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Physiological Standardisation.

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(*Read at the Meeting, April* 4, 1923.)

The discovery of the value of medicinal substances, and their later development, was based very largely on physiological, or, rather, pharmacological observations. During more recent years these have been looked upon as almost the sole means for a rational selection of remedies, and for the establishing of correct dosage.

It is only in comparatively recent years—since 1895—that pharmacology has been recognised scientifically as a method of ascertaining the value of a medicinal preparation. Prior to such recognition, if no chemical method existed for standardisation, entire dependence was placed, for the most part, on the standard methods for extraction, and on certain physical tests. Later, when it was recognised that a worthless sample of a medicinal drug would make an extract not differing in any apparent respect from one from an active sample, it was very evident that a physiological assay process was a necessity. The necessity for standardisation is also clearly shown in the table on p. 304, in which the highest and lowest results obtained with 18 different drugs are given, together with the official standards of the United States Pharmacopœia.

It will be obvious that physiological assay cannot be applied to any drug which induces no typical reaction when administered to an animal, or applied to living tissue, and, of course, it is unnecessary to apply it to drugs possessing an active constituent with well-marked chemical characteristics.

In general, therefore, it may be said that physiological standardisation is indicated for such drugs as are not amenable to chemical assay. Objection has been voiced against biological standardisation, not so much against the method, as against the limited application of the method, viz. that it is qualitative only.

The question is whether the test can be made quantitative, and the value of the substance be measured to establish the dosage.

In the course of my remarks I hope to be able to show how it is possible to arrive at this dosage fairly accurately, although the subject is so big that my observations must necessarily be somewhat sketchy, and my description of the processes employed somewhat superficial.

CANNABIS SATIVA.—This drug, which, when grown in India, is also designated as *Cannabis indica*, is notoriously unstable, perhaps more so than any other member of the materia medica. It is best standardised by determining its effect upon the dog; terriers are very suitable for the purpose, although this kind does not appear to be absolutely necessary. Generally the animals are starved twenty-four hours before being dosed with a sample of the standard drug, the doses usually being in the proportion of 0·01 grm. of extract, 0·1 c.c. of fluid extract, or 1 c.c. of tincture per kilo. of dog weight. The effect of the drug is to produce incoordination in the muscles, generally preceded by some degree of preliminary excitement, drowsiness and a fall in temperature. It is practically impossible to kill an animal, even with an overdose of *Cannabis*, although it is not a difficult matter to render it insensible.

I.

THE NECESSITY FOR STANDARDISATION.

	Lowest Assay Per Cent.	Highest Assay Per Cent.	U.S.P. Standard Per Cent.	
Aconite root ...	0·272	0·80	0·5	Aconitine
Belladonna leaf	0·253	0·515	0·3	Mydriatic alkaloids
Belladonna root	0·34	0·724	0·45	Mydriatic alkaloids
Cantharides, Russian	0·292	1·13	0·6	Cantharidin
Cinchona, Red	5·36	10·28	5·0	Total anhydrous alkaloids
Coca leaf ...	0·353	0·928	0·5	Ether-soluble alkaloids
Digitalis ...	0·213	0·45	0·25	Digitoxin
Ergot ...	0·107	0·347	0·15	Cornutine (of Keller)
Gelsemium ...	0·178	0·64	0·4	Alkaloids
Hyoscyamus ...	0·043	0·234	0·08	Mydriatic alkaloids
Hydrastis ...	2·90	4·09	2·5	Hydrastine
Ipecac ...	1·73	2·63	1·75	Alkaloids
Jalap ...	4·53	9·66	7·0	Total resin
Mandrake ...	3·64	5·98	4·0	Resin
Nux vomica ...	0·523	1·39	1·25	Strychnine
Opium gum ...	10·50	12·27	9·0	Crystallised morphine
Sanguinaria ...	2·52	6·04	2·5	Alkaloids
Stramonium leaf	0·17	0·618	0·25	Mydriatic alkaloids

A considerable degree of skill is necessary in determining the amount of *Cannabis* intoxication within a definite time, but it is surprising how accurate this ocular method of physiological standardisation has become. Chemical standardisation is inadequate, since it bears no obvious relationship to the physiological activity.

THE DIGITALIS SERIES OF HEART TONICS.—Digitalis, together with strophanthus, squills, convallaria, and similar drugs, contains glucosides of great physiological activity, and such drugs do not lend themselves to chemical methods of standardisation, as shown in Table I. They form an extremely important part of the cardiologist's armoury, and digitalis and strophanthus, in particular, require careful examination. The effect of a therapeutic dose of tincture of digitalis is to increase the amplitude of the heart beat, which is also slowed, but the contractions are still quite regular; the blood pressure is also raised, but within the limits of safety even in a serious cardiac conditions.

Dried digitalis leaves vary considerably in therapeutic activity. Some batches are quite often met with containing at least four times the amount of activity which should be possessed by a standard sample.

For instance, in Table II. showing the assay of 10 samples of digitalis leaves, the first sample is shown to have no less than 4 times the standard activity. Every specimen examined was well over strength, the average of the 10 samples being no less than $2\frac{1}{2}$ times the U.S.P. Standard.

II.

ASSAY OF TEN SAMPLES OF DIGITALIS LEAVES.

Sample No.		Volume of Tincture per grm. body weight of frog		Potency in terms of U.S.P. Standard taken as 1·00
1	...	0·0015 c.c.	...	4·00
2	...	0·0018 c.c.	...	3·33
3	...	0·0019 c.c.	...	3·16
4	...	0·0025 c.c.	...	2·40
5	...	0·0025 c.c.	...	2·40
6	...	0·0025 c.c.	...	2·40
7	...	0·0030 c.c.	...	2·00
8	...	0·0030 c.c.	...	2·00
9	...	0·0035 c.c.	...	1·71
10	...	0·0035 c.c.	...	1·71
	Average	0·00257 c.c.	...	2·51

The accepted standard is a tincture of such strength that 0·006 c.c. per grm. frog weight shall cause *systolic* stoppage of the heart in 60 minutes.

The effect of administering a tincture made from such a specimen as No. 1 in Table II. would be to raise the blood pressure to a dangerous degree and, instead of the digitalis diminishing conductivity (*i.e.* by acting directly on the conductile tissue, and so producing a slower and independent ventricular rhythm), as it does in such a condition as auricular fibrillation, the use of such a tincture would practically mean the administration of a toxic dose, and the heart would be made to beat so irregularly as seriously to endanger life.

The physiological method of standardisation adopted by the United States Pharmacopœia is to determine the minimum systolic dose on frogs. What is

probably the better method is to determine the minimum lethal dose for these animals, although there is very little to choose between these two methods. The minimum lethal dose is based on the grm. body weight of tincture necessary to kill the frog after the drug has been injected into the abdominal lymph sac. The tests for digitalis or strophanthus are generally carried out in a series of five, as shown in the Tables III.—A, B, C. In every case the result is compared with the effect of a standard product of known strength.

STROPHANTHUS.—While it is a common experience to meet with digitalis leaves over-active, it is generally the reverse as far as strophanthus is concerned. The examination of six separate samples of some of the best strophanthus seeds obtainable showed that in no one instance did the specimen reach beyond 80 per cent., and in some cases it was as low as 50 per cent. standard (see Table IV.).

III.A.

MINIMUM LETHAL DOSE DETERMINATION = PRELIMINARY TEST.

(Tincture Strophanthus.)

Frog No.	Weight grms.	Dose per grm. weight of frog	Total dose c.c.	Result
1	18	0·00006	0·11	Alive
2	18	0·00008	0·14	Alive
3	19	0·00010	0·19	Alive
4	21	0·00012	0·25	Alive
5	20	0·00014	0·28	Alive
1	21·5	0·00016	0·34	Dead
2	23	0·00018	0·41	Dead
3	21	0·00020	0·42	Dead
4	19·5	0·00022	0·43	Dead
5	22	0·00024	0·53	Dead

Result:—M.L.D. is between 0·00014 and 0·00016.

III.B.

M.L.D. DETERMINATION—SECOND TEST.

(Tincture Strophanthus.)

Frog No.	Weight grms.	Dose per grm. weight of frog	Total dose c.c.	Result
1	19	0·00013	0·25	Alive
2	17	0·00014	0·24	Alive
3	18	0·00015	0·27	Dead
4	20	0·00016	0·32	Dead
5	19·5	0·00017	0·32	Dead

Result:—0·00015 is apparently the M.L.D., to be verified by a third test.

III.C.

M.L.D. DETERMINATION—FINAL TEST.

(Tincture Strophanthus.)

Frog No.	Weight grms.	Dose per grm. weight of frog	Total dose c.c.	Result
1	18	0·00014	0·25	Alive
2	18	,,	0·25	Alive
3	19·5	,,	0·27	Alive
4	20	,,	0·28	Alive
5	20·5	,,	0·29	Alive
1	18	0·00015	0·27	Dead
2	18·5	,,	0·28	Dead
3	17	,,	0·26	Dead
4	20	,,	0·30	Alive
5	22	,,	0·33	Alive
1	20·5	0·00016	0·33	Dead
2	22	,,	0·35	Dead
3	20	,,	0·32	Dead
4	19	,,	0·30	Dead
5	24·5	,,	0·39	Dead

Result:—0·00015 c.c. is the M.L.D. of the tincture tested, because a majority (3/5ths) of frogs were killed by this dose.

IV.

STRENGTH OF SIX LOTS OF TINCTURE STROPHANTHUS (U.S.P.) MADE FROM BEST STROPHANTHUS SEED OBTAINABLE.

Tincture No.	M.L.D.	Per Cent. of Standard
1	0·00022	70
2	0·0003	50
3	0·00023	67
4	0·00022	70
5	0·00019	85
6	0·00025	60

Standard is tincture (U.S.P.) with M.L.D. = 0·00015.

Note.—This standard is the average strength of hundreds of tinctures tested during a period of 15 years.

ERGOT.—Ergot and ergot preparations are official in the British Pharmacopœia, but, as in the case of many other drugs in this official publication, no reference is made to a physiological test. Ergot has defied all attempts to standardise its products by chemical methods, and a physiological test would appear to be absolutely necessary, especially when it is realised that the drug is used in serious conditions of hæmorrhage, and on its timely use may depend the life of the patient. For many years it has been known that ergot will constrict the peripheral vessels of animals, and may even produce gangrene. The effect of ergot on the comb of birds was known as far back as the year 1884, but it was not until 1898 that Houghton

proposed applying the cock's comb method for the routine assay of commercial ergot preparations. The method suggested was that of giving the crude drug by means of a catheter, introducing fluid preparations into the rooster's crop. The best birds for the purpose appear to be white leghorn cocks, not over one year old, and sufficiently susceptible to the action of ergot for 1 c.c. of the fluid extract to blacken the comb in the typical manner, and to a reasonable extent, in one hour. The present-day method is to inject the preparation deep into the breast muscles, and the effect of the sample is compared very carefully with the blood stasis produced by a similar dose of the standard.

There are a number of difficulties associated with the standardisation of ergot by the pressor test, notably the fact that histamine, which is invariably present in ergot, lowers the blood pressure of most anæsthetised animals, and thus obscures the pressor effect of the ergot, and consequently makes the blood pressure test of the drug an uncertain measure of its value as a hæmostatic agent. The cock's comb reaction, on the other hand, is not obscured by counteracting substances, and with our present knowledge appears to be the most satisfactory of the various tests proposed.

In 1894, Keller (*Schweiz. Woch. Chem. Pharm.*, 1894, **32**, 121) devised a chemical method of standardisation, based on the estimation of cornutin. This method has been compared with the physiological method by the U.S. Public Health and American Marine Hospital Service (*Hygienic Laboratory*, Bull. No. 76), and the following conclusions are drawn from the results:—(1) The results of chemical methods for the assay of ergot show little relation to those obtained by biological methods, and the latter should be used as a means of ensuring to those who require this drug a remedy both potent and of uniform strength. (2) For this purpose the method of using the cock's comb is recommended, on practical grounds, rather than using the uterus. (3) Ergot preparations should be marked with the date of manufacture. (4) The fluid extracts of ergot examined varied in strength greatly, some being only about a quarter as strong as the preparation freshly made from Spanish ergot. Non-pharmacopœial preparations showed even greater discrepancies between the strengths claimed and those actually found.

Blood Coagulants.—Preparations of this description, notably hæmostatic serum, normal horse serum, thrombin, etc., are widely used at the present time in order to control hæmorrhage, particularly in hæmophiliac subjects. One of the best known of these products is hæmostatic serum. The standard adopted for this preparation, which contains prothrombin, thrombokinase, and a third substance known as anti-antithrombin, is a shortening of the coagulation time to one-third or one-fourth the normal time for the test animal. Briefly, the method consists in anæsthetising a dog with chloretone by intra-peritoneal injection. The femoral vein and carotid artery are opened and a glass canula inserted for injecting the solution and drawing off samples of blood. A convenient amount of blood—about 3 c.c.—is drawn into a clean test tube and immediately placed in water at 40° C. The beginning and progress of coagulation are observed at intervals of one

minute, and, when coagulation is complete, the tube can be inverted without disturbing the clot. In some cases a tough film forms on the surface, while the volume of blood below remains fluid. This film should be broken so that correct observations can be made.

Several samples are withdrawn and tested to determine the normal coagulation time before making the injection. Usually it is advisable to test a sample of blood every 15 minutes. If no effect from an active coagulant is observed in one and a half hours, the dog is probably highly resistant to a hæmostatic effect and must be discarded.

Clinical tests have repeatedly verified the correctness of the results by this test, and seem to establish the fact definitely that this is a dependable means of assay.

THE PITUITARY GLAND.—While the extracts of this gland (posterior lobe) have widely different effects, as, for example, pressor, oxytocic, diuretic, cathartic, etc., no evidence has yet been forthcoming to demonstrate the presence of more than one active constituent. Some authorities hold that the pressor and oxytocic (uterus-constricting) constituents of pituitrin are one and the same, seeing that, when tested physiologically, more often than not they run parallel. Others, however, hold that there are probably at least four active principles present in pituitrin, two of which are concerned with blood pressure, one is inert, and still another is responsible for the effect of pituitrin on non-striated muscle. In view of this conflicting evidence, the importance of a physiological method of standardisation becomes apparent, and at least two such methods have been suggested. The first is concerned with determining the rise in blood pressure produced by injecting a standard dose of pituitrin intravenously into a dog or cat, preferably anæsthetised with chloretone dissolved in oil. The carotid artery is connected up with a mercury manometer, and this in turn with a kymograph. The rise in blood pressure produced by a standard dose of pituitrin is compared with the effect produced by a similar dose of the sample under examination. The other method of standardisation, and the one which will probably be adopted when an extract of the posterior lobe of the pituitary body becomes official, is the oxytocic test. This has received considerable attention recently at the hands of the Medical Research Council. It depends upon the well-known effect of pituitrin on the freshly isolated uterus of a young virgin guinea-pig. A suitable guinea-pig's uterus is divided into two, being bicornuate, and is suspended in a modified Locke-Ringer solution. Oxygen is allowed to bubble through, and the whole immersed in a water bath. The introduction of a small quantity of pituitrin is sufficient to produce a marked contraction, which is recorded upon the kymograph. The solution is then changed and a small quantity of pituitrin under examination introduced, and compared with the standard. By this method it is possible to get to within 5 per cent., or certainly 10 per cent. of activity (see Figs. I. and II., p. 310).

ADRENALIN.—This drug was made official for the first time in the British Pharmacopœia of 1914. With the possible exception of thyroxin, it is the only

hormone yet isolated from the ductless glands. It is present in the medullary portion of the suprarenals, being first prepared in pure form by Takamine in the year 1901. The standardisation of adrenalin is carried out by determining the rise in blood pressure which a known dose of adrenalin will produce in an anæsthetised dog when compared with the injection of a standard amount, generally 1/6500 grain. Adrenalin tends to oxidise fairly rapidly, and there is no chemical method which can be regarded as absolutely satisfactory. It is a matter of interest that natural adrenalin is about twelve times as active as the dextro-rotatory substance. Chemically, the natural substance is optically active, rotating a ray of polarised light to the left, whereas the synthetic product is relatively inactive, containing, as it does, equal parts of the lævo-rotatory and dextro-rotatory substances.

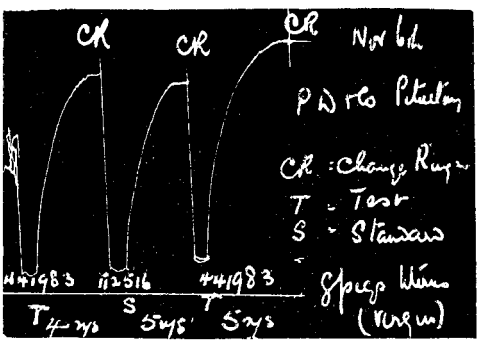

Fig. 1

Effect of pituitrin on excised uterus of virgin guinea-pig. The centre tracing is the standard with which the pituitrin was compared.

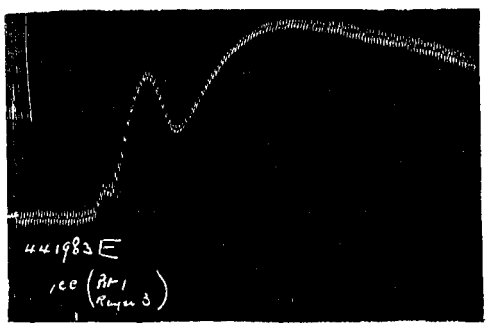

Fig. 2

Injection of 1 c.c. 1 in 4 pituitrin into decerebrate cat showing increase of blood pressure 78 mm. Hg.

An approximate estimation of the strength of adrenalin solution may be made by means of the following colorimetric test:—Two c.c. of the 1:1000 solution of adrenalin to be tested are made up to 100 c.c. with water. To 5 c.c. of this are added 5 c.c. of potassium iodate (1:500), and the solution warmed to boiling point. It is allowed to stand for fifteen minutes, after which it is diluted to 50 c.c. and compared with the standard colour. The solution for this purpose is as follows: Potassium platinic chloride, 20 grms.; cobalt chloride, 12 grms.; hydrochloric acid, 100 c.c.; and water to 1000 c.c. As indicated, this test can only be approximate, and where accurate data are required a physiological estimation should be carried out as described above.

