, if ever, in this country contains potato starch.

The other point to which exception can be taken is on page 210, where it is startling to find that the author openly and unblushingly supplies information relative to the sophistication of sago. A process is described to prepare potato starch to resemble sago, and receives this recommendation—"even an expert eye is unable to detect (the product) as artificial sago—still less are the general public capable of telling the difference." There is here no mention of England; so perhaps the author is speaking of other countries when he says—"a large proportion of the commercial product known as sago consists of potato starch alone." The present reviewer in a fairly long experience, dealing with hundreds of samples, can say that he has found no shred of evidence to sustain such an indictment against manufacturers of the sago used in this country, and he believes these statements to be absolutely incorrect, so far as England is concerned.

The type, paper, and binding are good. Proof reading has been carefully done; the errors are few.

J. R. STUBBS.

VINEGAR: ITS MANUFACTURE AND EXAMINATION. By C. A. MITCHELL, M.A., F.I.C. 2nd edition. Pp xvi + 211. London: Chas. Griffin & Co., Ltd. 1926. Price 10s. 6d. net.

It is a little surprising that there should be sufficient demand for a book on vinegar to make a new edition necessary, and evidently the subject must interest a wider circle of readers than those connected with the industry.

In the review of the first edition (ANALYST, 1916, 41, 324), attention was called to several omissions and a few inaccuracies; these have been corrected, but it is to be regretted that much of the new matter has been put into an appendix instead of being incorporated in its proper place in the text. It annoys a reader to have to refer to the end of a book for the continuation of the subject.

The historical portion, which is practically identical with that in the first edition, is interesting and throws light on the development of the industry.

A good account is given of the acetifying bacteria and the theories of acetification, and the modern scheme of classification has been described (also in an appendix). It is useful to have all this information in a book on vinegar-making, since former works (Brannt, etc.) do not attempt to discuss the action of different types of bacteria. As the author rightly points out, much remains to be done in this direction.

The analytical portion gives a useful summary of tests, including some devised by the author, and the composition of different types of vinegar is shown in a series of tables.

Considered as a whole, the book is a valuable contribution to industrial technology.

H. DAVIS.

COAL AND ALLIED SUBJECTS: COMPENDIUM II. By N. SIMPKIN, M.Sc., A.I.C., F. S. SINNATT, M.B.E., M.Sc., F.I.C., and Associates. London: H. F. and G. Witherby. 1926. Price 15s.

This compendium comprises Bulletins XI to XVI of the Lancashire and Cheshire Coal Research Association, and, as explained in the prefatory note, has been issued in order that others engaged in the coal industry might share in the knowledge gained by the work of the Association. The subjects dealt with are all of decided importance to those employed in the practical supervision of collieries, as a synopsis of the contents will indicate.

Bulletin XI contains a concise, but comprehensive, discussion of the principles underlying the problem of the stone dusting of mines, with special reference to the use of shale for this purpose. It is only by a microscopical examination of the shales that it is possible to determine the condition of the silica present, and hence, to forecast the effects of inhalation on lung tissue. Bulletin XII describes an investigation of the chemical composition of iron pyrites from coal. This research is part of a comprehensive study of the spontaneous ignition of coal as it applies to the seams in the Lancashire coalfield. In this connection it is interesting to note that the complete oxidation of pyrites present to the extent of 1 per cent. in coal evolves sufficient heat to raise the temperature of the coal 74°C. Bulletin XIII is a brief account of the methods available for the determination of small volumes of carbon monoxide in vitiated mine air. Bulletin XIV deals with the preparation of transparent sections of coal for microscopical investigations, and Bulletin XV with the melting point of coal ash. The conclusion is reached that chemical analysis is of little value as a criterion of this constant. Bulletin XVI gives a general account of the waters available at collieries for steam raising purposes, and of the methods and principles of water softening, and should be of interest to many outside the Coal Industry. With regard to the use of "boiler compounds," the authors aptly remark that a boiler is primarily intended for raising steam, and not for performing chemical reactions.

The publication of such a compendium, at a time when the need for the application of scientific methods to the Coal Industry is all too apparent, is greatly to be welcomed, and the Research Association are to be congratulated on it.

T. S. WHEELER.

THE PROBLEM OF PROOF. By A. S. OSBORN. 2nd edition. Pp. xxiii + 539. U.S.A.: The Essex Press. London: Sweet Maxwell, 1926. Price 25s.

This stimulating work has deservedly reached its second edition within the short space of four years. The first edition was reviewed in the ANALYST (1922, 47, 543), and the great value of the book to all who are concerned with the demonstration of the truth in courts of law—whether as judge, advocate, or witness—was then pointed out. All too often it is difficult for the legal mind to grasp the scientific point of view, and *vice versa*; but a study of the two chapters: "Cross-Examination from the Stand-point of the Witness" and "Cross-Examination from the Stand-Point of the Lawyer," would go a very long way towards bridging this gulf.

Much of the book is practically the same as in the first edition, but the subject matter has been re-arranged so that the more technical portions now run continuously in the later chapters, and a new chapter on "Thought and Reasoning" has been added. Here Mr. Osborn once more shows his gift of writing in pithy and arresting aphorisms. For instance, to cite only one or two of these—in referring to the undigested accumulation of facts, he remarks: "There are many, not altogether unintelligent human beings, who have the notion that when they are not reading or talking, or doing something with their hands, or learning something, that they are doing nothing, and, sad to say, they often are. Many individuals are wholly unable to be busy with the mind alone. . . . . Unwise and persistent reading is a refuge for the unthinking, and often is a sign of ignorance and unintelligence." And again: "Simple knowing is static, while thinking is dynamic;" and, "Thinking is not learned by reading about thinking, but is only learned by thinking."

Even the bibliography at the end of the book arrests attention, ranging, as it does, from Tyndall and Osler to Scott's novels, and from Archbishop Whately to Dickens and Mark Twain, and having Mr. Osborn's shrewd comments after each entry.

This is a book not merely to have upon one's shelves, but to read and re-read, for it affords an excellent training in logical precision, and, apart from its practical value as a guide to the expert witness, it well repays study for its own sake. The reading of the new edition has but served to strengthen the opinion I formed of its predecessor. It shows on every page the outlook of an original mind.

EDITOR.