ANTITOXINS.—The principle of physiological standardisation applies with particular cogency in the case of antidiphtheria serum and antitetanus serum. Both the diphtheria bacillus and the tetanus bacillus elaborate soluble or exo-toxins during the process of growth, which can be collected, standardised and used for the production of antitoxins. Practically nothing is known chemically about these toxins and, consequently, they have to be standardised in the first instance by comparing their lethal effect on a guinea-pig which has received a standard

dose of known strength antitoxin. Actually, the object is to determine the minimum dose of toxin which will kill a guinea-pig in four days. Having arrived at the strength of a particular batch of diphtheria or tetanus toxin, as the case may be, horses are gradually immunised with increasing doses prior to the animal being bled with the object of obtaining the antitoxin. This is standardised in terms of antitoxin units. The test of each batch of antitoxin consists in determining the minimum amount of antitoxin which will protect a guinea-pig against a standard dose of toxin. Generally, a series of at least six guinea-pigs is taken, each animal receiving in turn a standard dose of known strength toxin together with varying amounts of the antitoxin under examination (see Table V.). Briefly stated, one antitoxin unit represents that amount of serum which will protect 100 guinea-pigs, each from a minimum fatal dose of toxin.

V.

SERUM TEST.

Serum: Anti-diphtheria.

Cage No.	Colour of Guinea Pig	Weight Grms.	Test Toxin Amount	Serum Amount	Injected	Death	Release
333	Normal	250	0·35	0·0033	17/10/16	—	21/10/16
,,	Red	,,	,,	0·0025	,,	—	21/10/16
,,	Blue	,,	,,	0·0020	,,	—	21/10/16
,,	Yellow	,,	,,	0·00166	,,	—	21/10/16
,,	Red & blue	,,	,,	0·00143	,,	20/10/16	—
,,	Red & yellow	,,	,,	0·00125	,,	19/10/16	—

Result:—Antitoxic unit is that quantity of serum that will exactly *neutralise* one L+dose of toxin.

In the above test 0·00143 c.c. of serum did *not* neutralise the L+dose of toxin, but 0·00166 c.c. did neutralise the L+dose.

Therefore 0·00166 c.c. of this serum is one antitoxic unit.

1 c.c. of serum contains 10000÷166, or 602+antitoxic units.

The Ministry of Health is now recommending that antidiphtheria serum, both for prophylactic and curative purposes, should be in a concentrated form. This may be done by taking advantage of the fact that the antitoxin element of the serum is associated with the globulin fraction. By a process of precipitation with ammonium sulphate the globulins may be separated from the albumins, and the serum thus concentrated. Such serum has the advantage of containing less protein and, consequently, there is considerably less chance of associated effects, such as rash, urticaria, serum-sickness, etc.

The majority of pathogenic micro-organisms do not elaborate any appreciable amount of soluble toxins and, consequently, in preparing antistreptococcus serum it is necessary to immunise the horse first with pure cultures of the streptococci, and later with the live organisms. It is for this reason that antistreptococcus serum cannot be standardised in terms of antitoxin units.

Tuberculins, for the most part, are standardised by weight, whereas in the case of bacterial vaccines standardisation is arrived at by determining the number of organisms actually present in the emulsion. In fact, a vaccine really represents

a standardised, sterilised suspension of pathogenic micro-organisms in physiological salt solution, whereas a tuberculin is a suspension of ground comminuted tubercle bacilli containing so many mgrms. per c.c.

VITAMINS.—As practically nothing is known regarding the chemistry of the vitamins, it is necessary to adopt physiological methods in order to determine the activity of concentrated extracts.

In the case of the fat-soluble *A* a curative test is the method chosen, albino rats being the most satisfactory for the purpose. These animals are fed upon a synthetic diet, complete and properly balanced in all respects except that it does not contain vitamin *A*. In a few weeks the rats develop the symptoms due to the deficiency, namely, poor condition, harsh, rough coat, protuberant hairs, general sluggishness, and a peculiar eye condition known as xerophthalmia. At this stage the substance to be tested is added to the diet given to these rats; if the substance is active the animal will speedily recover, when it may be assumed that vitamin *A* is present. The test, an outline of which is given in Table VI., requires several weeks for completion.

It is now possible to standardise certain preparations containing fat-soluble vitamins in terms of units. This applies especially to cod-liver oil and preparations containing it. The quantitative method is one devised by Zilva and Miura (*Biochem. J.*, 1921, **15**, 654–659), and modified somewhat by Hjort (*Proc. Roy. Soc. Biol. Science*, 1922, **93**, 440–449), and is based on the belief that the fat-soluble vitamin potency of any substance such as cod-liver oil can best be determined by finding the least quantity of the material which, when given daily over a period of three to five weeks, will induce definite growth in white rats that have ceased to grow on a diet deficient in the particular fat-soluble vitamin. While this value will always show some variations, due to slight differences in technique, yet the method, on the whole, has been found satisfactory.

In the case of the water-soluble *B* a curative test is also adopted, both white rats and pigeons being used for the purpose. When rats are employed they are fed with a synthetic diet complete in all respects except that vitamin *B* is absent. The remainder of the test is as in the case of the fat-soluble *A*, that is, the animal develops the symptoms of deficiency disease as indicated by polyneuritic symptoms, viz., the animal stands with difficulty, develops muscular spasms, and, finally, partial paralysis in the hind legs. The test substance is then added to the diet; if the animal recovers, vitamin *B* is assumed to be present; but if it does not recover, vitamin *B* is absent.

If pigeons are used for the test, the birds are given a synthetic diet deficient in vitamin *B*; a diet of polished rice answers the purpose admirably. In from three to four weeks birds fed on such a diet develop a condition of polyneuritis, or, as it is sometimes called, avian beri-beri. If the substance to be tested contains vitamin *B*, one dose administered to the pigeon will cause recovery in from six to eighteen hours. In the event of vitamin *B* being absent from the food, recovery does not take place. Here, again, the tests require several weeks for completion (see Table VII.).

The test for the water-soluble *C* vitamin differs from the two just described in that, instead of being curative, it is a prophylactic test. In this case the object is to try to prevent the disease from developing by feeding the test animals with the substance supposed to contain vitamin *C*; guinea-pigs on a scurvy-producing diet are used for the test. A synthetic diet, complete in all respects except for the absence of vitamin *C* is employed. The animals are divided into two sets respectively "A" and "B." Those in Set "A" are fed on a diet lacking in vitamin *C*, with the result that the animals develop scurvy in about thirty days. Those in Set "B" are fed with the same diet, to which is added some of the substance to be tested. If these animals do not develop scurvy it is assumed that vitamin *C* is present, since the other animals which received the same diet except for the test substance developed the disease. On the other hand, if the second set of animals (B) develop scurvy, it is obvious that vitamin *C* is not present in the test substance, because its administration did not prevent the development of scurvy. This test requires about two months for completion (see Table VIII.).

VI.

TESTS FOR VITAMINS. FAT-SOLUBLE *A*. (Curative Test.)
(Albino rats used for test.)

1. Synthetic diet complete except for absence of Vitamin *A*.
2. Animal developed symptoms due to the deficiency.
3. Substance tested now added to diet.
4. If animal recovers, Vitamin *A* is present in substance.
5. If animal does *not* recover, Vitamin *A* is *not* present.

This test requires several weeks for completion.

VII.

TESTS FOR VITAMINS. WATER-SOLUBLE *B*. (Curative test.)
(White rats or pigeons used for test.)

1. Rats:—
   a. Synthetic diet complete except for absence of Vitamin *B*.
   b. Remainder of test same as for Vitamin *A*.
2. Pigeons:—
   a. Synthetic diet deficient in Vitamin *B*.
   b. Polyneuritis develops in three to four weeks.
   c. One dose of substance containing Vitamin *B* causes recovery in 6 to 18 hours.

VIII.

TESTS FOR VITAMINS. WATER-SOLUBLE *C*. (Prophylactic test.)
(Guinea pigs on scurvy-producing diet for test.)

1. Synthetic diet complete except for absence of Vitamin *C*.
2. Animals divided into two sets "A" and "B."
3. Set "A" fed on diet as in No. 1.
4. Animals developed scurvy in about 30 days.
5. Set "B" fed on same diet plus substance tested.
6. Set "B" do not develop scurvy—Vitamin *C* is present.
7. If the "B" animals *do* develop scurvy, Vitamin *C* is *not* present.

This test requires about two months for completion.

In conclusion, I wish to thank Messrs. Parke, Davis & Co. for their permission to publish this communication.

DISCUSSION.

Dr. G. W. MONIER WILLIAMS asked whether the animals showed tolerance to the drugs.

Mr. E. HINKS wished to know how the original standards with which the samples were compared were arrived at.

Mr. E. T. BREWIS asked whether the author had made any comparative examination of *Cannabis indica* as grown in India and Madagascar as against the American plant, and whether he had found any marked difference in the activity of the plants as grown in different countries. He would like to know how the activity of the digitalis leaf, as grown in the United States of America, compared with the Continental (South-East European) varieties and that grown and dried in England. In his opinion, the most active was the English variety, due to the great care with which it was collected and dried, and he considered that with digitalis in particular, unless it were stored in what he would call a "super-dry" condition, as compared with the manner in which drugs were usually stored, it was inclined to deteriorate. He enquired if the author had observed any deterioration of the liquid preparations of digitalis—such as the tincture—on storage.

Mr. CHASTON CHAPMAN said that he had noticed that the author had mentioned that one of the vitamins was destroyed by boiling, and he would like to know whether milk was seriously affected by pasteurisation.

Dr. STANLEY WHITE, replying, said that in the case of *Cannabis indica* the animals did become tolerant, and had, of course, to be changed. The question of standards for physiological tests was certainly one of great difficulty. In the United States of America the Government had made standards for antitoxins, supplied the antitoxin in a dry state, and were responsible for that standard. In the case of pituitary extract, pituitary glands were passed by the anatomist and histologist as perfect, care was taken in removing fat, and a standard dry extract was made. In the case of digitalis the Medical Research Council were having difficulty, and he could not say what were the relative values of the plant as grown in different countries. He was acquainted with only two varieties of *Cannabis indica*—the Indian and the American.

The question of deterioration was an important one; some products tended to deteriorate, probably 10 per cent. per annum, and the practice had recently been introduced of stating on the label that the preparation should be used before a certain date; this procedure was being adopted all over the country with preparations of pituitary gland.

As regards the effect of heating milk, the anti-scorbutic vitamin was destroyed by boiling. In the case of dried milk much of the value (*i.e.* water-soluble vitamin *C*) was absent, and the makers themselves advised that orange juice should be given in addition. Vitamin *A* was destroyed by continued heat, but was probably not affected to any extent by pasteurisation; "*B*" would be less likely to be destroyed; but "*C*" would most probably be destroyed.

Presence of Barium and Strontium in Natural Brines.

By A. G. FRANCIS, B.Sc., F.I.C.

(*Read at the Meeting, May* 2, 1923.)

Introduction.—Barium and strontium in small proportion have been detected in waters from numerous sources, as, for example, in the springs of Harrogate, and Llangammarch Wells in this country, of Ems, Wiesbaden, Carlsbad, Meinberg, Aix-la-Chapelle, and Pyrmont in Europe, and of Varennes and St. Leon in North America, but the presence of these elements in quantities greater than a few parts per 100,000 is a much rarer phenomenon. It seems therefore, desirable to place on record the results of the analysis of some samples of water lately received by this Department from boreholes in Staffordshire, Derbyshire, and Scotland, where boring for oil has been in progress, together with an account of the method of analysis and a simple explanation of the presence of large quantities of the chlorides of barium and strontium in natural brines.

Previous Analyses.—Bedson (*J. Soc. Chem. Ind.*, 1887, 712), White (Analyst, 1899, **24**, 67), and Richards (Analyst, 1901, **26**, 68) have reported the presence of barium in water in this country to the extent of 137·2, 40·7 and 41·0 parts of barium chloride per 100,000 respectively, but they did not find it associated with strontium. In the Ohio valley district of West Virginia and Ohio weak brines occur that have long been known to contain barium and strontium, and some recent analyses of them (*J. Ind. Eng. Chem.*, 1917, 18) show that the chlorides of these elements are present in approximately equal quantities, the brines at Malden containing 60 and 55, and those at Pomeroy 32 and 42 parts per 100,000 of barium chloride and strontium chloride respectively.

Results of the Analyses.—The following table gives the results (expressed as grms. per 1000 grms. of water) obtained:—

	Derbyshire				Staffordshire		Scotland
	Renishaw		Heath	Brimington	Apedale		West Calder
Depth	3198 ft.	4185 ft.	2870 ft.	1350 ft.	3570 ft.	4245 ft.	3910 ft.
Spec. gravity at 15·5/15·5° C.	1·127	1·116	1·049	1·059	1·178	1·167	1·063
Calcium (Ca)	15·20	13·85	2·58	7·15	35·97	28·67	4·85
Strontium (Sr)	0·67	0·36	0·21	0·16	0·50	0·45	0·88
Barium (Ba)	0·93	—	1·12	0·06	—	—	0·48
Magnesium (Mg)	2·30	2·96	0·84	1·26	0·12	0·47	1·49
Sodium (Na)	40·45	37·40	19·35	20·21	42·23	47·08	24·62
Potassium (K)	0·15	0·29	0·17	0·12	0·27	0·32	0·20
Lithium (Li)	—	Trace	Trace	Trace	0·007	0·005	Trace
Ammonium (NH_4)	0·085	0·11	0·039	0·035	0·012	0·016	0·06
Iron (Fe)	0·023	0·05	0·10	Trace	—	0·010	—
Aluminium (Al)	0·009	—	0·02	0·02	—	—	—
Manganese (Mn)	0·016	—	Trace	0·006	—	—	—
Zinc (Zn)	—	—	—	0·017	—	—	—
Chlorine (Cl)	98·40	90·61	39·00	47·76	131·55	125·89	50·70
Bromine (Br)	0·965	0·86	0·35	0·35	0·76	0·63	0·62
Iodine (I)	Trace	0·006	—	—	0·004	0·004	—
Carbonate (CO_3)	—	0·016	—	0·03	—	0·003	0·04
Sulphate (SO_4)	—	0·45	—	—	0·27	0·35	—
Nitrate (NO_3) —	—	—	—	0·01	—	—	—
Silica (SiO_2)	0·002	0·002	0·005	—	Trace	Trace	—

If the barium and strontium are calculated, for the sake of comparison, as chlorides in parts per 100,000 (w:v), although in the Renishaw sample taken at 4185 ft. the strontium is present almost wholly as sulphate and in the two Apedale samples partly as sulphate, the figures are:

	Renishaw		Heath	Brimington	Apedale		West Calder
Depth	3198 ft.	4185 ft.	2870 ft.	1350 ft.	3570 ft.	4245 ft.	
Barium chloride ..	159·0	—	178·2	9·6	—	—	77·4
Strontium chloride ..	136·7	75·7	39·9	30·7	106·6	95·1	169·4

These proportions of barium and strontium chlorides are exceptionally high.

METHOD ADOPTED FOR THE SEPARATION OF CALCIUM, STRONTIUM, BARIUM, AND MAGNESIUM.—If, after the removal of iron and aluminium by ammonia, the barium is precipitated as sulphate by dilute sulphuric acid before removing calcium and strontium as oxalates, spectroscopic examination of the barium sulphate so formed shows that the precipitate is contaminated with both calcium and strontium sulphates. Such contamination takes place even when the brine is diluted with 10 or 20 times its volume of water. It is necessary, therefore, first to precipitate calcium and strontium as oxalates, and subsequently to precipitate the barium as sulphate. Magnesium is then estimated in the filtrate from the barium in the usual manner. As a rule, when only small quantities of barium are present in the brine none of the barium is precipitated with the oxalates; but when, as in the case of the brine from Heath, much barium is present, the oxalate precipitate may contain a few mgrms. of barium oxalate.

The oxides obtained by ignition of the oxalates were converted into nitrates, and the anhydrous nitrates separated by treatment with mixed absolute alcohol and ether in the usual way. Traces of barium were separated as chromate from the strontium nitrate, and the strontium was precipitated first as carbonate and finally as sulphate.

The barium sulphate, obtained as described, was converted into carbonate, the carbonate was dissolved in dilute acid, and the barium re-precipitated as chromate. From the filtrate traces of strontium were recovered by precipitation, first as carbonate, and finally as sulphate, the weight being added to that of the main quantity of strontium sulphate.

Double separations were carried out in all cases, and the precipitates finally ignited and weighed were examined spectroscopically by means of a spectrometer.

EXPLANATION OF THE PRESENCE OF THE CHLORIDES OF BARIUM AND STRONTIUM IN BRINES.—Bischof (*Chemical and Physical Geology*, 1853, Vol. 1, p. 378, footnote) suggested an explanation of the presence of barium chloride in the above-mentioned American brines by assuming the reduction of barium sulphate to sulphide by means of dissolved organic matter and the subsequent decomposition of the sulphide with strong solutions of calcium or magnesium chloride, with the formation of barium chloride, calcium or magnesium hydroxide and hydrogen sulphide. White (*loc. cit.*) put forward a similar explanation of the presence of barium chloride in a water from Ilkeston, but suggested that the decomposition of the barium sulphide might have been effected by sodium chloride.

If either of these explanations is correct, it becomes necessary to account for the complete absence of sulphur in some form in those brines rich in barium chloride. This could possibly have taken place in one of three ways:

(1) Hydrogen sulphide may have escaped completely into the atmosphere.

(2) The brine containing sulphide sulphur may have met with water containing in solution the bicarbonate of a metal, such as lead, iron or zinc, capable of precipitating the sulphide sulphur and in such quantity as to remove the sulphur completely.

(3) The sulphide sulphur may have been re-oxidised completely to sulphate and the sulphate again precipitated as barium sulphate.

It is also conceivable that more than one of these causes may have operated at different times. For example, part of the hydrogen sulphide may have escaped into the air and the remainder may have been precipitated either as insoluble sulphide, or, after oxidation, as barium sulphate. In this last case the barium found in the brine would be less than that originally present. But, since some of the brines examined come from such great depths as 3,000 and 4000 feet, it is difficult to believe that explanations (1) and (3) can operate. If explanation (2) is the cause of the complete removal of the sulphur, then, owing to the small solubility of the bicarbonates of the heavy metals, great dilution of the brines must have taken place; but this has not occurred in the case of the Renishaw brine which is an approximately 17 per cent. solution.

The explanations put forward by Bischof and White appear to be cumbersome, and a simpler one based upon the permutit reaction is now suggested. It is supported by some experiments made with two permutit filters, one of which was of German origin and the other of British make.

The base-exchanging property of the natural zeolites [hydrated alkali (lime) alumino-silicates] has been known for many years. Bischof (*loc. cit.*, Vol. II., p. 157) demonstrated the conversion of analcite and natrolite into prehnite, involving the replacement of soda by lime, and was aware of the relationship of chabasite (lime-soda zeolite) to gmelenite (soda chabasite with little lime). He suggested that a similar reaction takes place in nature during the conversion of analcite and natrolite into prehnite. The synthesis of artificial zeolites also has been known for many years (*vide* Clarke, *Data of Geochemistry*, p. 366 and 413), but it is only in the last two decades that a hydrated sodium alumino-silicate of the type $Na_2O.Al_2O_6.2SiO_2.6H_2O$ has been manufactured and used commercially for softening water under the name "permutit." As is well known, the reaction is reversible, and the lime permutit can be reconverted into soda permutit by passing a 10 per cent. solution of sodium chloride through the filter.

The filters used for the present experiments were glass tubes, 23 mm. in diameter, and contained columns of permutit 500 and 470 mm. in length respectively.

After the filters had been regenerated by means of 10 per cent. brine and washed with distilled water until the wash water showed a chlorine content of 0·3 part

per 100,000, they were charged at first by passing through them 20 litres of water containing 10 parts of barium or 10 parts of strontium per 100,000, made up in the following manner. Some grms. of barium carbonate or strontium carbonate were placed in a Winchester quart bottle nearly full of distilled water, and carbon dioxide was passed into the mixture for some time. After the carbonates had settled completely the clear supernatant liquid was titrated with 0·1 *N* sulphuric acid, methyl orange being used as indicator. The clear liquid was then siphoned off and diluted with distilled water until the barium or strontium content was 10 parts per 100,000. The filters were charged at the rate of 200 c.c. per hour, and the effluent liquors were quite free from barium or strontium.

Owing to the length of time consumed in charging the filters in this manner, it was later decided to make a solution of strontium nitrate containing 200 parts of strontium per 100,000, and to pass this solution through the filter at the rate of 200 c.c. per hour. It was found that the filter completely removed the strontium, and in this way it was quickly charged with 4 grms. of strontium.

One litre of a solution of sodium chloride of definite strength was then allowed to percolate through the charged filters at the rate of 100 c.c. an hour, and the filtrates were collected in four portions of 250 c.c. each.

With barium permutit it was found that a 10 per cent. solution of sodium chloride had no regenerative action, a 20 per cent. solution dissolved only traces of barium, a 25 per cent. solution dissolved 0·12 grm. of barium per litre, but a 30 per cent. solution of sodium chloride removed the barium completely. It was further observed that more than half of the barium was taken out by the first 250 c.c. of the strongest brine, and that this portion contained barium equivalent to 520 parts of barium per 100,000, when the filter had been charged with 2 grms. of barium.

Next, with strontium permutit a 10 per cent. solution of sodium chloride was found to dissolve a trace of strontium, a 15 per cent. solution 0·36 grm. strontium per litre, whilst a 20 per cent. solution removed the strontium completely. The first quarter of the 20 per cent. brine filtrate proved to have about one half of the strontium, and this portion contained strontium equivalent to 804 parts of strontium per 100,000 when the filter had been charged with 4 grms. of strontium.

Since a 10 per cent. solution of sodium chloride will readily regenerate a filter charged with calcium, these results show a gradation in the strengths of brine required to remove calcium, strontium and barium completely from a permutit filter charged with these elements.

The presence of large quantities of the chlorides of barium and strontium in natural brines may now be simply explained by considering the following train of events. Water charged with carbon dioxide falls upon igneous rocks containing minerals such as felspar, hyalophanes, biotite and muscovite in which these elements are widely distributed (Clarke, *loc. cit.*, p. 14 and 20; Washington, *Chemical Analysis of Rocks*, p. 20). More or less of the barium and strontium passes into solution as bicarbonates, and the water subsequently percolates through

a rock or stratum containing zeolites or minerals of a kindred character. Here the barium and strontium are removed from the water and retained by the rock or stratum. Thresh (*Memoirs of the Geological Survey of England and Wales: Water Supply of Essex*, 1916) has shown that the Thanet sand and sand from the Blackheath beds possess the power to act in some degree as a permutit filter. If, now, a strong brine percolates through a stratum charged in this manner with barium and strontium, these elements will pass into the brine, and the amount of these elements taken up by the brine will depend, other things being equal, on the depth of the stratum through which the brine passes. In the case of a sufficiently deep stratum, therefore, the brine, after percolation, will contain large amounts of barium and strontium chlorides. Such a brine, after dilution with a water which either is free from, or contains only very small quantities of sulphate, would yield, according to the degree of dilution, brines comparable with those from Renishaw, Heath and West Calder.

SUMMARY.—(1) The results of the analysis of some brines containing the chlorides of barium and strontium are given. (2) An explanation based upon the permutit reaction is put forward to account for the presence of large quantities of the chlorides of barium and strontium in natural brines.

In conclusion, I wish to thank Sir Robert Robertson for permission to publish the results of the analyses given in this paper, and also Mr. W. T. Burgess for advice and for the loan of the permutit filters with which the experiments were carried out.

GOVERNMENT LABORATORY,
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DISCUSSION.

Mr. C. C. ROBERTS enquired what effect this solvent action would have on the estimation of strontium when it had been precipitated, and in what particular form the estimated strontium was obtained.

Mr. W. T. BURGESS said that when the author told him about these waters, which came from such great depths, and that he was puzzled to account for the presence of the relatively large quantities of strontium and barium, he thought the matter over and suggested that it might be possible to imitate nature by the base-exchanging properties of the artificial zeolite, "permutit." He accordingly placed two experimental permutit filters at the author's disposal, and the account that the author had given of his experiments afforded an explanation of the presence of the unusual elements in the waters.

The author had found that the 10 per cent. solution of salt, which would readily regenerate permutit charged with lime, failed to have any effect on permutit containing strontium and barium, but that 20 and 30 per cent. solutions were respectively required. It was curious that these concentrations were approximately proportional to the atomic weights of the three elements—calcium, strontium and barium.

The waters were "geological," having been imprisoned for ages until they were tapped by the borings. Water might possibly have found its way through millstone grit, and he had found that this rock, after crushing and "regeneration" with salt, had a slight softening effect on hard water passed through it; in other words, it acted like permutit.

Mr. FRANCIS, in reply, said that it was impossible to precipitate strontium completely by means of dilute sulphuric acid in the presence of large quantities of sodium chloride. The strontium was first thrown down as oxalate and, after the precipitate had been washed and ignited, it was dissolved in dilute hydrochloric acid. The strontium was then estimated as sulphate in the usual manner.

Titanium in Nile Silt.

BY E. GRIFFITHS-JONES.

(*Read at the Meeting, February* 7, 1923.)

IN the published analyses of the Nile silt titanium appears not to have been separately estimated. Lucas, in *The Chemistry of the River Nile*, Survey Department Laboratories, Cairo, 1908, gives 17 analyses of the Nile silt and states that titanium was present in the silt. It appeared of interest to estimate the titanium quantitatively. The figures are higher than was expected, titanium being stated by one authority to be present in clays up to one per cent.

As is well known, hydrogen peroxide gives a yellow to orange colour with solutions of titanium in presence of sulphuric acid. The colorimetric estimation based on this reaction was employed. One grm. of the sample was treated in a large platinum crucible with about 20 c.c. of 40 to 60 per cent. hydrofluoric acid, together with 1 c.c. of strong sulphuric acid. The crucible was heated on the water bath and finally on a hot plate, until the residue was quite free from sulphuric acid. A further 3 c.c. of sulphuric acid were then added, and the heating on the hot plate repeated. Silica was thus eliminated, and the residue left free from hydrofluoric acid.

The residue was fused with about 15 grms. of potassium hydrogen sulphate, the resulting fused mass dissolved in warm water containing 5 per cent. of sulphuric acid, and the solution cooled and made up to 1 litre. Twenty-five c.c. of this solution were transferred to a Nessler glass, 5 c.c. of hydrogen peroxide (10 vol.) added, and the whole made up to the 50 c.c. mark and matched with a standard solution of titanium.

The standard was prepared by fusing 0·2 grm. of Kahlbaum's titanic acid with potassium hydrogen sulphate, dissolving the fused mass in 5 per cent. sulphuric acid, as in the case of the sample, and diluting the solution to 1 litre. One c.c. of this standard contained 0·2 mgrm. of titanium dioxide (TiO_2).

The following results, expressed as percentage of TiO_2, calculated on the silt dried at 100° C., were obtained:—

	Titanium dioxide Per Cent.
Nile silt collected from the river bank at Maadi, near Cairo ...	2·35
Silt from the River Atbara taken 10 miles upstream from the junction with the main river:—No. 1, a grey silt of fine texture	2·2
No. 2, a coarse sand	1·3
Silt from Nile at Hassanat Discharge Site about 5 miles upstream from the junction with the Atbara:—No. 1, fine silt, brown in colour ...	2·4
No. 2, fine silt, grey in colour ...	2·55
No. 3, fine silt, brown in colour ...	2·1
No. 4, fine sand	1·7

It seemed of interest to ascertain whether or not titanium was present in any appreciable quantity in plants grown in Egyptian soil. Straw was selected as being likely to contain titanium in view of its high percentage of silica.

The following figures were obtained with a sample of chopped straw (Arabic "Tibn"):

	Per Cent.
Total ash	6·27
Silica, calculated on ash...	82·4
Titanium dioxide, calculated on ash	0·4

This ratio is very different from that existing in the Nile silt. The Maadi silt had a silica content of 47·5 per cent.

It may be added that no titanium was detected in the residue from 10 litres of filtered and evaporated Nile water.

The silica present in filtered Nile water is about 20 parts per million.

PUBLIC HEALTH LABORATORIES,
CAIRO.

Note.

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.

A GALLOTANNIN APPARENTLY FREE FROM GLUCOSE.

IN Mitchell's colorimetric method of estimating tannins (ANALYST, 1923, 2) the standard gallotannin upon which the comparison was based was a substance with $[\alpha]_D = +51·8$ in aqueous solution, but which apparently yielded practically no glucose after acid hydrolysis with sulphuric acid or enzymic hydrolysis with *Penicillium*. As these results were directly opposed to the widely-accepted theory, which represents gallotannin as galloyl-glucose, and, if confirmed, must necessarily affect that theory, I asked Mr. Mitchell to procure for me, if possible, a supply of the same commercial tannin, and I wish to thank him for the trouble he has taken to obtain it.

I am now engaged in a detailed investigation of this gallotannin, but even at this stage I am in a position to confirm the results recorded by Mitchell.

I have subjected samples of this tannin to acid hydrolysis by the method of Fischer and Freudenberg (*Ber.*, 1912, **45**, 923); and by that of Feist and Haun (*Arch. Pharm.*, 1913, **251**, 500), and have tested the products of hydrolysis both polarimetrically and by Bertrand's volumetric reduction method (*Bull. Soc. Chim.*, 1906, **1** [3], **35**, 1285). I have also hydrolysed the tannin for 14 days with the tannase from *Aspergillus Luchuensis*, and have tested the products by the same methods. The exact results obtained in each experiment will be recorded later, but meanwhile it may be stated that in no instance did the amount of glucose exceed 0·6 per cent. by the polarimetric method or 1·2 per cent. by the reduction method.

I do not attach much weight to the results obtained by the reduction method after the acid hydrolysis, for it has been shown that, under the conditions of such hydrolysis, gallic acid by itself yields a product which reduces Fehling solution (*Ber.*, 1914, **47**, 801). The maximum amount of glucose which the reduction method indicated after the tannase hydrolysis was, however, only 0·6 per cent.

The optical behaviour of this gallotannin cannot, therefore, be attributed to its small sugar content. It is possible that it is a sugar-free tannin the fundamental basis of which is optically active leucodigallic acid (*cf.* Nierenstein, *Annalen*, 1912, **388**, 223), and experiments are in progress to determine the point.

In the meantime I should be pleased to send a small quantity of this tannin to anyone who wishes to examine it.

M. NIERENSTEIN.

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Notes from the Reports of Public Analysts.

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

CITY OF BIRMINGHAM.

ANNUAL REPORT OF THE CITY ANALYST FOR 1922.

DURING the year 5108 samples were analysed, of which 4503 were taken under the Food and Drugs Acts, the number purchased being at the rate of 486 for every 100,000 persons living in the city. Of these samples 4138 were bought informally, and 365 formally. The adulterated samples numbered 238, of which 146 were milks, 10 sponge cakes and 11 sausages.

MILK.—The average composition of the 2349 samples examined was:—Fat, 3·65; and solids-not-fat, 8·76 per cent.

MUSTARD.—Seventy-five samples were genuine, but one contained 35 per cent. of wheat flour. Seven genuine samples gave the following average results:—Nitrogen, 5·1; ash, 4·49; aqueous extract, 23·4; methylated spirit extract, 20·8; and moisture, 4·76 per cent.

ALE AND BEER.—Salicylic acid was detected in 4 out of 55 samples examined. Two contained 1 grain; one, half a grain; and one, a quarter of a grain per pint.

NATIONAL HEALTH DISPENSING.—Thirty insurance prescriptions were taken to 24 panel pharmacists, with the result that 13 medicines, dispensed by 8 different pharmacists, did not comply with the prescriptions.

Alkaline Mixture.—This should have contained 260 grains of sodium bicarbonate made up to 8 ozs. with peppermint water. Five of 12 informal samples were incorrectly dispensed. One contained only 180 grains of sodium bicarbonate; another also contained 17 grains of magnesium carbonate; a third contained neither sodium bicarbonate nor peppermint mixture, but, in place of them, 135 grains of potassium citrate, and about 2 fl. ozs. of solution of ammonium acetate and camphor water; the fourth was deficient in sodium bicarbonate, and also contained liquid extract of nux vomica; and the fifth contained 90 grains of magnesium carbonate and 266 grains of magnesium sulphate.

SEIDLITZ POWDERS.—Ten of 14 samples had been carefully prepared. One contained 20 per cent. of sodium bicarbonate and of Rochelle salt in excess.

RHUBARB.—Six samples were passed as genuine.

Other samples included white mixture, Gregory's powder, Glauber's salt, bicarbonate of potash, tincture of iodine, eucalyptus oil, and white precipitate ointment. (See also Quarterly Reports, ANALYST, 1922, **47**, 295, 431, 512; 1923, 170.)

———— J. F. LIVERSEEGE.

CITY OF BIRMINGHAM.

REPORT OF THE CITY ANALYST FOR THE 1ST QUARTER, 1923.

THE number of samples analysed was 1385, of which 1201 were under the Sale of Food and Drugs Acts (1049 informal of which 56 were adulterated, and 152 formal of which 19 were adulterated).

TREACLE AND GOLDEN SYRUP.—Six out of 9 samples were adulterated with glucose syrup in amounts ranging from about 25 to about 90 per cent. A circular was sent to the members of the grocery trade calling their attention to the necessity for such mixtures being suitably labelled.

DISPENSING.—Five different prescriptions were dispensed by 18 pharmacists, and 15 were practically correct. In one case when the dose was a *tea*spoonful, a bottle graduated in *table*spoonfuls had been used, and this would have meant a serious over-dose of quinine if the patient had accepted the marks on the bottle to represent doses.

POTASSIUM CARBONATE.—The B.P. requires that this drug shall contain not more than 18·5 per cent. of moisture. An informal sample contained 23·9 per cent. of moisture, and another informal sample 23·2 per cent. of moisture and 270 parts of lead per million.

Proceedings were taken in connection with a sample which contained 17·9 per cent. of moisture, 10 parts of arsenic, and 80 parts of lead per million (see ANALYST, 1923, 215, 260). The sample in question was dry in appearance, and had not adhered to the sample bottle. I stated in Court that I had great difficulty in believing that a dry powder would act on glass. The magistrates dismissed the case on the ground that the drug was "unavoidably mixed with some extraneous matter in the process of collection or preparation," and that "collection" included storage.

By the courtesy of the defence I have seen part of the shop bottle in which the drug was kept, and find that the inside surface had been corroded. I understand that the bottle had been in use some years. Since the case I have proved that wet potassium carbonate will remove lead from glass containing it. It seems

probable that previous samples of potassium carbonate had acted on the glass and formed a coating of lead-contaminated potassium carbonate on the interior surface of the shop bottle, and that when the bottle was filled up with the last sample some of this lead-contaminated potassium carbonate mixed with the pure drug, and so accounted for the large proportion of lead present in the sample.

Experiments are in progress to ascertain if potassium carbonate complying with the British Pharmacopœia requirements as to the amount of moisture has any action on glass containing lead.

J. F. LIVERSEEGE.

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.

LIMITS OF ACCURACY IN DISPENSING.

ON May 1, F. O. Finn appeared at the South-Western Police Court to answer a summons by the Battersea Borough Council for selling a compound drug deficient in potassium iodide to the extent of 17 per cent.

Mr. C. A. Hackman, Public Analyst for Battersea, stated that the prescription was:—"Potassium iodide, 5 grains; liquor hydrargyri perchloridi, ½ dram; chloroform water to ½ an ounce. Send 8 ounces of the mixture; ½ ounce to be taken 3 times a day."

He found that each fluid ½ ounce contained 4·15 grains of potassium iodide, or the whole sample 66·4 instead of 80 grains, which would mean a daily deficiency of 2·6 grains. In his opinion, the error was greater than should arise in the ordinary course of dispensing practice.

In cross-examination, he agreed that the B.P. allowed a purity of 98 per cent. of potassium iodide. His certificate was based on a purity of 100 per cent., which would reduce the deficiency in the sample to 15 per cent. He had estimated the mercuric chloride colorimetrically and judged it to be correct. His laboratory balance was sensitive to 1/10 milligram, and he would expect a pharmacist's scales to be correct to at least ¼ grain. When analysing such prescriptions he would allow a margin of error of 10 per cent., though a good pharmacist should work to within 5 per cent. The size of the bottle should have nothing to do with the error. The mixture should be made in a measure. He could not agree that what the doctor meant was that the ingredients should be put into a "reputed" 8-ounce bottle.

Dr. G. Q. Lennane, Medical Officer of Health, Battersea, stated that he considered the error in this case, avoidable and due to careless dispensing. Even if he were unable to observe any difference between the action of 4¼ grains and 5 grains, he would object if the exact quantity were not given.

Mr. Glynn-Jones, counsel for the defence, suggested to this witness that it was not usual to put "½ ounce three times a day." The average patient did not know what half an ounce was, and so it was usual to say a tablespoonful. As tablespoons varied, would it not have been more accurate to say one-sixteenth part? If 80 grains of potassium iodide and 1 oz. of mercury perchloride liquor had been put in and the bottle then filled up with chloroform water, there would have been no ground for complaint.

He submitted that the analyst's certificate must give full particulars and contain such data as would enable the Court to judge what offence had been committed. There was no standard of accuracy to which they could adhere (the Magistrate here disagreed, saying "We have been told that there are 4·15 grains instead of 5·0"). They were not told what was the composition of what was sold. It was quite possible that the proper quantity of potassium iodide was present, and that there was too much water, in which event it should have been treated as a case in which a foreign ingredient was present. As regards a single ingredient being deficient, the analyst had admitted that there was a standard which the certificate did not mention, and that that standard reduced the deficiency by 2 per cent. If a standard existed, the analyst must say on his certificate that he was aware of that standard, and that he based his calculations in reference to it. For aught that appeared on the certificate the analyst might not have known of the B.P. standard. The proper form of certificate dealing with this particular point should contain the words "satisfying the requirements of the British Pharmacopœia."

Medical evidence was then given to the effect that although three-quarters of a grain was an inaccuracy, it was not of great importance. If the right ingredients were put in the bottle it was perfectly safe to fill it up with water. There was a great variation in common tablespoons, and to take one-sixteenth part was more accurate. A pharmacist also gave evidence that, owing to variations in the sizes of bottles, he regarded the method of mixing the ingredients in the bottle as the common sense way, and as more desirable.

Mr. Glyn-Jones, in concluding his argument, said that his contention was that the purchaser intended that the prescription should be made up according to the accepted practice, a practice accepted both by doctors and pharmacists. There was no evidence that the purchaser did not get the doses the doctor prescribed. The analyst had admitted that an inaccuracy of 10 per cent. was permissible. He submitted that there was some doubt whether it was possible to measure without error. The prosecution would not contend that 100 per cent. efficiency was possible, and the question was where did "de minimis" begin to operate. Another point was that possible variations in the size of the bottles would account for an error of at least 5 per cent.

The Magistrate (Mr. Marshall) said that he did not agree with the arguments of Mr. Glyn-Jones. He thought that sufficient care had not been taken in dispensing this prescription, and imposed a fine of 40s., with £3 3s. costs.

NUT CREAM BUTTER.

On May 4th, H. Charnley, grocer, was summoned at Douglas, Isle of Man, for selling a substance labelled "Nut Cream Butter," containing no butter, and being a product made solely from nuts, without labelling it "Margarine."

The analyst's certificate showed that the substance contained: Fat or coconut oil, 90·40; non-fatty solids, 9·20; moisture, 0·40 per cent.; butter fat, none. The inspector stated that he had previously assisted in a prosecution in Bolton against a number of shopkeepers for selling the same product without labelling it "Margarine." One was fined £5, and the others made to conform with the law.

The defendant stated that he sold the product to regular customers—vegetarians—who knew perfectly well what they were getting. The substance did not resemble butter in appearance or taste. It was white and tasted of coconut.

It was not made "in imitation of butter," and it was stated on the wrapper that it was made "purely from sweet nuts."

The High Bailiff thought that, on the whole, an offence had been committed, and fined the defendant £2.

"BEEF AND MALT WINE."

On May 9, D. Gouldman, described as the proprietor of the Liebig Standard Wine Co., was summoned at the Salford Police Court, in three instances, in respect of Liebig's Beef and Malt Wine, which was alleged to be not of the nature, substance and quality demanded. It was sold at 5s. a bottle.

The certificate of the Public Analyst, which was practically the same in each case, gave the composition as follows:—Water, 75·63; total sugars, 21·80; alcohol, 1·50; other extractive matter, 1; and salicylic acid, 0·07 per cent. The "other extractive matter" contained 0·01 per cent. of nitrogen calculated on the original liquid, which indicated the possible presence of not more than 0·2 per cent. of equal parts of meat and malt extracts. This opinion was based on the fact that a mixture of equal parts of meat and malt extracts contained about 5 per cent. of nitrogen. The certificate continued: "This is not a beef and malt wine. Its composition is similar to that of a flavoured cordial, which usually contains up to 0·01 per cent. of nitrogen derived from sources other than beef or malt extracts."

For the defence it was submitted that if there were no meat and malt in the preparation it would not, of course, be of the substance demanded, but that there was some was admitted; therefore it became a question as to quantity. The defendant made two wines, one alcoholic and the other non-excisable. If made as a non-excisable preparation there was a limit to the alcohol. The amount of beef and malt in the wine was the maximum that could be placed with less than 2 per cent. of alcohol and with the quantity of salicylic acid that could be put in with safety.

The Stipendiary Magistrate remarked "This does not affect me. I am influenced by the words 'Beef and Malt Wine'."

The defendant stated that he had frequently tried to increase the quantity of beef and malt extract, but unsuccessfully, for, as soon as an attempt was made, fermentation was set up.

Medical evidence was given that, as a non-alcoholic wine, the preparation was fairly beneficial, but not so beneficial as a standard preparation containing more alcohol.

The Stipendiary Magistrate, remarking that it was a bad case, imposed a fine of £20 in each of the three cases, and allowed £10 10s. costs. He added: "I have said nothing about the price. There was no cross-examination about value, but I have thought about it."

TIN IN CANNED FOODS.

On May 11th the International Tea Co's Stores were summoned at Dover for selling a tin of asparagus containing 2·9 grains of tin per lb. of asparagus.

Dr. A. B. McMaster, Medical Officer of Health for Dover, stated that tin was an irritant poison, which, when taken by certain individuals, gave rise to acute symptoms, although other individuals could take a small quantity of tin without appreciable effect. As tin was a cumulative poison its effect increased as time went on.

In cross-examination by Mr. Cecil Whiteley, K.C., he agreed that any food, fruit, or vegetable which had a natural acidity was specially liable to be contaminated with tin. He had not personally known of a case of food poisoning

traced to chemical poison in the unopened tin, but there were records of cases in the report of the Government enquiry in 1902, and in recent cases. He agreed that the tin found in this asparagus was in an insoluble form, but he had known cases where the tin had been in the liquid as well.

In re-examination, Dr. McMaster stated that the standard in the Government report quoted was the usual standard. Anything above 2 grains to the lb. would be recognised as exceeding the safety line.

Dr. W. G. Savage, Medical Officer of Health for Somerset, stated that he was present on subpœna. He agreed that tin was found in all canned foods in varying quantities, and that it was particularly liable to be found in an acid food, such as asparagus. He did not consider 2·9 grains to the lb. could be harmful in any way. His experience was all against it. He had made a special study of food poisoning for 18 years, and in all that period had never heard of a case of food poisoning due to tin in canned foods. His later investigations seemed to prove conclusively that where tin got into canned foods a great deal of it became insoluble, and most of it passed through the body and did not do any harm. In the past, with insufficient knowledge, it was a natural assumption that chemical poisoning was the cause of the trouble in the reported cases, but, with later experience, it had been shown that in practically all the cases it was not a question of chemical poisoning at all, but of bacterial poisoning.

The Chairman announced that the Bench had given full consideration to the evidence given on both sides and had decided to dismiss the case.

Department of Scientific and Industrial Research.

PRESERVATION OF STONE WORK.

THE problems involved in the prevention of serious decay and gradual demolition of tooled surfaces of main structures, especially of historic buildings, are very complex, and need to be approached from different angles, with the help of wide scientific knowledge.

Accordingly it has been decided to set up, under the Department of Scientific and Industrial Research, a special Committee of the Building Research Board to report on the best methods by which decay in building stones, especially in ancient structures, may be prevented or arrested.

The Chairman of the Committee will be Sir Aston Webb, P.R.A., and the other members will be:—Mr. R. J. Allison, F.R.I.B.A., Professor C. H. Desch, F.R.S., Mr. A. W. Heasman, Mr. J. A. Howe, Sir Herbert Jackson, F.R.S., Dr. Alexander Scott, F.R.S., and Mr. H. O. Weller, M.I.C.E.

All communications should be addressed to the Secretary, Department of Scientific and Industrial Research, 16, Old Queen Street, S.W.1.

Grass Sickness in Horses.

THE following report is an excerpt from the full memoir presented to the Highland and Agricultural Society of Scotland concerning researches into "grass sickness" in horses carried out by the Director of the Investigation, Dr. J. F. Tocher and his co-workers—Mr. W. Brown, Dr. J. W. Tocher and Mr. J. B. Buxton.*

* The report in this form was given by these investigators at a meeting of the Scottish Branch of the National Veterinary Medical Association, held at Perth, December 15, 1922, and subsequently published in *The Veterinary Record* of January 20, and February 3, 1923.

Dr. TOCHER said that in 1918 the belief was prevalent that "grass sickness" in horses was probably produced by alsike clover, and a thorough survey of the infected areas in Forfarshire and Perthshire was made, with the object of identifying toxic plants and clovers, but with negative results. Horses were fed on alsike clover for an entire season, but without ill-effects. Alcoholic extracts from that clover also proved non-injurious, and no toxic substances could be isolated from the extract.

In 1919 a complete tour of the whole of the infected area was made, and a series of *post mortem* examinations carried out. The results indicated the probability of the animals having died from acute toxæmia of bacterial origin, since vegetable toxins had been experimentally excluded. In the same year a large anaerobic bacillus was isolated by Drs. J. F. and J. W. Tocher from portions of the stomach and intestines collected in the course of the tour, and this bacillus was found to have the morphological characteristics and toxigenic properties of van Ermengen's *B. botulinus*, and it was then noticed that the clinicial symptoms closely resembled those of botulism.

During 1920 the investigation was conducted on the assumption that *B. botulinus* was the probable cause of "grass sickness," and a report giving scientific evidence in support of this conclusion was sent to the Highland and Agricultural Society. As the result, funds were raised for continuing the research on bacteriological lines, and the association of the disease with botulism has been established.

Dr. Tocher also produced maps showing the distribution of "grass-sickness" since 1911, and its extension northwards from Forfarshire and Perthshire. In 1921 seven cases were recorded in the Elgin and Banff areas.

Mr. W. BROWN, in describing the symptoms and *post mortem* appearances of "grass sickness," pointed out that it was a seasonal disease which occurred among horses shortly after they are put on to grass, its incidence ranging from about May to September. Two distinct types of the disease were met with (*a*) the acute, and (*b*) the sub-acute, and there were distinct differences in the symptoms of those two types. The *post mortem* appearances were comparatively few but characteristic, and in the sub-acute cases, which might last for 2 or 3 weeks, there were certain features not observed in the acute cases. A full description of these and also of the diagnosis and treatment of the disease was given.

Dr. J. WILLIAMSON TOCHER, describing the bacteriological investigation, said that in acute cases the cerebro-spinal fluid was sterile, but that in sub-acute cases several organisms had been isolated. These included (1) staphylococci; (2) a diphtheroid-like organism; (3) streptococcus; (4) a strepto-bacillus or cocco-bacillus. Of these, the only organism pathogenic to laboratory animals was the streptococcus, but the horse did not react in any way to the sub-cutaneous injection of this organism. In all sub-acute cases of "grass sickness" there is a secondary meningitis of a mild type produced by organisms normally present in the naso-pharynx of the horse.

The method of isolating the causal anaerobic organism was to plunge the spleen in boiling water for 3 to 5 minutes and then to cut it, with aseptic precautions, into small pieces. These were placed in test tubes containing 2 per cent. glucose broth, heated for 20 minutes at 80° C., and, incubated anaerobically in McIntosh and Fildes' jars. In all cases slightly alkaline broth was used. The tubes were incubated for 48 hours, and were then examined for their reaction, and those showing formation of acid and gas selected for further tests. Tubes in which no reaction was shown after an additional 48 hours' incubation were rejected. It was noticed that there was an odour of butyric acid in the case of several of the tubes.

From the selected tubes sub-cultures of anaerobes were prepared in the

following manner: Two per cent. neutral glucose agar was poured in a thin layer on to the bottom of a Petri dish and allowed to cool. Thinly-seeded shake cultures of the suspected organisms were made either in 2 per cent. lævulose agar or 2 per cent. lactose agar, the agar being faintly alkaline to neutral red. The tube of agar was then poured on to the surface of the glucose agar and allowed to solidify. When solid, a third layer of glucose agar was poured on to this second layer to prevent access of air, and good anaerobiosis was obtained with the resulting medium.

The chief contaminating organism in these spleen cultures was *B. sporogenes*. It was distinguished from *B. botulinus*, *B. Welchii*, and *V. septique* by not fermenting lactose, whereas lævulose is fermented by *B. sporogenes*, but not by *B. botulinus*. The filtered cultures from *B. sporogenes* appeared to be non-toxic to laboratory animals. The organisms isolated in these cultivations agreed in morphological characteristics with *B. botulinus*, and the bacillus and its toxin produced in experimental animals and in horses lesions known to be associated with botulism, and identical in character with the lesions found in horses suffering from "grass sickness."

Mr. BUXTON gave a description of his diagnosis by serological and immunological methods. He said that when, in 1919, he had agreed to make a series of serological experiments to test the hypothesis of Drs. J. F. and J. W. Tocher that "grass sickness" was really botulism, he had told them quite frankly that he was unable to accept their hypothesis. At that time botulism was only known to be a primary intoxication, for the characteristic symptoms were only known to have been produced by direct administration of the toxin of *B. botulinus* which had been produced under abnormal conditions outside the animal body. He could not imagine the existence of suitable conditions for the production of the toxin in the field or during the process of preparing or handling horse-fodder.

Samples of the blood from acute and from chronic cases and from recovered animals were tested for the presence of botulinus antitoxin by means of toxins prepared from known strains of *B. botulinus*. These tests showed that botulinus antitoxin was present in the blood of horses which were suffering from the chronic form of "grass sickness," or had recovered from the disease, whilst the blood of a normal horse did not contain a similar anti-body. As was expected, the blood of acute cases did not contain antitoxin. The unstable nature of botulinus toxin was well known, and it was inconceivable that it could have been ingested with the pasture or taken in water. The only way of accounting for its presence in these animals was the assumption that in these cases one was dealing with an *infection* and not with a *primary* intoxication.

Toxin prepared from an organism isolated from a case of "grass sickness" was found to be neutralised by botulinus antitoxin, and this toxin, when injected subcutaneously into horses, reproduced the typical symptoms of an acute case of "grass sickness."

The active immunity produced by means of botulinus toxin-antitoxin mixtures appeared to have had some influence in decreasing the incidence of "grass sickness."

Dr. J. F. TOCHER pointed out that the prevential serum was not prepared from "grass sickness" cultures, but from known strains of the *B. botulinus* obtained from an external or non-grass-sickness source. The death rate among uninoculated horses amounted to 9·3 per cent., whilst the death rate among inoculated horses, on the same farms, was 2·8 per cent. There was, therefore, a reduction due to inoculation of 6·5 per cent. The odds in favour of inoculation reducing the death rate were 1,750,000 to 1. If grass sickness were not identical with botulism, this reduction in the death rate, by inoculation of a botulinus mixture from a non-grass-sickness source, could not have occurred.

These results were therefore conclusive as proving that grass sickness was botulism. Horses should therefore be protected by the use of the preventive serum prepared by Mr. Buxton. A supply was now available on a commercial basis (*cf.* ANALYST, 1923, 118).

Parliamentary Notes.

HOUSE OF COMMONS.

JUNE 13.

LABELLING OF DRIED MILK.—The Minister of Health, replying to a question by Mr. Hurd, said that he was aware that skimmed dried milk was being sold without sufficient indication of its being unsuitable for children, such as is required in the case of condensed milk. He was considering regulations to provide for the proper labelling of dried milks.

JUNE 25.

APPOINTMENT OF GLOUCESTERSHIRE PUBLIC ANALYST.—The Minister of Health, replying to Mr. Garland, stated that his attention had been drawn to the terms and conditions offered by the County and City of Gloucester for the appointments of their respective Public Analysts. He was aware that those authorities had appointed a person as Public Analyst whose qualification did not appear to be within the terms of the regulations as to the competency of Public Analysts, and he was therefore in communication with those authorities on the subject.

Ministry of Health.

THE following circular has been sent to County Councils and Sanitary Authorities (England and Wales):

MILK (SPECIAL DESIGNATIONS) ORDER, 1923.

Circular 408.

SIR,

I am directed by the Minister of Health to refer to Circulars 356 and 362,* and to state that representations have been made to him as to the working of the Milk (Special Designations) Orders, and that after consideration it appears to him that certain amendments of the detailed provisions of the Orders are desirable. He has accordingly decided to issue a new Order which embodies these amendments and revokes the existing Orders as from the 1st July next.

The new Order does not alter the general scheme of grading at present in operation, but it provides for some relaxation of the conditions under which licences may be granted for the sale of "Grade A" milk and "Pasteurised" milk.

The alterations in regard to "Grade A" milk will be found in Article 2 of the Order and Part III. of the Third Schedule, and affect the conditions of production only. It is now provided that only the milch cows of the herd need be subjected to veterinary examination, and consequently only these animals will need to be registered. If a cow is suffering from a disease which is not of a permanent character it will be sufficient for the cow to be isolated (the fact being reported to the Licensing Authority), but in the case of a disease whose injurious effect on the milk is likely to be of a permanent character the cow must as at present be removed from the herd. Further, to meet difficulties which have arisen as to the economical disposal of dry cows, the provision as to separation of the herd has been altered so as to require only the cows in milk belonging to the herd to be kept separate from all other cows in milk. But in this connection

* See ANALYST 1923, 25.

it should be noted that paragraph (2) (*a*) of the Second Schedule to the Order, as in the case of the existing Orders, requires the licensee to take such measures as the Licensing Authority may require to ensure that "Grade A" milk is kept separate at all stages from all other milk.

The following alterations have been made in the conditions applying to "Pasteurised" milk:—

(1) The bacteriological condition has been relaxed so as to remove the test for coliform bacillus and to provide that the number of bacteria shall not exceed 100,000 per c.c. Until the end of the present year this limit is further increased to 200,000 bacteria per c.c. But it will be observed that the bacteriological test for milk sold as "Grade A Milk Pasteurised" will remain as provided in the existing Orders.

(2) The day of pasteurisation is not required to be shown on the labels of vessels containing the milk.

(3) The period during which the milk may be treated by a suitable heating process other than that specified in paragraph (1) of Part IV. of the Third Schedule to the Order (provided the other conditions are satisfied) is extended till the end of the present year.

A number of minor alterations have also been made with a view to removing doubts which had been raised as to the proper interpretation of the former Orders.

A copy of the new Order, which is entitled the Milk (Special Designations) Order, 1923 (Order 68, 444), is enclosed herewith. Although this Order does not come into operation until the 1st July next Licensing Authorities will no doubt regard it as sufficient, in matters where the new Order relaxes the conditions formerly applying, that licensees should comply with the new conditions from the date of this Circular.

I am at the same time to refer to the question of the sampling of graded milk which is mentioned on pages 2 and 3 of Circular 356. As there stated it is thought to be desirable that samples of "Certified" milk should be taken on the average at the rate of about one sample a month for each producer. A scheme has been drawn up in the Department by which the sampling of the milk of each producer is divided between two or three Authorities, the samples being taken more frequently in the summer than in the winter months. Communications have been sent to the Authorities whose participation is desired, and in view of these arrangements it is not necessary that samples of "Certified" milk should be taken except at the request of the Department. In some cases where milk of any other grade from the same source is sold in a number of areas it may be desirable for the Licensing Authority issuing the principal licence (*i.e.* the licence for the bottling establishment or the pasteurising establishment, as the case may be) to formulate a similar scheme.

Further copies of this Circular and of the Order can be purchased through any bookseller or directly from His Majesty's Stationery Office at the addresses shown above.

I am, sir, your obedient servant,

The Town Clerk or
The Clerk to the Council.

A. K. MACLACHLAN, *Assistant Secretary.*

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS.

Food and Drugs Analysis.

Detection of Maize Meal in Bread, Pastry, etc. K. Fricke and O. Luning. (*Zeitsch. Untersuch. Nahr. Genussm.*, 1923, **45**, 69–78.)—The method described depends on the presence of characteristic proteins in maize and on their separation from the proteins contained in wheat flour. Fifty grms. of the dry powdered bread, etc., are boiled with 100 c.c. of 0·3 per cent. alcoholic potassium hydroxide solution for one hour under a reflux condenser; the hot liquid is filtered, the filtrate evaporated until a turbidity appears, and the liquid is then poured into 1 litre of cold water. Twenty grms. of alum are added and dissolved in the solution and, after twenty-four hours, the precipitate formed is collected on a

filter. The moist precipitate is transferred to a basin, mixed with sand, dried, powdered, and boiled with 10 c.c. of amyl alcohol for one hour under a reflux condenser. The hot mixture is filtered through a paper moistened previously with hot amyl alcohol, the filtrate is cooled and treated with three times its volume of benzene and a small quantity of kieselguhr. After a few hours, the precipitate is collected on a filter, washed with benzene until all amyl alcohol has been removed, the filter and its contents are dried, placed in a flask, and digested with 10 c.c. of cold 3 per cent. potassium hydroxide solution (aqueous) for one hour. The mixture is then filtered, and the clear filtrate tested with Esbach's reagent (picric acid, 10 grms.; citric acid, 20 grms.; water, 970 c.c.). A turbidity or precipitate appears immediately if the sample contained maize flour. The presence of milk, fat, or eggs in the flour product does not interfere, and the test is capable of detecti ng as little as 5 per cent. of maize flour in the products. W. P. S.

Estimation of Starches by the Weights of their Grains. L. Lindet and P. Nottin. (*Ann. Falsificat.*, 1923, **16**, 134–137.)—Estimation of the weight of starch grains affords a useful method of checking quality and detecting adulteration of farinaceous substances. About 60 grms. of the starch are moistened with water and, after standing an hour or two, are vigorously shaken in a flask of water, while a narrow capillary tube, closed at one end by the finger, is plunged to the bottom, and an average sample of the starch obtained by allowing the tube to fill. The contents of the sample tube are run on to a microscope slide, this is examined with uniform motion and the diameter of 150 or 200 successive granules is measured by the micrometer; it is necessary to employ an assistant to write down the diameters as if the eye is removed from the microscope during the measurement some of the smaller granules will be overlooked. The average diameter is then found, and the results are calculated to the weight per million grains. Lenticular grains are found to have thickness 3/5 of their radius, and the density of starch is 1·54, so that the simplified formulæ for calculating volume and weight are $V=2\cdot12R^3$ and $W=3\cdot26R^3$. The weights per million of different-sized grains are as follows:

Diameter	Mgrms. per million	Diameter	Mgrms. per million.
2 μ	0·004	40 μ	26·10
5 ,,	0·05	60 ,,	87·80
10 ,,	0·40	80 ,,	207·40
20 ,,	3·24	100 ,,	405·00

The weight of one million grains of potato starch varies, according to kind, from 5 to 14 mgrms. H. E. C.

Composition of Eastern Sweetmeats. A. Heiduschka and P. Zywnev. (*Zeitsch. Untersuch. Nahr. Genussm.*, 1923, **45**, 61–64.)—Various sweetmeats known as "Halwa" are prepared in Turkey and the neighbouring countries, and consist of mixtures of partially inverted sugar, saponin, nuts, and meal. A sample of "white halwa" examined contained 14·5 per cent. of insoluble nut kernels, whilst the water-soluble portion consisted of sucrose, 50·55; invert sugar, 37·49;

saponin, 0·10; ash, 0·11; and water, 11·80 per cent. "Sesame halwa" contained sesame flour, 56·30, and sesame oil, 22·18 per cent., together with the usual saccharine constituents. W. P. S.

Isolation of Ethylgalactoside-β in the Presence of a large Proportion of Reducing Sugars. J. Charpentier. (*J. Pharm. Chim.*, 1923, **27**, 368–371.)—In order to eliminate the presence of sugars which interfere with the isolation of the galactoside, use has been made of the work of Bougault and Perrier (*J. Pharm. Chim.*, 1920, **22**, 119) and of Perrier (*Thèse de Doctorat en Pharmacie*, Paris, 1921), who showed that the action of hydrocyanic acid on reducing sugars, in the presence of small quantities of ammonia, resulted in the formation of a nitrile hydrolysing to an acid containing one atom more carbon than the original sugar, and which was completely precipitated by lead subacetate. In order to extract, for example, the ethylgalactoside from hydrolysed gum arabic (obtained by Bourquelot and Bridel's biochemical emulsin method (*J. Pharm. Chim.*, 1920, **22**, 209) the procedure is as follows:—The alcoholic solution, separated from the emulsin by filtration, is evaporated under reduced pressure, and the extract dissolved in water and clarified by animal charcoal, the same volume (300 c.c.) of hydrocyanic acid solution (1·171 in 100) and 15 drops of ammonia solution being then added. The polarimetric reading falls from 3°2′ at the beginning to +14′ after 24 hours, and ceases at +6′. Lead subacetate is then added, drop by drop, till no further precipitate is formed, the solution filtered, and hydrogen sulphide passed through the filtrate to eliminate the lead. The filtered liquid is evaporated to dryness, and the residue taken up several times with ethylacetate. The solution deposits transparent crystals having a rotatory power of $\alpha_D = \frac{0{,}2 \times 10}{2 \times 0{\cdot}227} = -4°40$, (where $\alpha = -12$; $l = 2$; V = 10 c.c.; $p = 0{\cdot}227$ grm.), and the properties of ethylgalactoside-β. D. G. H.

New Reagent for Alkaloids. C. and E. Viel. (*Comptes rend.*, 1923, **176**, 1156–1159.)—The reagent contains antimony trichloride 5 grms., concentrated hydrochloric acid 20 c.c., and potassium iodide 40 grms. in 100 c.c. of water. Four c.c. of this reagent are added to 1 c.c. of an alkaloid solution containing 20 per cent. of hydrochloric acid, after which a few drops of fresh 1 per cent. neutral sodium sulphite solution are run in and the characteristic golden green precipitate is immediately formed. This reaction is given by 0·001 per cent. solutions of quinine, hordenine, cinchonine, strychnine, emetine, sparteine, pilocarpine, morphine, veratrine, atropine, caffeine, cocaine or theobromine. A similar result is given by solutions of aromatic amines in which the concentration exceeds 0·2 per cent., and the precipitates obtained in stronger solutions separate in the crystalline form. The authors have prepared several of these alkaloidal compounds in the form of crystals, and have shown by analysis that they consist of double iodides of antimony and the alkaloid, analogous to the compounds formed by Mayer's and Dragendorff's reagents. The compounds are readily decomposed by alkalis, with liberation of the alkaloid in the free state. T. J. W.

Nicotine Content of the Leaves and Smoke of Untreated German-grown Tobacco. H. Rhode. (*Zeitsch. Untersuch. Nahr. Genussm.*, 1923, **45**, 112–115.)—The five brands of tobacco examined contained from 0·68 to 2·37 per cent. of nicotine calculated on the dry substance. When the samples were smoked in a pipe, attached to an aspirator with an intermittent action, from 15·4 to 28·8 per cent. of the total quantity of nicotine was found in the smoke. The amount of the alkaloid present in the smoke was not proportional to that in the tobacco itself. W. P. S.

Detection of Oxymethylanthraquinones in Aloes and Rhubarb. M. Lestage. (*Ann. Chim. anal.*, 1923, **5**, 112.)—A modification of Bornträger's method for detecting oxymethylanthraquinones in purgative drugs such as aloes, rhubarb, cascara, etc., is as follows:—Two or 3 grms. of the substance (an anthraquinone glucoside) are hydrolysed, and 1 to 2 c.c. of pure pyridine added. After shaking, and decanting if necessary, 3 or 4 drops of ammonia solution are added. The glucoside gives an intense red colour; dilute solutions of rhubarb a pale pink; and aloes a yellow coloration turning to bichromate yellow and then to chromate yellow. The reaction can be applied to preparations with a basis of rhubarb, aloes or cascara. D. G. H.

Biochemical, Bacteriological, etc.

The Sourness of Acids occurring in Foods and in Wine. T. Paul. (*Zeitsch. Nahr. Genussm.*, 1923, **45**, 83–101.)—Previous methods of estimating the sourness of the taste of acids have been based upon the following principles: (*a*) Determination in the case of each acid of the weakest solution in which the sour taste can still be distinguished from that of pure water (dilution method); (*b*) Determination of the concentrations at which solutions of different acids have the same sourness of taste; (*c*) Arrangement of solutions of different acids of equal concentration in a series according to their sourness. Of these methods the dilution method is the most satisfactory, but it is shown by a series of experiments that the results do not run parallel with the dissociation constants of the respective acids, and that the method is not well suited for quantitative estimations.

The "constant" method, which has proved trustworthy in the estimation of sweetness (ANALYST, 1922, **47**, 261), has also given good results in comparative tests of the sourness of different acids, and the comparison is made in a similar manner. If, for example, a solution of acetic acid containing 8 millimols of $CH_3.COOH$ per litre (the "constant" solution) is under examination, two hydrochloric acid solutions are prepared, one of which tastes distinctly sourer and the other less sour than the acetic acid solution ("extreme solutions"). Between these limits is inserted a series of further hydrochloric acid solutions which always show the same difference of concentration from one another ("intermediate solutions"). If, for example, the two "extreme solutions" contain 3·0 and 15·0 millimols of hydrogen chloride per litre, and the difference in concentration is 1·5 millimols, the following series is obtained:—3·0, 4·5, 6·0, 7·5, 9·0, 10·5, 12·0,

13·5, and 15·0 millimols of hydrogen chloride. The different results—"stronger," "equal" and "weaker"—thus obtained are plotted as curves, and the values calculated by the method of Spearman and Wirth (*Zeitsch. f. Elektrochem.*, 1921, 27, 537). In order to obtain good average results, from 20 to 30 persons should make the test on each occasion.

The following definitions of conceptions and units of measurement are given:—(*a*) "*Taste tone*" is the specific acid taste which belongs to many acids, in addition to the purely sour taste. (*b*) *Sourness* (souring capacity, strength or intensity of acid taste) is the property of an acid to taste sour. It is measured by the concentration of a hydrochloric acid solution with an equal sourness of taste. (*c*) *Iso-acid* liquids are those which show an equal sourness of taste. (*d*) *Specific sourness* (degree of acidity, acidifying power) is the value which gives the number of grms. of hydrogen chloride which must be dissolved in a definite volume of water to give a solution which tastes exactly as sour as a solution of 1 grm. of the substance in the same volume of water. (*e*) *Molar sourness* (molar degree of acidity) is the value which gives the number of molecules of hydrogen chloride which must be dissolved in a definite volume of water to give a solution which tastes exactly as sour as the solution of 1 molecule of the substance in the same volume of water. Specific and molar sourness are expressed by the quotients of the iso-acid solutions C_1/C_2, where C_1 represents the concentration of iso-acid hydrochloric acid and C_2 the concentration of the acid in question. (*f*) The *unit of sourness* (acidity unit) is the reciprocal value of the specific sourness. It is the value which shows the number of grms. which must be dissolved in a definite volume of water to give a solution tasting exactly as sour as a solution of 1 grm. of hydrogen chloride in an equal volume of water. (*g*) *The Molar unit of sourness* (molar unit of acidification) is the unit of sourness in relation to the molar quantities. *Acidity ratio* is the ratio of the acidity, *i.e.* of the hydrogen ion concentration of iso-acid liquids.

The following table gives the molar sourness and the acidity ratio of several organic acids in comparison with hydrochloric acid (hydrogen chloride):

Concentration of Acids millimols per litre	Acetic Acid		Lactic Acid		Acetyllactic Acid		Tartaric Acid		Hydrogen potassium tartrate	
	Molar sourness	Acidity ratio	Molar sourness	Acidity ratio	Molar sourness	Acidity ratio	Molar sourness	Acidity ratio	Molar sourness	Acidity ratio
1	0·75	1:8·3	0·81	1:2·9	0·87	1:1·9	1·35	1:2·2	0·60	1:12·2
2	0·75	1:10·0	0·81	1:3·6	0·87	1:2·1	1·31	1:2·7	0·60	1:17·4
3	0·75	1:10·8	0·80	1:4·2	0·87	1:2·2	1·27	1:3·0	0·59	1:21·5
4	0·73	1:11·5	0·80	1:4·7	0·86	1:2·3	1·27	1:3·3	0·58	1:24·6
5	0·69	1:12·1	0·78	1:5·1	0·85	1:2·4	1·26	1:3·6	0·57	1:26·6
6	0·67	1:12·6	0·74	1:5·4	0·83	1:2·4	1·23	1:3·7	0·55	1:27·8
7	0·64	1:13·0	0·72	1:5·6	0·80	1:2·5	1·20	1:3·8	0·54	1:29·0
8	0·62	1:13·2	0·70	1:5·7	0·76	1:2·5	1·17	1:3·9	0·53	1:30·2
9	0·60	1:13·3	0·68	1:5·8	0·73	1:2·5	1·13	1:4·0	0·52	1:31·4
10	0·59	1:13·3	0·66	1:5·9	0·70	1:2·6	1·10	1:4·0	0·51	1:32·6
11	0·57	1:13·4	0·64	1:6·0	0·68	1:2·6	1·06	1:4·1	0·49	1:33·8
12	0·56	1:13·4	0·63	1:6·1	0·67	1:2·6	1·02	1:4·1	0·48	1:35·0
13	0·55	1:13·5	0·61	1:6·2	0·65	1:2·6	0·99	1:4·2	0·47	1:36·2
14	0·54	1:13·6	0·60	1:6·3	0·64	1:2·7	0·95	1:4·2	0·46	1:37·4
15	0·54	1:13·6	0·60	1:6·3	0·63	1:2·7	0·92	1:4·2	0·46	1:38·6

Hence, the molar sourness of acids compared with hydrochloric acid varies, though not in constant ratio, with the concentration, decreasing as the concentration rises. The hydrogen ion is of primary importance for the excitation of sourness of taste. The fact that the molar sourness of mixtures of acetic and lactic acids is less than that of the sum of the molar sourness of the two constituents can only be due, in the main, to the reduction of the hydrogen ion concentration, which results, by the law of mass action, from the mixing of solutions of weak acids with those of acids of medium strength. Acids with almost the same dissociation constant, such as acetyllactic acid and tartaric acid, may show very different sourness; from which it would seem that acid anions, *i.e.* undissociated molecules, also play a part in the excitation of sourness of taste. The electrolytic dissociation theory does not, of itself, afford a sufficient explanation of sourness. The results of experiments (described in detail) appear to justify the conclusion that sourness of taste also depends upon the vapour pressure of acids. The acids examined may be ranged in the following series in accordance with their specific or molar sourness: —(1) Carbonic acid, (2) hydrogen potassium tartrate, (3) acetic, (4) lactic, (5) acetyllactic, (6) hydrochloric, and (7) tartaric acid. This arrangement differs in many respects from the arrangement of the acids in accordance with their dissociation constants. Certain analogies between sweetness and sourness of taste support the view that it will eventually be found that all four categories of taste (sweetness, sourness, salinity and bitterness) have similar relationships.

Determination of the Velocity of Digestion of Albumin. A. Friederich. (*Chem. Zeit.*, 1923, **47**, 61–62.)—Since digestion of albumin is accompanied by solution of the products of the digestion, the course of the process may be followed by periodic determinations of the concentration of the liquid, conveniently by means of Loewe's interferometer. In a 250 c.c. flask, 2·5 grms. of the material to be investigated are distributed in 150 to 200 c.c. of water containing 10 c.c. of dilute hydrochloric acid, the mixture being left in an incubator, with frequent shaking, until all soluble matter is dissolved; this point is indicated by identity of the interferometer readings of two successive small filtered samples of the liquid, taken at hourly intervals. A solution of 0·5 grm. of pepsin in a little water is then added, and the liquid made up to 250 c.c. with water at 37° C. and well mixed, about 1 c.c. being immediately filtered and examined in the interferometer; the reading thus obtained is taken as the zero reading of the experiment. The flask is kept in the incubator at 37° C., and hourly readings taken on filtered samples until no further change occurs. A larger volume is then filtered, and the dissolved albumin in the clear filtrate estimated by Kjeldahl's method, the following semi-micro-method giving good results. Into a round Jena glass flask having a diameter of about 3 cm. and a neck 13 cm. long are introduced 2·5 c.c. of the filtered liquid, 25 drops of concentrated sulphuric acid, 5 drops of hydrogen peroxide solution (perhydrol), a portion of potassium sulphate as large as a pea, and a little copper oxide. The liquid is heated over a small flame until clear, 2 to 3 drops of alcohol and a few drops of hydrogen peroxide solution being added to the cold liquid, which is then again heated until clear and transferred to a 150 to 200 c.c. boiling flask. By means of a narrow pipette drawn out from a test-tube, 10 c.c. of concentrated

sodium hydroxide solution are passed into the bottom of the flask, and the ammonia distilled off in a current of steam and collected, after cooling, in $N/70$ acid. The excess of the latter is then determined by titration in presence of methyl-red. The total weight of digested protein per 100 grms. of substance, and also the amounts digested at the end of each hourly interval, can then be calculated.

T. H. P.

The Newfoundland Cod-liver Oil Industry. S. S. Zilva and J. C. Drummond. (*Chem. Trade J.*, 1923, **72**, 541–542.)—This paper is the outcome of a visit paid by the authors to the industrial centres for the production of cod-liver oil in Newfoundland, and their examination of the samples collected. The oil is obtained under hygienic conditions, and the second fraction, which is allowed to be used only for industrial purposes, possesses a vitamin potency almost equal to that of the first fraction. Oils obtained in 1919, 1920 and 1921 showed no variation in vitamin content. The method adopted for refining the oil consists in chilling and allowing the stearin to settle out. This stearin, comprising from 3 to 5 per cent. of the oil, although used only for technical purposes, has a vitamin potency considerably higher than that of butter. For this reason the unrefined oil contains a larger proportion of vitamin than the same material after refining, and the former would probably be of greater medicinal value, and could be employed in the form of emulsions. Owing to their greater purity the Newfoundland oils are superior to those produced in Norway, although the opinion of the medical profession is in favour of Lofoten oils. The imports of Norwegian oils into this country are approximately five times the quantity of those from Newfoundland (*cf.* ANALYST, 1922, **47**, 445). T. J. W.

Use of Cod-liver Oil in the Feeding of Farm Animals. J. C. Drummond S. S. Zilva and J. Golding. (*J. Agric. Sci.*, 1923, **13**, 153–162.)—The administration of cod liver oil to farm animals is followed by highly beneficial results, and no taint of milk or meat is occasioned if the oil is of proper quality and is given in suitable quantity. The effects are due to the large proportion of vitamin *A* present in the oil. Oil from stale and decomposing livers—known as cod oil or coast cod oil—has a deep brown colour and an objectionable odour; it is also marked by high acid value and high content of protein derived from the decomposition of the liver tissue. The table shows the relation between the growth constants and the chemical characters of the oils; that used for cattle feeding need not be of the best quality, but should be clear and bright, and have an acidity not greater than 10 per cent., and a colour not deeper than golden yellow. It should also give a strong purple colour when 3 drops of the oil are dissolved in 3 c.c. of petroleum spirit (b.p. 40° to 50° C.) and one drop of sulphuric acid added; it is found that response to this old test is roughly parallel to the vitamin value of the oil, as the chromogenic substance and vitamin *A* undergo oxidation in a somewhat parallel manner. For pigs $\frac{1}{4}$ to 1 oz. daily is a suitable quantity, and affords complete protection against rickets. Sows in pig receiving $1\frac{1}{2}$ to 2 ozs. daily secrete milk rich in vitamin *A*, and for cows and oxen $\frac{1}{2}$ to 2 oz. may with advantage be given.

Analyses and Growth Values of Cod-liver Oils.

No.	Origin	Remarks	Colour	Acid value	Saponification value	Iodine value	Total nitrogen Per Cent.	Total phosphorus Per Cent.	Colour test	Growth dosage* Mgrms.	Quality
K1	Norway, Finmarken	Cod & haddock	Very pale	1·71	191·8	147	nil	trace	8	12	Medicinal
K3	,, ,,	,, ,,	Lemon-yellow	1·73	190·7	159	nil	trace	10	12	,,
K22	,, ,,	2nd fraction oil by draining residues	,,	2·60	189·4	149	0·008	0·004	12	5	Medicinal or good quality cattle
K24	,, ,,	,, ,,	,,	2·11	190·2	152	0·007	0·004	6	25	,, ,,
K5	,, ,,	2nd fraction from rotting of liver residues	Pale golden brown	8·76	195·2	142	0·010	trace	4	12·5	Cattle oil or industrial
H2	,, ,,	,, ,,	Golden-brown	4·35	184·4	153·5	0·03	0·002	10	8	Cattle oil
L3	,, Lofoten	,, ,,	,,	7·44	191·5	167·7	0·015	0·005	4	50	Cattle oil or industrial
HL1	E. Coast, England	From putrid livers	,,	14·4	184·5	147·2	0·02	0·002	6	50	Industrial
HL2	,, ,,	,, ,, ,,	Dark brown	21·4	189·5	155·3	0·03	0·003	8	25	,,
HL3	,, ,,	,, ,, ,,	Very dark brown	21·5	179·8	186·2	0·05	0·006	6	50	,,
N3	Newfoundland	,, ,, ,,	Deep golden brown	26·0	193·3	156·7	0·025	0·005	8	12	,,
N4	,, ,,	From liver pulp by rotting	Golden-brown	1·95	188	156	0·012	trace	8	12	Cattle or industrial
G1	E. Coast, England	From putrid livers	Deep golden-brown	23·11	184·1	175·6	0·036	0·0026	5	50	Industrial
G2	,, ,,	,, ,, ,,	,, ,,	25·64	178·9	179·4	0·040	0·0028	4	50	,,
J4	Newfoundland	2nd fraction from fresh livers	Golden yellow	1·48	190·2	156·2	0·007	trace	13	8	Cattle
R1	?	Commercial cattle oil	Deep brown	17·40	—	—	0·025	0·004	13	8	,,

* Growth dosage represents weight in mgrms. of oil which just maintains steady growth in a test rat of 100 grm. body weight. The figure is therefore inversely proportional to the vitamin concentration in the oil.

H. E. C.

Cod-liver Oil in the Winter Feeding of Milch Cows. J. C. Drummond, K. H. Coward, J. Golding, J. Mackintosh, and S. S. Zilva. (*J. Agric. Sci.*, 1923, **13**, 144–152.)—By feeding cows with cod-liver oil it is confirmed that the presence of vitamin *A* in milk depends upon an adequate supply thereof in the diet, but the amount of the vitamin supplied in the diet does not considerably affect the milk yield or its fat content. Neither does increase of the vitamin content produce a rise in the quantity of lipochrome pigment, as does the turning of the cows out to grass. Administration of cod-liver oil up to at least 4 ozs. per diem does not impart any fishy flavour to the milk or butter. Stall feeding in winter always produces a milk of lower vitamin content than that of the summer milk from grass-feed, even when quantities of silage are included in the diet. H. E. C.

Bios Requirement of Baker's Yeast. J. J. Willaman and A. G. Olsen. (*J. Biol. Chem.*, 1923, **55**, 815–836.)—By growing pure cultured baker's yeast in a synthetic medium containing ammonium chloride as the source of nitrogen, and estimating the reproduction by microscopic counting of the cells, the authors have shown that MacDonald and McCollum's method, involving repeated precipitation of aqueous sucrose solution by absolute alcohol, whilst effecting the removal of vitamin *B*, has little effect upon the bios present. This substance may, however, be extracted from the sugar by recrystallisation three times from 80 per cent. alcohol. A series of 19 organic and inorganic nitrogenous substances was tested for their effects upon yeast reproduction, both with and without the addition of ammonium chloride and of beer wort, but in no case were the results obtained with the substances alone commensurate with those yielded by beer wort only. By correlation of these results with those obtained by numerous other workers the following conclusions are drawn:—Bios is not identical with vitamin *B*, since marked differences are shown in their solubility in alcohol, resistance to alkalis, adsorption by fuller's earth, and precipitation by phosphotungstic acid and by mercuric chloride. Bios is of vitamine nature, and without it normal growth of yeast is impossible. The rate of yeast growth is proportional to the amount of bios present up to an optimum concentration of this substance. No direct action upon yeast growth may be exercised by bios, which may, however, act by assisting enzymic action in the yeast cell. Over 60 nitrogenous compounds tested failed to give results similar to those obtained by the addition of bios. T. J. W.

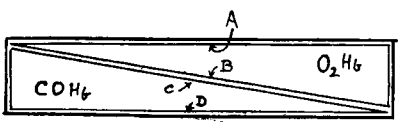
Utilisation of Carbohydrates by Rats Deprived of Vitamin *B*. H. A. Mattill. (*J. Biol. Chem.*, 1923, **55**, 717–727.)—Estimations of the respiratory quotients of rats provided with a vitamin-free basal diet and of others supplied with a similar feed, but containing dried yeast, both before and after the administration of sucrose and dextrose, showed that practically no difference was detectable between the two groups of animals in their ability to assimilate these carbohydrates. Basal quotients were similar in both groups, but non-fasting quotients were slightly lower in the animals deprived of vitamin *B*. A description is given of a simple apparatus by means of which the respiratory quotients of small animals may be determined in from 20 to 40 minutes. T. J. W.

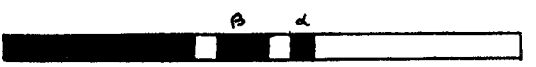
Potency of Commercial Vitamin Preparations. E. M. Bailey, H. C. Cannon and H. J. Fisher. (*Rep. Connecticut Agric. Exp. Station, Bull.* 240, 1922.)—The authors have examined 20 preparations on the American market for their value as sources of vitamin *B* in comparison with dried brewer's yeast as a standard. The method adopted was to compress the material into tablets containing 50 or 100 mgrms. of dry substance, and to supply them or an equivalent weight of pasty material daily to albino rats fed upon a standard diet free from vitamin *B*. The animals were weighed twice weekly, and their growth, physical condition, etc., recorded. The rats continuing to decline in weight after being supplied with the commercial preparation were provided with dried yeast, in order to eliminate specimens whose condition was due to causes other than the absence of vitamin *B*. The preparations showing a vitamin efficiency equal to, or exceeding, that of dried yeast were also tested in smaller quantities of 25 mgrms. To justify the claim to a superior therapeutic value the authors have assumed that such preparations should show a vitamin value at least equal to that of a corresponding weight of dried yeast under similar conditions. Partial analyses of the preparations indicated, in some cases, the addition of filling materials and the presence of various drugs, including cathartics and vegetable tonics. Of the preparations examined 15 per cent. were superior to, 20 per cent. closely approximated, and 25 per cent. were inferior to dried brewer's yeast, whilst the remaining 40 per cent. were useless. Numerous curves showing the results obtained and descriptions of the preparations and their biological results are given. T. J. W.

Abstractor's Note.—No details are given of the dried brewer's yeast employed as the standard in these experiments. Since the vitamin content of this material varies greatly according to the age, method of drying, etc., the results given above are of relative value only.

Presence of Vitamin *C* in some Oriental Fruits and Vegetables. H. Embrey. (*Phillipine J. Sci.*, 1923, **22**, 77–82.)—Guinea pigs were fed upon a basal ration containing whole oats, rice bran and salts, and the control animals died of acute scurvy in from nineteen to twenty-one days. The remaining animals were supplied with weighed portions of different fruits, etc., mixed with their ration, and the anti-scorbutic effect noted. A daily 10 grm. portion of either pomelo (*Citrus maxima*, Merr.), pepino (*Cucumis sativus*, Linn.), chico (*Achras sapota*, Linn.) or guava (*Psidium guajava*, Linn.) was sufficient to prevent the onset of scurvy during nine weeks, and complete protection was also given by 15 grms. daily of banana (*Musa Cavendishii*, Lamb.). The administration of 10 grms. of lansones (*Lansium domesticum*, Corr.) daily failed to prevent a steady decline in weight of the animals during a period of four weeks, after which this fruit was out of season and unobtainable. Complete protection was given by a daily dose of 15 grms. of kangkong leaves (*Ipomoea reptans*, Linn.) or of camote leaves (*Ipomoea batatas*, Linn.) during a period of from seven to nine weeks. The clinical symptoms and post mortem appearances of scurvy in the guinea pig are given, and the paper is illustrated by numerous diagrams of curves showing the results obtained (*cf.* ANALYST, 1922, **47**, 216). T. J. W.

Calibration of the Reversion Spectroscope for the Estimation of Carbon Monoxide in Blood. H. Hartridge. (*J. Physiol.*, 1922, **57**, 47–51.)—A special wedge-shaped cell is described for use with the reversion spectroscope in the estimation of carbon monoxide in blood (*cf. J. Physiol.*, 1912, **44**, 1 and abstract, p. **351**), the internal dimensions of which are $10 \times 2{\cdot}2$ cm. and depth $2{\cdot}5$ cm., and the diagonal partition 2 mm. thick. The thickness of the liquid at various points is determined by sprinkling the inside of the cell with lycopodium and measuring the points A, B, C, and D for various positions from one end of the trough to the other by means of a travelling microscope, and the results are tabulated for different positions showing the value of $\frac{D-C}{D-C+B-A}$. The calibration of the spectroscope is carried out by placing a solution of filtered blood (20 c.c. in 400 c.c. of water containing a trace of potassium hydroxide) in one compartment of the wedge and water in the





other, and adjusting the wedge before the spectroscope until the *a*-band appears sharpest; reference to the table already made shows the values of A, B, C, and D for this position, and $100 \times \frac{C-A}{B-A}$ c.c. of water are added to each 100 c.c. of blood solution to bring it to the correct strength. The mean micrometer readings for O_2Hb are now taken for this position, and the temperature noted. The water in the one compartment is now replaced by carboxy-hæmoglobin solution, prepared by saturating the blood solution with the gas (coal gas may be used), and adding a few drops of ammonium sulphide. Different thicknesses of the two solutions are now set opposite the slit, and a number of readings are taken and the position of the bands tabulated. The calibration curve is then given by plotting $\frac{D-C}{D-C+B-A}$ against the spectroscope readings.

H. E. C.

Use of Potassium Persulphate for Accelerating Digestion in Kjeldahl's Nitrogen Estimation. S. Y. Wong. (*J. Biol. Chem.*, 1923, **55**, 427–430.)—The author has determined the most effective method for employing potassium persulphate as an accelerator of digestion, by which means the time is reduced from $3\frac{1}{2}$ hours to about 40 minutes. Five c.c. of urine, blood or milk or $0{\cdot}5$ grm. of dry protein are transferred to a Kjeldahl flask, followed by 2 c.c. of 5 per cent. copper sulphate solution, 5 grms. of potassium sulphate and 20 c.c. of concentrated sulphuric acid. The mixture is at first heated gently, and afterwards boiled

vigorously, until of an amber colour, after which the flask is allowed to cool for 10 minutes, and 3 c.c. of water are added, followed by 10 grms. of potassium persulphate. The flask is rotated to mix its contents, and again heated until the mixture becomes green, this occurring in about 15 minutes, after which the liquid is cooled and diluted, and the distillation is carried out in the usual manner Results obtained by this are slightly higher than those yielded by the usual method. T. J. W.

Use of Potassium Persulphate in Folin's Method for Nitrogen Estimation. S. Y. Wong. (*J. Biol. Chem.*, 1923, **55**, 431–435.)—Slight modifications of the procedure previously described (see preceding abstract) are adopted for the estimation of nitrogen in small quantities of urine, blood and milk by Folin's "nesslerisation" method, in which an ammonium sulphate solution of known strength is used as standard. This modification avoids the interference produced by silica, if present, and yields clear solutions when testing substances which are oxidised with difficulty. The results obtained agree well with those obtained by Kjeldahl's method and by Folin's original method. T. J. W.

New Colorimeter for the Estimation of Hæmoglobin. H. S. Newcomer. (*J. Biol. Chem.*, 1923, **55**, 569–574.)—This instrument is provided with two vertical cups containing plungers actuated by a rack and pinion, the latter bearing a rotating drum calibrated to read percentages of hæmoglobin. The two optical fields are brought into juxtaposition in the eyepiece by means of a double rhomboidal prism, and the fine dividing line is practically invisible when the two fields are of the same depth of tint. Owing to the difficulty encountered in satisfactorily matching the colour of oxyhæmoglobin the author dilutes 10 or 20 cb.mm. of blood to 502 c.c. with 1 per cent. hydrochloric acid, thus producing a yellow solution of acid hæmatin which is readily matched by a standard yellow signal glass 1 mm. in thickness, fitted to the top of the plunger immersed in water. The standard adopted for the calibration was the average content of hæmoglobin contained in normal adult male blood, this being equal to 16·92 grms. per 100 c.c. of whole blood, and this value was adopted as 100 per cent. on the scale of the instrument. Since the colour of the acid hæmatin solution deepens rapidly immediately after mixing, it is allowed to stand at least 30 minutes before reading, or a correction may be made from a table supplied with the instrument. Exact readings may be readily made and the results obtained are accurate to approximately 1 per cent. T. J. W.

Colorimetric Estimation of Iron in Hæmoglobin and Blood. S. Y. Wong. (*J. Biol. Chem.*, 1923, **55**, 421–425.)—One c.c. of blood is transferred to a test tube containing 4 c.c. of water and, after mixing, the pipette is rinsed with the dilute solution. One c.c. of this solution is run into a hard glass tube, in which is also put 1 c.c. of concentrated sulphuric acid and a glass bead, and the mixture is boiled rapidly over a micro-burner until the tube is filled with white fumes, after which the open end of the tube is closed with a watch glass and the boiling is

continued for 3½ minutes. After cooling for 20 seconds, 1 c.c. of sodium chlorate solution is added, drop by drop, and boiling is renewed for 3 minutes, after which 0·3 c.c. of the sodium chlorate solution is added and boiling is continued for 2 minutes. The tube is then again closed with the watch glass, and the mixture is heated until white fumes again appear, and, after cooling, the liquid is diluted to about 16 c.c. One c.c. of a standard solution containing 0·1 mgrm. of ferric iron is transferred to a similar tube, 1 c.c. of sulphuric acid added, and the mixture diluted to about 16 c.c. To each tube 5 c.c. of 3 *N* ammonium or potassium thiocyanate solution are added, the contents of each tube diluted to 25 c.c., and the colours compared in a colorimeter. The results obtained agree well with those given by Wolter's volumetric method (*Biochem. Z.*, 1910, **24,** 108), by calculation from a standard solution of pure methæmoglobin, and by Palmer's carbon monoxide method (*J. Biol. Chem.*, 1918, **33,** 119). T. J. W.

New Method for the Estimation of Sugar in Blood. Denigès. (*Ann. Chim. anal.*, 1923, II., **5,** 71–72.)—Provided that the concentration of the sugar does not exceed 4 grms. per litre, glucose may be estimated colorimetrically by means of its phenylosazone. Use is made of a phenylhydrazine acetate solution, containing a little sodium bisulphite and stored in yellow or black glass flasks. In the application of this method to estimating sugar in blood, from 4 to 6 c.c. of the latter are mixed with an equal volume of a 1 :5 aqueous trichloroacetic acid solution, and the mixture either filtered or centrifuged. Five c.c. of the clear liquid thus obtained are mixed with 1 c.c. of the phenylhydrazine reagent in a test-tube, which is immersed in a boiling water-bath for exactly 5 minutes, and then in cold water; the turbidity of the liquid is destroyed by the addition of 1·5 grms. of glacial acetic acid, and the yellow colour of the solution then compared with those of potassium dichromate solutions of known concentrations, chosen to correspond in tint with the liquids given, in the above way, by 0·02, 0·04, etc., per cent. glucose solutions. A more exact result may then be obtained by comparison with a glucose solution of known strength. T. H. P.

Whole Culture Method for Testing the Virulence of Diphtheria Bacilli. C. G. Bull and C. M. McKee. (*Amer. J. Hygiene*, 1923, **3,** 103–108.)—The authors have tested the method previously described by Havens and Powell (ANALYST, 1922, **47,** 359) with nearly 4000 nose and throat cultures from school children, basing the diagnoses on the morphological characteristics and the formation of diphtheric lesions by subcutaneous injection into guinea pigs. The results obtained were checked by the isolation of pure strains of the bacilli from the original whole cultures. In this manner 94 per cent. of the unqualifiedly positive cultures were found to be correctly diagnosed, whilst 22 per cent. of the questionably positive cultures yielded positive results, and the remainder negative. Of the questionably negative cultures, 5 per cent. gave positive, and 95 per cent. negative results, but no virulent diphtheria bacilli were isolated from the avirulent whole cultures. The results obtained are in accordance with the observations of Havens and Powell, and the advantages of the whole culture method are (*a*) the number of

cultures that may be tested on one animal, *viz.* from 4 to 6; (*b*) the rapidity with which tests can be made; (*c*) the isolation of pure cultures is unnecessary; and (*d*) cultures from which pure cultures cannot be obtained may be tested. The method is particularly suitable in dealing with convalescents and contacts and in the detection of carriers. T. J. W.

Toxicological and Forensic.

Toxicity of Methyl Alcohol. G. Reif. (*Chem. Zeit.*, 1923, **47**, 367.)—In view of recent statements in chemical journals that "pure methyl alcohol" is non-poisonous, and that it is only the presence of impurities that makes it injurious, the author has made a series of experiments for the Reichsgesundheitsamt (*Deut. Med. Woch.*, 1923, **49**, 183). From the analytical results obtained in the examination of methyl alcohol, the drinking of which caused 10 deaths in Hamburg, the conclusion is drawn that even "practically pure methyl alcohol" will produce fatal results. No impurity, such as allyl alcohol, which could cause poisoning, was present in the Hamburg alcohol, and acetone was only present in negligible traces. These results, therefore, confirm those of an extended experimental pharmacological investigation of methyl alcohol.

Note on the Localisation and Diffusion of Pyramidon. R. Pronzergue. (*J. Pharm. Chim.*, 1923, **27**, 372–374.)—In view of the fact that pyramidon is frequently prescribed in daily doses varying from 0·3 to 1 grm., and its distribution in the human system never appears to have been recorded, results are here given which were obtained from the examination of two fatal cases of poisoning with large doses of about 30 grms. In the case of viscera, after macerating and digesting with hot water, the filtrate showed the presence of pyramidon by giving a characteristic violet colour with 10 per cent. ferric chloride, and a blue-violet colour with 10 per cent. potassium persulphate solution. Body liquids were first cleared with lead subacetate and sodium sulphate, and, on testing as above, showed the presence of pyramidon. By immediately comparing the violet colour obtained with 1 per cent. ferric chloride solution and (1) a 0·1 per cent. solution of pyramidon, and (2) the solution under examination, an approximate idea of the amount present was deduced, and it was found that the stomach contents contained in one case 6 grms. of pyramidon for 200 grms. of contents, and in the other 5 grms. for a content of 150 grms. It is concluded that when large doses of the drug have been taken the pyramidon is rapidly diffused, as it can be found in practically all the viscera and body liquids. Its elimination appears to be through the kidney, for no pyramidon was found in the urine, but in each case a large proportion of antipyrin was present, which is explained by the fact that, chemically, pyramidon is dimethylamino compound of antipyrin. D. G. H.

Water Analysis.

Detection of Sugar in Condensed Waters by means of Cresol. G. E. Stevens. (*J. Ind. Eng. Chem.*, 1923, **15**, 363.)—The reagent is prepared by dissolving 6 grms. of Castile soap in 100 c.c. of water and adding 15 c.c. of cresol.

About 5 c.c. of the water to be tested are placed in a test-tube, 10 drops of the reagent are added and 5 c.c. of concentrated sulphuric acid are run down the side of the tube so as to form a layer below the aqueous solution. The tube is then rotated gently; if sugar is present, a pink or reddish black ring appears at the junction of the two liquids. One part of sugar in 500,000 parts of water yields a reaction, and the presence of traces of oil, or of iron and other mineral salts does not interfere with the test. W. P. S.

Agricultural Analysis.

Investigation by means of Pyridine of the Humus and Fatty Matters of the Soil. M. Piettre. (*Comptes rend.*, 1923, **176**, 1329–1331.)—The humus may be extracted from soil, together with certain other substances, by means of aqueous pyridine. From 20 to 30 grms. of the fine soil, placed in a Durieux thimble, are extracted in a reflux apparatus with pyridine diluted with its own volume of water until the solvent is no longer appreciably coloured by the soil. The solution is then distilled as completely as possible, and the residue dried in a tared basin on a water-bath, the viscous skin forming on the surface being frequently broken with a platinum wire. After being dried for 12 hours at 105–110° C., the residue is weighed; from this weight the free humus is obtained by deducting the ash and the substances soluble in a mixture of alcohol and ether.

To ascertain the combined humus, the residual soil is dried to eliminate the pyridine and treated for some hours, with repeated shaking, with hydrochloric acid of 5 to 10 per cent. strength, according to the content of lime and phosphoric anhydride. The soil is then dried and extracted with aqueous pyridine as described above. The humus thus obtained contains an admixture of fatty substances, which vary in amount with the character of the soil and with the treatment to which it has been subjected, and may be extracted from the dried humus by a mixture of alcohol and ether in equal volumes. These fatty substances, which are almost entirely free from fatty acids containing double linkings, have been regarded as part of the so-called toxins of the soil. T. H. P.

Nature of the Pigment of Silage. H. E. Woodman. (*J. Agric. Sci.*, 1923, **13**, 240–243.)—It is shown that the pigment of silage is phæophytin, the magnesium-free derivative of chlorophyll, produced by the action of organic acids and carbon dioxide on chlorophyll. The colour of silage is not a safe guide to its quality, as this only depends upon the state of molecular aggregation of the colloidal pigment. Although phæophytin has the characters of an ester, it is present in too small amount to affect the accuracy of the estimation of the amino-acids. Phæophytin is soluble in hot alcohol or in acetic acid, but insoluble in petroleum spirit; copper acetate gives with the solution an olive brown colour, ferric chloride gives a green, and zinc acetate gives a blue-green colour with a red fluorescence. H. E. C.

Organic Analysis.

Estimation of Formaldehyde in Presence of Copper Sulphate. M. Jakes. (*Chem. Zeit.*, 1923, **54**, 386.)—The estimation of formaldehyde in preparations containing copper sulphate (*e.g.* those used for treating plant diseases) cannot be carried out by the ordinary direct methods, because these require an alkaline medium, which causes a reducing action by the formaldehyde on the copper compound; also, titration of acid or iodine is not possible in presence of copper. The method recommended is to treat the solution with potassium ferrocyanide, which forms Hatchett's brown, and this can adsorb considerable quantities of excess of potassium ferrocyanide. A solution of the preparation is made up so that the copper content, after dilution, shall not exceed 3 grms. per litre. The quantity of potassium ferrocyanide necessary to produce the compound $Cu_2Fe(CN)_6$ is calculated and then increased by about 20 per cent. This is dissolved in water and added slowly, with frequent shaking, to the formaldehyde-copper solution, diluted to the mark, well shaken, poured into a conical flask, and left over-night. It is then filtered, the first runnings being tested to ensure absence of $Cu^{\cdot\cdot}$ and $Fe(CN)_6^{\prime\prime\prime\prime}$ ions, and an aliquot part of the remainder taken for the formaldehyde estimation by the usual hydrogen peroxide method. R. F. I.

Estimation of the Iodine Value of Fats by Aschmann's Method. B. M. Margosches, R. Baru and L. Wolf. (*Zeitsch. anal. Chem.*, 1923, **62**, 178–184.)—Further investigation of this method (*cf.* ANALYST, 1921, **45**, 465) shows that it will yield trustworthy results in the case of oils, even when a solvent is not used and the mixture is not shaken, provided that the time of contact of the oil with the reagent is not less than twenty-four hours. W. P. S.

Action of Molybdic Acid on the Optical Rotation of Tartaric and Malic Esters. E. Darmois. (*Compt. rend.*, 1923, **176**, 1140–1142.)—On the addition of aqueous solutions of molybdic acid to the esters of tartaric or malic acid the optical rotation and dispersion of the ester gradually increase, the change being accelerated by an increase in temperature. A mixture of solutions of molybdic acid and tartaric acid in molecular proportions immediately shows a rotation and dispersion corresponding with the final results obtained with the tartaric esters. Similar results are obtained by the use of ammonium molybdate in place of the free acid, but no action is observed with molybdic acid and small increments of potassium hydroxide until the added alkali is one-fourth the amount required to neutralise the acid; but with larger quantities the reaction is rapid. By a similar method the author has prepared and identified ammonium dimolybdomalate. The results obtained indicate that the mutarotation is not due to progressive esterification of the alcohol group, but probably to hydrolysis of the ester group of ethyl malate. T. J. W.

Method for the Estimation of Cyanamide. A. Nanussi. (*Giorn. Chim. Ind. Appl.*, 1923, **5**, 168.)—The silver salt of cyanamide reacts with ammonium

chloride in accordance with the following equations, the resulting ammonia and guanidine being titratable with acid: $Ag_2N.CN+2NH_4Cl=2AgCl+N{:}C.N(NH_4)_2$ and $N{:}C.N(NH_4)_2=NH_3+NH{:}C(NH_2)_2$. On this reaction is based the following method for estimating cyanamide either in solution or in calcium cyanamide:

In the latter case, 1 grm. of the finely ground material is treated, with continual shaking and cooling, with 200 c.c. of water and sufficient dilute nitric acid to give a slight acid reaction. The carbon and so-called insoluble nitrogen are filtered off and thoroughly washed, the filtrate being rendered faintly alkaline with dilute ammonia solution, and the cyanamide is precipitated by means of ammoniacal silver acetate solution; if cyanamide in solution is to be estimated, this precipitation is carried out at once. The liquid is filtered, and the silver cyanamide washed until free from ammonia, the filter and precipitate being then transferred to a half-litre Erlenmeyer flask, mixed with 100 to 200 c.c. of water and treated with excess of ammonium chloride solution, and, immediately, with a measured volume, in excess, of 0·5 *N* sulphuric acid. After 10 minutes, during which time the liquid is thoroughly shaken and pounded with a glass rod to break up the filter paper, the excess of acid is determined by titration with 0·5 *N* sodium hydroxide in presence of methyl orange. The results yielded by this method agree well with those obtained by the Kjeldahl method. T. H. P.

Estimation of Benzene in Coal-Gas by means of Active Charcoal. A. Krieger. (*Chem. Zeit.*, 1923, **47**, 357–358.)—The active charcoal method for the estimation of benzene in gas (*cf.* ANALYST, 1921, **46**, 253) is criticised and considered unreliable on the ground that the charcoal gradually loses its efficiency as an absorbent. It is usual, after the absorption, to expel the benzene by heating by means of superheated steam, and experiment shows that the moisture retained by the charcoal after the steam distillation much reduces its absorbing power for further quantities of gas. Different samples of charcoal also vary much in their absorptive power. It is also suggested that local over-heating of the absorbent by the superheated steam may cause the charcoal to become gradually inert. The paraffin method is preferred for such estimations. H. E. C.

Action of Iodine on certain substituted Semi-carbazides and its Application to their Estimation. A. Doucet. (*J. Pharm. Chim.*, 1923, **27**, 361–365.)—As the result of a series of experiments it is concluded that the action of iodine on phenylsemicarbazide results in phenylazocarbonic amide being first formed (C_6H_5–N=$CONH_2$), and that this substance, in the presence of excess of iodine, then forms a more complex crystalline, but unstable, additive compound, which readily gives up its iodine to sodium thiosulphate, re-forming phenylazocarbonic amide. For the estimation of phenylsemicarbazide a known excess of iodine is added and titrated with sodium thiosulphate and, in order to obviate the inconvenience of the formation of the phenylazocarbonic amide in the liquid, excess of sodium thiosulphate is added and titrated back with iodine solution. For example, 0·2 grm. of phenylsemicarbazide and 1 grm. of sodium acetate are dissolved in 10 c.c. of alcohol, and, after ten minutes, 50 c.c. of 0·1 *N* sodium

thiosulphate are added and the liquid titrated with 0·1 *N* iodine solution. The two atoms of iodine are equivalent to one molecule (151) of phenylsemicarbazide. *Metabenzamino-semicarbazide* acts in a similar way with iodine, forming the derivative $NH_2CO.C_6H_4$–N=N–CO_2NH_2, which does not appear to have been described hitherto. It melts at 186° C., without decomposition, and is stable in air; its solubility in 100 grms. of the following solvents is:—Boiling water, 3·25 grms.; boiling 95 per cent.; alcohol, 2·2 grms.; acetone at 15° C., 0·8 grms.; 95 per cent. alcohol at 15° C., 0·7 grms.; water at 15° C., 0·07 grms. Unlike phenylazocarbonic amide it is insoluble in ether, chloroform or benzene. It may be estimated in a similar way to phenylsemicarbazide. D. G. H.

Estimation of Various Monohydric Phenols by the Phenol Reagent of Folin and Denis. (*J. Ind. Eng. Chem.*, 1923, **15**, 406–407.)—Certain monohydric phenols may be estimated colorimetrically by means of phosphotungstic-phosphomolybdic acid reagent. About 0·5 mgrm. of the phenol and 50 c.c. of water are placed in a 100 c.c. flask, 10 c.c. of the reagent are added, the mixture is heated to 30° C., and 20 c.c. of 20 per cent. sodium carbonate are added. After thirty minutes, the mixture is diluted to 100 c.c., and the coloration obtained compared with that produced by a known amount of a standard phenol under similar conditions. β-Naphthol is recommended for use as the standard, since it is a solid and can be weighed directly. The method yields trustworthy results with *m*-cresol, thymol, isoamyl-phenol, ethyl-phenol and butyl-phenol; in the case of resorcinol, the coloration obtained is of so different a shade that comparison is impossible. W. P. S.

Inorganic Analysis.

Use of Phosphorus in Gas Analysis. A. Holmes. (*J. Ind. Eng. Chem.*, 1923, **15**, 357.)—The author prefers to use phosphorus in place of pyrogallol for the absorption of oxygen in gas analysis; the absorption is rapid, particularly if the gas has been treated previously in a bromine pipette, and the disappearance of white fumes indicates that all the oxygen has been removed from the gas.
W. P. S.

Detection of Hydroxylamine. W. M. Fischer. (*Chem. Zeit.*, 1923, **47**, 401.)—The following reaction serves for the detection of as little as 0·00000047 grm. of hydroxylamine per c.c. of solution, but is not given by phenylhydroxylamine, diacetyldioxime, or other organic hydroxylamine derivative. From 1 to 5 c.c. of the liquid to be tested, when shaken with 1 to 2 drops of 2·5 per cent. yellow ammonium sulphide solution and 1 to 2 c.c. of 10 to 25 per cent. ammonia solution, assumes a purple colour if hydroxylamine is present. If the concentration of the hydroxylamine is very low, continuance of the shaking for two minutes may be necessary, but this time is shortened if 1 to 2 drops of *N*/10 manganese chloride or sulphate are added beforehand to the hydroxylamine solution. The coloration is highly unstable and may disappear within one or two minutes, but, if the proportion of hydroxylamine is not too small, reappears on addition of a drop of the

ammonium sulphide solution. Metals precipitable by hydrogen sulphide in acid solution, cadmium in particular, must be removed before application of the test, which is, however, not disturbed by small amounts of the cations, Ni$^{\cdot\cdot}$, Co$^{\cdot\cdot}$, Fe$^{\cdot\cdot}$ and Cr$^{\cdot\cdot\cdot}$. T. H. P.

Electrometric Titration of Selenium in Presence of Tellurium, Iron, and Copper. H. H. Willard and F. Fenwick. (*J. Amer. Chem. Soc.*, 1923, **45**, 933–939.)—When selenious acid is reduced to metal by titanous sulphate in a cold solution containing 25 to 75 per cent. of concentrated hydrochloric acid, and almost, or quite, saturated with sodium chloride, a good end-point is shown with the polarised bimetallic electrode system previously described (*ibid.*, 1922, **44**, 2516). The sodium chloride ensures rapid and uniform coagulation of the selenium hydrosol and increases the sharpness of the change in voltage at the end-point, and the use of a cold solution eliminates loss of selenium by volatilisation. The titration is carried out in a current of carbon dioxide.

After sufficient of the titrating solution has been added to give a constant potential, little change occurs until the end-point is nearly reached. The characteristic rise in voltage occurs, with the fall immediately following; the condition of the solution as regards acidity and foreign salts determines which of the two predominates. As in other titrations with titanous salts, the actual voltage change may not be large, and the galvanometer used should give a distinct deflection for a change in e.m.f. of 1 millivolt between the electrodes. The coagulation of the colloidal selenium close to the end-point is quite distinct, and affords a fairly close visual check on the titration. It is inadvisable to clean the electrodes between titrations other than by washing them with water, as the small amount of adherent selenium increases the sharpness of the end-point.

Tellurium is not reduced under the above conditions, and, even in large amounts, does not interfere with the titration of selenium, although it alters somewhat the character of the end-point; the fall in potential as the end-point is passed does not always occur, but the rise is fully as sharp as in absence of tellurium. Moderate proportions of sulphuric acid exert no deleterious effect on the determination of the end-point, and no volatilisation of selenium occurs at the fuming temperature of this acid. The titration of selenium is independent of the concentration of iron present, although ferrous iron is formed in the reaction. The reducing action of trivalent titanium on mixtures containing copper and selenium is selective, the latter being reduced first; both elements may be estimated by a single titration. T. H. P.

Estimation of small quantities of Molybdenum in Tungsten. W. J. King. (*J. Ind. Eng. Chem.*, 1923, **15**, 350–354.)—The colorimetric method described depends on the solubility of molybdenum thiocyanate in ether. Tungsten metal is dissolved in hydrofluoric acid with the addition of nitric acid, the solution is evaporated, the residue treated several times with nitric acid, and evaporated each time. The residue is then ignited at a temperature below 550° C. Five grms. of the oxide thus obtained are dissolved in 25 c.c. of 3·3 per cent. sodium hydroxide

solution and 75 c.c. of hot water; the solution is filtered through asbestos, the filter washed with 5 per cent. sodium chloride solution, and the filtrate diluted to 250 c.c. Seventy-five c.c. of this solution are nearly neutralised with dilute hydrochloric acid, and 20 c.c. of 6 per cent. tartaric acid solution are added, followed by 25 c.c. of dilute (1:5) hydrochloric acid and 5 c.c. of 50 per cent. potassium thiocyanate solution. The mixture is diluted to about 350 c.c., cooled to 5° C., treated with 20 c.c. of stannous chloride solution (crystallised stannous chloride, 150 grms.; concentrated hydrochloric acid, 100 c.c.; and water, 250 c.c.) and shaken with 90 c.c. of ether. The ethereal solution is separated, and the extraction repeated as long as any colour is removed. The colour of the ethereal solution is then compared with that of a standard solution prepared in a similar way from pure tungsten and a known quantity of molybdenum. W. P. S.

New Method of Detecting Nickel in Solution. C. G. Vernon. (*Chem. News*, 1923, **126**, 200.)—On passing hydrogen sulphide through a nickel solution to which concentrated ammonia solution has been added, and boiling, nickel is deposited in the form of a bright mirror with darkening of the solution, or, in quite dilute solutions, as an iridescent film. The presence of cobalt does not interfere, and the test may be introduced into an ordinary qualitative analysis by adding excess of strong ammonia solution before precipitating the metals of the nickel group with hydrogen sulphide. D. G. H.

New Test for Nitrates. I. G. Nixon. (*Chem. News*, 1923, **126**, 261–262.)—Since the addition of nitric acid to a solution of a sulphonic acid in concentrated sulphuric acid produces nitrosulphonic acids of persistent and intense red or yellow colours, a test for nitrates (and also nitrites) has been devised, which is without the disadvantages of the ordinary ring test. Approximately equal volumes of concentrated sulphuric acid and the suspected solution are mixed, and five times the volume of a 1 per cent. solution of "G. salt" (2:6:8-naphthol-disulphonic acid) slowly added. An intense wine-red colour forms in the presence of nitrates or nitrites. If preferred, the nitrate solution may be mixed with that of the sulphonic acid, and concentrated sulphuric acid run in down the side of the test tube, when a red ring forms at the junction of the liquids, the whole mixture becoming red on mixing. "Gamma acid" (2:6:8-aminonaphthol-sulphonic acid), "Schaffer's acid" (2:6-naphthol-sulphonic acid), and the "Cleve's acids" (1:6: and 1:7:-naphthylamine-sulphonic acids) may all be used, but negative results were obtained with naphthalene-sulphonic acid. D. G. H.

Colorimetric Estimation of Traces of Thiosulphate, even in Presence of Sulphite. O. Hackl. (*Chem. Zeit.*, 1923, **47**, 62.)—Thiosulphate in solutions containing between 0·1 and 2 mgrms. of S_2O_3 per 100 c.c., may be estimated colorimetrically by means of silver nitrate and, if the liquid is rendered slightly acid by addition of sulphuric acid, the reaction is not affected by the presence of sulphite. After addition to the solution of from 4 to 10 drops of silver nitrate solution, the development of the coloration to its full intensity occupies several minutes. The

coloration is matched by means of a solution containing 0·1 mgrm. of S_2O_3 per c.c., this being added in small amounts at intervals of a few minutes to a volume of water equal to that of the solution to be tested and containing the same quantity of silver nitrate. T. H. P.

Physical Methods, Apparatus, etc.

Coincidence Method for the Measurement of the Wave-Length of Absorption Bands. H. Hartridge. (*Proc. Roy. Soc.*, 1923, A. **102**, 575–587.)—In order to eliminate the error arising in the measurement of the mean wave-length of absorption bands due to the breadth of the bands and the indefiniteness of their margins, a spectroscope is described in which two spectra are seen side by side but reversed in direction. The observer has then only to set one edge of a band coincident with the same edge of the one above it—a proceeding which can be done with accuracy—and observe the micrometer reading, which gives at once the mean wave-length with a limit of error of about 0·6 A.U. The optical construction of the instrument, which should preferably have a diffraction grating, and the method of calibration are described in detail. An accurate method for the quantitative estimation of two pigments, either separate or in the same solution, depends on the principle that when two substances together cause absorption of light the mean wave-length varies proportionately to the relative concentrations, provided that the mean wave-lengths of the substances separately do not differ by more than the apparent width of the bands. For such estimations the coincidence method is most suitable, and for this purpose a purely empirical micrometer scale will suffice. Known mixtures of the two pigments are examined, and the mean wave-length or micrometer readings noted for the most convenient band and the results plotted against relative concentrations, or the wedge trough (see abstract, p. 341) may be used. An unknown amount of pigment may then be estimated by mixing it with a known amount of the other pigment and observing the mean wave-length. A useful application of the method is for the estimation of carbon monoxide in blood, the gas shifting the absorption bands about 60 A.U. towards violet. Into one compartment of the wedge-shaped trough is put diluted blood saturated with oxygen, and into the other blood saturated with carbon monoxide; the beam of light then encounters the same number of molecules of blood pigment in all parts of the trough, but encounters different numbers of those combined with oxygen and carbon monoxide. Wave-lengths are therefore measured by the reversion spectroscope corresponding to different known percentages of carbon monoxide and are plotted on a graph which is then available for the determination of the degree of saturation of unknown samples. Temperatures should be carefully noted as each rise of 1° C. shifts the α bands 0·28 A.U. towards the red. The limit of error for the estimation of carbon monoxide is about 1·6 per cent. (see also p. 341). H. E. C.

Absorption-Spectra of Tanning Extracts in the Ultra-Violet. De la Bruère. (*J. Soc. Leather Trades Chem.*, 1923, **7**, 121.)—Absorption spectra in the

visible region give characteristic results for dyestuffs, but not for tanning materials. The authors, however, show photographs in the ultra-violet zone, of quebracho extract (a catechol tan) and of chestnut extract (a pyrogallol tan), and also of varying mixtures of the two. The differences in these are sufficiently characteristic to warrant further investigation. R. F. I.

Control of Thermostats. D. J. and J. J. Beaver. (*J. Ind. Eng. Chem.*, **1923, 15,** 359–361.)—Owing to oxidation of the surface of the mercury, caused by the small electric arc produced when contact is made and broken, ordinary thermo-regulators fail to give good results when required to keep the temperature within very narrow limits for months at a time. This difficulty may be overcome by using a very small current across the mercury contact, and then amplifying it sufficiently by means of a vacuum-tube to actuate a magnetic relay or other current controlling mechanism. An apparatus of this type is described in detail. W. P. S.

Stalagmometry and Viscometry. F. V. von Hahn. (*Chem. Zeit.*, **1923, 47,** 402.)—By means of the apparatus shown in the figure, the viscosity and surface tension of a liquid at high temperatures may be measured successively on one and the same sample. The stopcock at the top of the side-tube being closed, the liquid is forced up into the bulb A, and the time required for the contents of the bulb to flow out through the capillary tube then measured; this time of efflux gives a measure of the viscosity. If, however, the stopcock is opened after the bulb has been filled, the level of the liquid sinks below the ground lower end of the capillary, from which the liquid drops. The number of drops corresponding with the volume of the bulb gives a measure of the static surface tension. T. H. P.

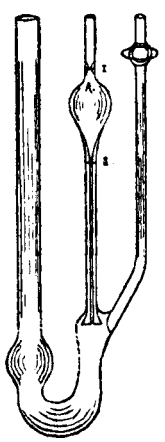


Abb 1

Preparation of Sections of Mammalian Hair. J. A. F. Roberts. (*J. Textile Inst.*, **1923, 14,** T114–116.)—The hair is tied with silk thread in bundles containing from 40 to 100 hairs, about 12 cm. in length, and these are shaken with ether for a few minutes to remove fat, etc., after which the hairs are stained by immersion in picroformol overnight, followed by washing in several changes of rectified spirit until the solvent is no longer coloured; the bundles are then dehydrated by immersion for a few hours in absolute alcohol. Clearing is effected by immersion in xylol, followed by heating for 12 hours in a mixture of xylol and melted paraffin wax, after which the bundles are transferred to a bath of melted paraffin wax (m.pt. 58° C.) for a period ranging from 12 hours to 10 days, the wax being changed at least three times in order to eliminate xylol. The bundles are embedded in the usual manner, and are immersed for at least a week in cold water before being cut. The sections are fixed on albumenised slides, treated

with a solvent of paraffin wax and finally mounted in Canada balsam. Illustrations are given of photomicrographs showing transverse and longitudinal sections of hairs from Welsh mountain sheep × 425. T. J. W.

Reviews.

THEOPHRASTUS BOMBASTUS VON HOHENHEIM CALLED PARACELSUS—HIS PERSONALITY AND INFLUENCE AS PHYSICIAN, CHEMIST AND REFORMER. By JOHN MAXSON STILLMAN, Professor of Chemistry, Emeritus; Stanford University. Pp. 184. Chicago and London (149, Strand): The Open Court Publishing Co. 1920. Price 10s. net.

Although this volume bears the date 1920, it appears that it has only recently been published in this country, which explains why this note is somewhat belated. Let it be said at once that chemists are indebted to Professor Stillman for his essay, as he modestly styles it, upon a man whose fame has been subjected to obloquy far beyond the measure of most famous men.

The part which English-speaking scholars have taken in the elucidation of the character, works and teaching of Paracelsus has been a very small one when compared with what has been done by Sudhoff, Strunz, Aberle, and Julius Hartmann. The late Miss Anna Stoddart's *Life of Paracelsus*, which appeared in 1911, was confessedly written as a popular book, and was not addressed to men of science. Professor Stillman's is a contribution for students to assist them in separating myth from fact in the life of Paracelsus, and he has attempted, quite successfully as it seems to one sympathetic reader, to bring to a focus the researches of the historians of science which have been carried on during the last thirty years. He gives a sufficient account of the life of his subject—his student days at Basle and under Trithemius; his year, or thereabouts, in the mines and laboratories of Sigismond Fugger at Schwatz; his travels to the Universities of Germany, France and Italy, his journeys in Denmark, Sweden and England; and his return to Basle, as the city physician and professor of medicine in the University, where his reforming zeal and unconventional lectures aroused so much opposition that his friends urged him to leave the city.

The experiences of Paracelsus at Basle were the expression of his deep antagonism to the teachings of the slavish followers of the Galenist traditions. His spoken word, supplemented by his writings, "shook," as Thomas Thomson so well said a century ago, "the medical throne of Galen and Avicenna to its very foundation."

Professor Stillman discusses the medical theory of Paracelsus, and his work as a reformer in chemistry, in medicine and in surgery. Perhaps the greatest service he has conferred by his book is the lucid insight he provides into the neoplatonism of Paracelsus, which has hitherto obscured the understanding of his writings for those who have lacked the philosophical outlook, and has, at the same time, given

theosophical mystics a special claim upon his work, a claim which has, perhaps, led many to look upon him as an obscurantist.

Professor Stillman's book can be confidently recommended to those to whom he has dedicated it, namely, "the builders of the sciences of chemistry and medicine whose labours have contributed to the realisation of the dream of Paracelsus of a science founded not upon dogma but upon observation and experiment." The book is illustrated with several portraits of Paracelsus, and contains a good bibliography of the principal recent authorities. WILLIAM KIRKBY.

ORGANIC CHEMISTRY, OR THE CHEMISTRY OF THE CARBON COMPOUNDS. By VICTOR VON RICHTER. Edited by Professor R. ANSCHUTZ and Dr. H. MEERWEIN. Volume III., THE CHEMISTRY OF THE HETEROCYCLIC COMPOUNDS, translated by E. E. FOURNIER D'ALBE, D.Sc. Pp. xviii.+326. London: Kegan Paul, Trubner & Co., Ltd. 1923. Price 25s. net.

The chief defect of this English translation of the 1912 German edition of Richter's Organic Chemistry (compare previous review, ANALYST, 1922, **47**, 326) is now remedied by the appearance of Volume III. containing, in an extended form, all those missing parts of what used to be Volume II. before the present edition was undertaken.

The extension into three volumes is now obviously a great advantage to the English chemist, both as regards the increased quantity of material at his command, and also owing to greater convenience for reference, emphasised by the separate Index of Heterocyclic Compounds which has, for the first time, been provided.

It is, unfortunately, inevitable that the work should become less interesting and less readable as it becomes increasingly efficient as a means of rapid reference, and it is also inevitable that this edition should lack mention of the great advances made since 1912.

It is a pity, however, that English and other "foreign" chemists should be reached only indirectly by its aid, through other German works of summarisation and reference, and it is hoped that in the next edition, even now urgently required, translation of such references (and perhaps also of the German system of ring-notation) may be accomplished as well as that of the text has already been.

ARTHUR FAIRBOURE.

THE CHEMISTS' YEAR-BOOK, 1923. Edited by F. W. ATACK, M.Sc., B.Sc. Eighth Edition. Two Vols. Pp. 1107 and Index. Manchester: Sherratt and Hughes. 1923. Price 21s.

Previous editions of this useful publication have been reviewed in the ANALYST (1917, **42**, 406; 1922, **46**, 43), and it is now so well known to chemists that a detailed review is scarcely necessary.

The arrangement and scope of the work follow very closely on the lines of the previous edition, certain sections of which have been corrected and revised,

and to which a short new section on leather analysis has been added, but, on the whole, so little alteration from the last edition is apparent that the review of that edition in the ANALYST (1922, **46**, 431) applies almost exactly to the present edition. In this previous review the following criticism was offered: "These analytical sections, many of them by authors who are admitted experts in their subjects, are unequal in merit. The section on water analysis is inadequate, that on the bacteriological examination of water so inadequate as to be useless; the section on spectrum analysis is very meagre. Such a subject as the 'adulteration of fats'—including oils—can obviously not be dealt with in two pages, . . . a chemist . . . would hardly be helped by the picture of the combustion furnace and accessories and letterpress attached to it." As far as can be judged by a reasonably careful perusal of the points criticised, no serious attempt has been made in the present edition to act on this very justifiable criticism of an earlier edition, and one is rather surprised to find how very closely the present edition follows a much earlier edition, *viz.* the fourth of 1918–1919.

Generally speaking, the tabular matter, which covers a very wide field and omits little of value to chemists in general—it might even be shortened without loss—is excellent, but the analytical sections are, to say the least, still "unequal in merit," and one hopes that this may be remedied in the next edition.

The editing of the analytical sections might be improved. For example, space in a work of this character does not allow any great detail in descriptions of analytical procedure or diagnosis, and therefore references to authoritative books and journals are of the utmost value; in some sections a fair number of references are given; in others, references are conspicuous by their absence. A "pocket-book" section on the analysis of any particular class of materials can only hope to give broad outlines, and should *always* direct the reader to sources of fuller detail.

In spite of the defects referred to, and obvious room for improvement, it is a most useful book, which will often save the chemist from long searches through books and journals which are difficult of access. R. G. PELLY.

THE NITROGEN INDUSTRY. BRITISH ASSOCIATION REPORTS (HULL MEETING, 1922), No. 14. By B. A. REYNOLD. London: British Association. 1923.

The discussion on the post-war progress of the nitrogen fixation processes at the British Association meeting at Hull last year was opened by Dr. J. A. Harker, F.R.S., and papers were also communicated by Mr. J. A. West, Dr. E. B. Maxted and Mr. E. Kilburn Scott. It is clearly evident that the Germans view the nitrogen problem in a national spirit, for since the armistice their production in fixed nitrogen has steadily risen to the astounding value of half a million tons per annum.

In other countries the problem of economical nitrogen fixation obscures the other and more important issues. It is difficult to ascertain which is the cheapest method of providing a nitrogenous fertiliser suitable for agricultural purposes. Undoubtedly, before the war, ammonia production by the Haber process was more

economical than cyanamide production. The increased price of coke, however, and the improvements effected in the conversion of cyanamide into suitable fertilisers, such as ammonia and urea, have swung the pendulum somewhat back towards cyanamide.

It is a sign of the times that electrolytic hydrogen now compares in price with water-gas hydrogen. On the other hand, Claude's high-pressure process is a distinct improvement on the Haber process, provided that the engineering difficulties are not insuperable.

The Haüsser process, described by Mr. Goodwin, must not be taken seriously as a method of manufacture, although, from the purely scientific aspects, in the light of Bone's recent experiments it is one of the most interesting processes.

It is a pity that Dr. Harker did no more than mention the cyanide process, on which a great deal of work is being expended. Prophecy is dangerous, but one might affirm that this is the process of the future. ERIC K. RIDEAL.

Publications Received.

LA TECHNIQUE INDUSTRIELLE DES PARFUMS SYNTHETIQUES. By R. SORNET. Pp. 135. Paris: Gauthier Villars et Cie. 1923. Price 10 fr.

COLLECTED PAPER FROM THE MEDICAL RESEARCH LABORATORIES. Parke, Davis & Co. Reprints Vol. 8 (1919–1920). Pp. 619. 1923.

BIBLIOGRAPHY OF COLLOID CHEMISTRY. By H. N. HOLMES. Preliminary Edition. Washington: National Research Council. 1923. Price $1.00.

MANUFACTURE OF ACIDS AND ALKALIS.

Vol. I.: RAW MATERIALS FOR THE MANUFACTURE OF SULPHURIC ACID AND THE MANUFACTURE OF SULPHUR DIOXIDE. By W. WYLD. Pp. 558. Price 36s. net.

Vol. V.: THE MANUFACTURE OF HYDROCHLORIC ACID AND SALT CAKE. By A. C. CUMMING. Pp. 425. Price 31s. 6d. net. London: Gurney & Jackson.

AN INTRODUCTION TO THEORETICAL AND APPLIED COLLOID CHEMISTRY. By W. OSTWALD. Translated from the 8th German Edition by M. H. FISCHER. Pp. 266. New York: Wiley; London: Chapman & Hall. 1923. Price 12s. 6d. net.

ANALYTICAL MICROSCOPY. By T. E. WALLIS. Pp. viii.+149. London: E. Arnold & Co. 1923. Price 6s.

SUPPLEMENTARY NOTES ON GRAVIMETRIC ANALYSIS. By W. LOWSON. Pp. v.+54. London: Longmans, Green & Co. 1923. Price 2s. 6d.

PRACTICAL BACTERIOLOGY FOR CHEMICAL STUDENTS. By D. ELLIS. Pp. 136. London: Longmans, Green & Co. 1923. Price 4s. 6d. net.